Stem Cell Production: Overcoming the Technical and Commercial Challenges

By Dave Thomas at TAP Biosystems

In order to meet the expanding market for stem cells in both clinical and drug discovery applications, automated, cost-effective, large-scale manufacturing processes are required to overcome the technical and commercial challenges posed by traditional cell culture methods.

The burgeoning numbers of stem cells required for cell therapy and drug discovery applications have created a strong market driver for the development of efficient manufacturing methods. Differentiating stem cells into desired cell lineages and then expanding them *ex vivo* is technically challenging and can be prohibitively expensive, particularly when produced manually using traditional cell culture methods.

If stem cells are to be used routinely for clinical or drug discovery applications, they need to be produced at an affordable cost of goods (COG). This means that cells have to be made on an industrial scale under optimal conditions and using as small amounts of expensive media and growth factors as is practical. Given these limitations, it is likely that manufacturing methodologies will use partially or fully automated systems. This article discusses methods of *ex vivo* expansion and differentiation of stem cells, describes the features of systems currently in use and reviews the cell culture strategies that may help to reduce manufacturing costs.

Drivers for Bulk Stem Cell Production

In the past decade, there has been increasing interest in the use of stem cells as therapies and also as tools for drug discovery. The use of haematopoietic stem cells (HSCs), first used in the 1950s, is a good example of successful stem cell therapy and has been widely utilised for treating diseases such as adult T-cell leukaemia-lymphoma and multiple myeloma (1). For regenerative medicine, mesenchymal stem cells (MSCs) are proving to be a popular cell type and are currently being utilised in 127 clinical trials to treat various conditions including stroke, graft versus host disease, multiple sclerosis and diabetes (2). MSCs are attractive because they can be isolated from a range of tissues, can differentiate into specific cell types and have a highly proliferative capacity *ex vivo*. They also secrete factors that promote tissue regeneration and exhibit low immunogenicity and immunomodulatory profiles. This means they have the potential to be safe and effective for addressing these therapeutic applications.

HSCs and MSCs are being applied in the clinic as either allogenic or autologous therapies. As an allogenic therapy, one batch of cells would be used to treat multiple patients. This production method, which mirrors the traditional pharmaceutical business model, is potentially easier to scale up and may therefore be lower in cost. The disadvantage is that there may be a risk of an immune rejection by the patient. With autologous therapy, a single patient-specific batch is created, with cells being taken from an individual, scaled up and then returned to the same individual. This represents a very different and more of a service-model of drug development, and may be more expensive to implement. However, the benefit with autologous therapies is that there may be less risk of adverse immune reactions.

In drug discovery applications, induced pluripotent stem cells (iPSCs) are becoming a popular option for studying disease pathogenesis and screening drug candidates. These stem cell types have unlimited self renewal capacity, can be differentiated into specific cell types and allow the production of a sufficient cell number to investigate the disease states of interest. This is particularly useful with neurological conditions, where patient brain tissue samples are rarely available in large quantities and animal models do not accurately reflect the specific human disease (3,4).

One barrier to the routine use of HSCs, MSCs or iPSCs as therapies or drug discovery tools is the cost of producing them at high densities (typically in the order of 10^9-10^{11} cell/ml) and in large volumes. This is because many of the culture processes used were originally

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developed at the laboratory scale using traditional cell culture methods, and are therefore very labour-intensive. Additionally, these processes have not been optimised to generate high cell yields using minimal amounts of expensive reagents; thus, when translated to large-scale manufacture, the COG becomes prohibitive and application of the cells in a large target market is not commercially viable.

Process Development

Stem cells can be cultured *ex vivo* and their populations expanded and differentiated in either adherent or suspension culture. Since allogenic stem cell therapies are generally meant to treat large patient numbers, this type of therapy may be more cost-effectively produced in bioreactors. Using bioreactors may yield greater cell numbers than the traditional flask- or plates-based methods. It may also reduce testing costs as only a single batch of cells is subjected to quality checks, whereas pooling of flasks/plates could result in multiple batches, each subject to its own quality checks. This may increase the cost of quality control and thus the overall COG.

The low *ex vivo* cell density of HSCs from current bioreactor manufacturing systems has been a hindrance to their adoption in the production of allogenic stem cell therapies. To improve cell numbers, many stem cell facilities believe the key is to identify and optimise those factors that affect cell differentiation and expansion.

One strategy being assessed is to use manual, rotary, orbital suspension culture, but this type of culture method is not as practical for bioprocess modelling as it is time-consuming and makes it almost impossible to evaluate a wide range of media and culture conditions in parallel (5). A newer approach is to use an automated bioprocess

mimic (see Figures 1 and 2). This can be used to study essential culture parameters such as pH, dissolved oxygen and metabolites, which can then be applied to the development of optimised media formulations and feed strategies (6). The result is cost-effective culture processes for growing stem cells to a high density, or decreasing the per-unit reagent cost.

