Downstream processing challenges and opportunities for cell-based therapies

Qasim A. Rafiq and Fernanda Masri

Abstract

With increasing numbers of advanced therapies including cell and gene therapies entering early and late-stage clinical development, significant focus has been directed towards the development of scalable, robust manufacturing processes. Much of the research and development activity in the sector has addressed the upstream challenges with particular emphasis on large-scale cell expansion, however equally important is the development of large-scale harvesting and downstream processing operations. This paper presents and summarises some of the challenges associated with the downstream processing of these advanced therapies and highlights recent advancements in the technologies suitable for such applications.

Introduction

The unavoidable transition towards an older aging population has become a significant medical challenge faced by many developed and developing economies. The increased demand for hospital and social care coupled with complex medical interventions, has led to spiralling costs for healthcare systems globally. Advanced therapies, including cell and gene-based therapies (CGTs) have the potential to address previously unmet patient needs, improving overall healthcare costs as well as tackling the social and economic effects of chronic and age-related conditions. However, before this can be realised, it is critical that robust, scalable and cost-effective manufacturing processes, that retain cell functionality, are developed. Unlike traditional manufacture of monoclonal antibodies (mAbs) and other biologics, the production and processing of cellular material, forms the basis of the product for CGTs [1] . As such, manufacturing processes need to be adapted to ensure that the cellular material retains the critical quality attributes (CQAs) throughout the entire process [2, 3].

As with the production of mAbs, significant research and development for CGT processes has focused on the intensification of upstream processes. For example, several studies have investigated the optimization of cell expansion processes of adherent human cells. Significant progress has been made in recent years with densities for human mesenchymal stem cells (hMSCs) cultured on microcarriers now reaching densities of $>1 \times 10^6$ cells mL^{-1} [4] and human pluripotent stem cells (hPSCs) reaching densities of $>2\text{-}3 \times 10^6$ cells mL^{-1} [5]. Whilst R&D activity should continue focusing on improving cell density, the shift of the manufacturing bottlenecks is inevitably leaning towards downstream processing (DSP) [6]. The DSP activities for cell therapies are those unit operations and processes which arise post after the cellular expansion stage and, depending on the therapy, can include harvesting, washing, cell concentration, formulation, final fill and finish and preservation.

Despite the fact that most CGT manufacturing processes include a clarification and concentration step of the cellular material derived from cell culture, DSP remains largely unaddressed. Typically, a swing-bucket rotor centrifugation step or a filtration step is included to remove cell debris, large aggregates, unwanted proteins, volume reduce (concentrate) and formulate the cellular suspension into a patient-friendly or preservation-friendly buffer [6]. In the vast majority of expansion studies, there is little or no mention of the harvest and subsequent DSP of the cells. For anchorage-dependent cells, dissociation into single-cell suspension whilst retaining the CQAs is essential. Current available DSP platforms generally offer either high specificity or high throughput, but rarely both. Whilst highly specific labelling separation systems may be suitable within low volume production (proof-of-principle, small scale studies or autologous applications), it is extremely unlikely these will meet the

demands in the allogeneic manufacturing environment. Equally, DSP techniques adapted from protein production for CGTs have the capacity to handle the scales required for allogeneic therapies but are hindered by the lack of specificity and sensitivity to target cell isolation. With increasing volumes, cell densities and more stringent target product profiles to meet regulatory requirements, the need for efficient, highly specific and scalable DSP techniques to effectively purify and formulate the final product, will be just as essential as cell expansion.

Harvest

The harvesting of cells is the process of removing the cells from the culture vessel (stirred-tank bioreactors, hollow-fibre/packed-bed reactors, gas-permeable membrane reactors, etc.) after expansion. Given the wide variety of CGTs, the use of both anchorage-dependent and suspension cells, in addition to a multiple array of culture devices are used. Therefore, the harvesting protocol is likely to be process-specific and require optimisation in each instance. In most instances, the process is likely to involve the use of an animal-free recombinant enzymatic dissociation reagent such as recombinant trypsin and some physical force exerted through an increase in impeller agitation, bioreactor rotation or increased flow rates depending on the culture vessel employed. For example, the harvesting of cells using anchorage-dependent hMSCs cultured in stirred-tank bioreactors on microcarriers requires a combination of enzymatic dissociation and mechanical force (through impeller agitation) to aid in the successful detachment of cells from the microcarriers *in situ* [7].