The use of a bioreactor for scale-up is dependent on the cell type being non-adherent and thus capable of being expanded in suspension culture. Unfortunately, a large number of cell types are not amenable to suspension culture. A technique that attempts to overcome this issue is the use of microcarriers, whereby stem cells adhere to microcarriers being maintained in suspension in the bioreactor. This has achieved some success with MSCs (7), but there is evidence suggesting stem cells cultured in conventional bioreactors show subtle preferences in differentiation towards certain lineages, temporal modulation of gene expression, and changes in the relative efficiencies of differentiated phenotypes (8).

An alternative is to use hollow fibre bioreactors for stem cells, and this

is achieving some success with expansion of HSCs (9). One drawback, however, is the difficulty in assessing cell morphology, confluence and viability *in situ* in real time, as cells either have to be harvested for

Figure 1 (above): The ambr automated micro-bioreactor workstation

Figure 2 (left): The ambr micro-bioreactor

Images: TAP Biosystems

Figure 3:
CompacT SC automated system for expansion and differentiation of stem cells in T-flasks

assessment or assessed using indirect measurement methods such as glucose consumption, albumin or urea production. Thus, scheduling the correct timing for addition of media, cytokines or growth factors can be problematic.

An alternative approach is to automate the traditional flask-based cell culture process. This allows stem cells to be cultured in parallel, thereby achieving high throughput and productivity while allowing them to be analysed for a number of parameters *in situ* in real time. Automated, flask-based stem cell culture is already being performed successfully on TAP's automated flask handling platform, the CompacT SelecT, and several examples of expansion and differentiation methods for human stem cells exist (10-14).

The platform was originally designed to automate manual cell culture processes for multiple different cell lines in T-175, Triple-layer and HYPERflasks. The system has a robotic arm that automatically transfers flasks between the integrated incubation and cell analysis equipment, and can make reagent additions or harvest cells according to cell-line-specific parameters. The system operates under negative pressure laminar airflow, thereby preventing any contamination, and is capable of continual unattended operation.

Process Optimisation

Maintaining pluripotent stem cells or inducing them to differentiate can be very labour-intensive as scientists have to constantly monitor the cells and add small volumes of growth factors or cytokines at specific times in order to mimic physiologically relevant conditions. In an ideal world, these manipulations would occur when it is optimal for the cells, but as this may be overnight or during weekends, the reality is that they often happen at a time that is more convenient for the scientist. This lack of consistency in process can lead to significant variations in cell quality.

To address these challenges, TAP worked with I-Stem (Institute for Stem cell Therapy and Exploration of Monogenic Diseases) in France to develop a CompacT SelecT derivative, the CompacT SC (Figure 3). This is more focused on addressing the issues associated with automated flask-based culture of stem cells.

Scientists at I-Stem are working with human skin, muscle, neuronal and retinal stem cells, and need automation to improve the reproducibility of cell culture processes by enabling the precise timing and execution of processing steps – such as measuring confluence, cell passaging and directed differentiation down specific cell line lineages.

The system at I-Stem has T75 flask processing, low volume reagent dispensing, integrated real-time imaging and cell viability monitoring capabilities. These features enable scientists at I-Stem to achieve optimal conditions for their stem cells by automatically monitoring cell growth, confluence and viability *in situ* and in real time. Automated imaging enhances quality control of the culture process and allows researchers to generate growth curves based on confluence measurement, as well as accurately determine population doubling times. This provides the information that the system needs to automatically passage flasks at pre-determined confluence levels without intervention from researchers. The system can also store a range of growth factors or cytokines, and be programmed to transfer low volumes of these into flasks at specific times, thus maintaining cells in a particular state of pluripotency or differentiation without any manual intervention.

Using this stem cell culture system, I-Stem scientists have optimised a process for expansion of human iPSCs to give a 130,000-fold amplification in cell number in 20 days, providing high cell yields of around 1×10^{11} cell/ml (15). They have also optimised a process for expansion of human MSCs, and have validated expansion of human MSCs into 6-, 24- and 96-well plates. Their automated process for MSC production generates a 10,000-fold amplification of cell numbers in 16 days to provide a yield of around 1×10^{10} cell/ml (15). The MSCs analysed by fluorescence-activated cell sorting (FACS) show well-differentiated phenotypes with the expression of appropriate surface markers.

The studies at I-Stem demonstrate that automated T-flask cell culture technology is capable of providing high cell densities and standardised batches of stem cells that could

be used to supply cell therapies in pre-clinical models or clinical applications, and high-throughput or high-content screening applications.

Conclusion

For large-scale, *ex vivo* production of stem cells to be both possible and affordable, automated systems are required that generate sufficient cell yields with the correct cell lineage, while using minimal amounts of expensive reagents. Traditional bioreactors may be the right solution for production of allogenic stem cell therapies, but scale-up protocols still require optimisation, and bioreactors cannot be used for adherent stem cells unless microcarriers become a viable technology.

Hollow fibre bioreactors offer promise for expansion of adherent stem cells, but do not currently have the capability for real-time analysis of many stem cell viability and lineage parameters. This makes it difficult to automate the addition of reagents at the optimal time required to maintain cells in a pluripotent or differentiated state.

Automated T-flask cell culture processing offers a high-throughput, parallel workflow, where stem cells can be analysed in real-time and optimally processed to facilitate expansion

Dr Dave Thomas is a Product Manager for Integrated Cell Culture Systems at TAP Biosystems (Royston, UK). He has worked at TAP since 2007; prior to this, he was a research scientist for several years and held positions in the pharma, biotech and CRO sectors, and in academic research at the University of Cambridge (UK). Dave's expertise is underpinned by a PhD in Cell Biology from the University of Cambridge, and a degree in Physiology & Pharmacology from the University of Southampton (UK). Email: dave.thomas@tapbiosystems.com

and/or differentiation. Using this technology, stem cells have been rapidly expanded to give high cell yields, making this an ideal technology for the cost-effective production of consistently high-quality stem cells for use in drug discovery programmes, as well as many critical clinical settings.

References

1. Thomas ED, Lochte HL Jr, Lu WC and Ferrebee JW, Intravenous infusion of bone marrow in patients receiving radiation and chemotherapy, *N Engl J Med*, 257(11): pp491-496, 1957
2. Clinical trials website, Visit http://clinicaltrials.gov
3. Mattis VB and Svendsen CN, Induced pluripotent stem cells: a new revolution for clinical neurology? *Lancet Neurol* 10(4): pp383-394,2011
4. Ito D *et al*, Accelerating progress in induced pluripotent stem cell research for neurological diseases, *Ann Neurol* 72(2): pp167-174, 2012
5. Kinney MA, Sargent CY and McDevitt TC, The multiparametric effects of hydrodynamic environments on stem cell culture, *Tissue Eng Part B Rev* 17(4): pp249-262, 2011
6. Ratcliffe E, Glen KE, Workman VL, Stacey AJ and Thomas RJ, A novel automated bioreactor for scalable process optimisation of haematopoietic stem cell culture, *J Biotechnol* 161(3): pp387-390, 2012
7. Eibes G *et al*, Maximising the *ex vivo* expansion of human mesenchymal stem cells using a microcarrier-based stirred culture system, *J Biotechnol* 146(4): pp194-197, 2010
8. Kowalczyk M, Waldron K, Kresnowati P and Danquah MK, Process challenges relating to hematopoietic stem

cell cultivation in bioreactors, *J Ind Microbiol Biotechnol* 38(7): pp761-767, 2011
9. Housler GJ *et al*, Compartmental hollow fibre capillary membrane-based bioreactor technology for in vitro studies on red blood cell lineage direction of hematopoietic stem cells, *Tissue Eng Part C Methods* 18(2): pp133-142, 2012
10. Thomas RJ and Ratcliffe E, Automated adherent human cell culture (mesenchymal stem cells), *Methods Mol Biol* 806: pp393-406, 2012
11. Thomas RJ *et al*, Automated, scalable culture of human embryonic stem cells in feeder-free conditions, *Biotechnol Bioeng* 102(6): pp1,636-1,644, 2009
12. Thomas RJ *et al*, Automated, serum-free production of CTX0E03: a therapeutic clinical grade human neural stem cell line, *Biotechnol Lett* 31 (8): pp1,167-1,172, 2009
13. McLaren D *et al*, Automated large-scale culture and medium-throughput chemical screen for modulators of proliferation and viability of human induced pluripotent stem cell-derived neuroepithelial-like stem cells, *J Biomol Screen*, epub ahead of print, 4 October 2012. Visit http://jbx.sagepub.com/content/early/2012/10/03/1087057112461446.abstract)
14. Hook L *et al*, Non-immortalised human neural stem cells as a scalable platform for cellular assays, *Neurochem Int* 59(3): pp435-444, 2011
15. Laabi Y, CompacT SelecT cell culture automation of human pluripotent stem cells and their progenies, ELRIG presentation, 18 April 2012. Visit www.elrig.org/index.php/events/drug-discovery-2012/975-yacine-laabi-istem