This procedure has been employed successfully for a range of anchorage-dependent cell types in a range of bioreactor systems and in all cases (Figure 1), importantly, demonstrated retention of the CQAs [8] despite concerns that the increase in agitation will result in cell-damaging shear forces. This harvesting method has demonstrated that it is possible to increase impeller agitation. This aids in the rate of cell detachment, whilst ensuring that the Kolmogorov scale remains greater than the size of the cell once it has been detached and therefore unlikely to cause cell damage. Such an approach led to > 95% harvesting efficiency with the cells demonstrating retention of CQAs, despite a five-fold increase in the impeller agitation (compared to the original culture agitation) and a Kolmogorov scale of ~ 30 μm, larger than the average diameter of the cells (~15 μm) [7]. This *in situ* detachment procedure should be scalable to larger, industrial-scale bioreactors having been established on well-understood theoretical mixing principles. Moreover, given the detachment procedure is undertaken *in situ*, it eliminates the need for an additional processing step outside of the culture vessel, reduces contamination risk and decreases time between process steps.

Although the procedure is likely to involve the use of a recombinant dissociation reagent, the harvest of a hollow-fibre or packed-bed bioreactor culture is likely to be different and may involve extended contact with the dissociation reagent, increased flow rates, and/or rotation of the culture vessel. In all instances of the harvesting procedure, it is important, particularly where mechanical forces are used to dissociate cells, that no additional debris or particulates arise due to the microcarrier/cell-substrate damage. Such an outcome would result in additional burden on the DSP operations to remove such particulates. Some groups have demonstrated successful culture of cells on biodegradable microcarriers [9] to overcome this concern, yet this may still pose a concern for final formulation and patient administration.

Advances in biomaterial formulation have facilitated the development of alternative approaches for cell harvesting with multiple studies demonstrating the potential to remove the need for enzymatic dissociation reagents and mechanical agitation to facilitate successful harvest. Such approaches include the use of environmentally responsive polymers to coat microcarriers which enable both the culture of anchorage-dependent cells and the subsequent detachment of the cells through a change

in the culture temperature and/or pH [10, 11]. Although promising, there is a concern that non-enzymatic methods for cell dissociation results in the formation of cell sheets rather than a single-cell suspension; this may be inappropriate for applications that require intravenous administration.

Volume reduction, wash and formulation

CGTs to date use swing-bucket rotor centrifugation for cell washing and concentration. A biological safety cabinet usually is needed for the subsequent resuspension step, which involves discarding spent medium and resuspending in a suitable buffer. This process may be repeated multiple times to ensure the complete removal of any unwanted reagents and/or particles. However, centrifugation has proven to be a difficult step to automate while maintaining sterility [1] and can pose a problem to achieve the required cell numbers for the manufacture of highly concentrated CGTs [12]. Alternatives to swing-bucket rotor centrifugation, include continuous counterflow centrifugation systems and filtration systems which can be more amenable to large-scale, contained and automated operations [6].

Counterflow centrifugation systems, such as kSep® (KBI Biopharma) and Elutra cell separation system (Terumo BCT), can keep the cells in suspension whilst washing them, clearing out residuals from the supernatant and exchanging the buffer. kSep® in particular can be fully automated and contained, uses a disposable flow path, is scalable from 0.1 L to >1000 L and can achieve high cell recoveries (>80%) and high cell viabilities (>90%). The capital investment needed, together with the cost of disposables, demands careful consideration when carrying out process development [6].

Tangential flow filtration (TFF) offers an alternative that can also be scaled, automated, fully contained and, generally, involves a lower capital investment and disposables costs, which may make it more amenable to process development activities [6]. For instance, for the anchorage-dependent cells, after the cells are enzymatically dissociated into a single-cell suspension (whether that is detachment from microcarriers, culture surfaces, colonies or embryoid bodies), these are generally quenched with culture media to preserve cell health by preventing the enzyme from further digesting cell components. More specifically, for the hMSC microcarrier-based expansion outlined previously, cell detachment from the microcarrier is only one aspect of the harvesting procedure. Assuming the microcarriers are not biodegradable and not part of the final product formulation, the next DSP step is likely to be the separation of cells from microcarriers by size exclusion techniques. For example, dead end filtration using a vacuum as a driving force was employed in the aforementioned hMSCs studies [7, 8]. However, this technique, although suitable for the purposes of the study, is not a scalable technique like TFF. Other groups have used TFF and expanded bed chromatography (EBC) as continuous, integrated alternative techniques for the improved washing and concentration of hMSCs [13, 14]. Cunha and colleagues showed high protein clearance (98%), high recovery of viable hMSCs (70%) with no impact on cell viability (95%) or other CQAs (morphology, immunophenotyped, proliferation, adhesion capacity and multipotent differentiation potential) [13, 14]. Furthermore, these studies show shorter processing times when using continuous filtration techniques as opposed to discountinuous operations.

Another recent technique that is showing promise for the DSP of CGTs is acoustic filtration [15, 16]. Acoustic filtration uses standing waves to capture, separate and concentrate particles in a fluid without having a physical barrier (membrane or filter) like traditional filtration systems. It is therefore considered suitable for shear-sensitive cell lines and potentially allows for higher throughput, together with less clogging issues than traditional membrane based systems, extending the lifetime of the disposables.

Addressing the DSP challenges

Due to the relative infancy of the CGT DSP space, a large proportion of the research and development activity needs to focus on effective process development and understanding of the critical process parameters (CPPs) at the small scale. The development of bench-scale technologies which better mimic the full scale will be paramount to the rapid development of robust DSP processes and allow cost-effective screening of the susceptibility of cell lines to operating conditions for CGTs [17]. To date, some efforts have focused on the development of ultra scale-down (USD) tools to mimic various large scale operations such as continuous centrifugation [18] dead end filtration [19, 20] and membrane separation [21, 22] (Table 1). USD devices allow the investigation of several manufacturing hydrodynamic environments [21] and geometries [23] with little material, increasing the throughput of the experimental phase in a cost-effective and time-efficient manner and ultimately, expediting process development of DSP operations.

Delivering effective DSP technologies and robust processes with the capacity to target specificity and throughput is required to ensure that advances in upstream processing are maximised. Effective scale-down models, adequately characterised, may facilitate a thorough understanding of the CPPs and expedite process optimisation and parameter screening studies in a cost-effective and timely manner. Moreover, further process understanding and knowledge of the product's CQAs will be key to develop effective and suitable DSP solutions with the capacity to scale and quality requirements of CGT products.

References

1. Mason, C. and M. Hoare, *Regenerative medicine bioprocessing: the need to learn from the experience of other fields.* Regen Med, 2006. **1**(5): p. 615-23.
2. Carmen, J., et al., *Developing assays to address identity, potency, purity and safety: cell characterization in cell therapy process development.* Regen Med, 2012. **7**(1): p. 85-100.
3. Bravery, C.A., et al., *Potency assay development for cellular therapy products: an ISCT review of the requirements and experiences in the industry.* Cytotherapy, 2013. **15**(1): p. 9-19.
4. de Soure, A.M., et al., *Scalable microcarrier-based manufacturing of mesenchymal stem/stromal cells.* Journal of Biotechnology, 2016. **236**: p. 88-109.
5. Kropp, C., et al., *Impact of Feeding Strategies on the Scalable Expansion of Human Pluripotent Stem Cells in Single-Use Stirred Tank Bioreactors.* Stem Cells Transl Med, 2016. **5**(10): p. 1289-1301.
6. Pattasseril, J., et al., *Downstream Technology Landscape for Large-Scale Therapeutic Cell Processing.* Bioprocess International, 2013. **11**(3): p. 38-47.
7. Nienow, A.W., et al., *A potentially scalable method for the harvesting of hMSCs from microcarriers.* Biochemical Engineering Journal, 2014. **85**(0): p. 79-88.
8. Nienow, A.W., et al., *Agitation conditions for the culture and detachment of hMSCs from microcarriers in multiple bioreactor platforms.* Biochemical Engineering Journal, 2016. **108**: p. 24-29.
9. Sun, L.Y., et al., *Cell proliferation of human bone marrow mesenchymal stem cells on biodegradable microcarriers enhances in vitro differentiation potential.* Cell Prolif, 2010. **43**(5): p. 445-56.
10. Yang, H.S., et al., *Suspension culture of mammalian cells using thermosensitive microcarrier that allows cell detachment without proteolytic enzyme treatment.* Cell Transplantation, 2010. **19**(9): p. 1123-32.
11. Hanga, M.P. and R.G. Holdich, *Membrane emulsification for the production ofuniform poly-N-isopropylacrylamide-coatedalginate particles using internal gelation.* Chemical Engineering Research and Design, 2014. **92**: p. 1664-1673.

12. Lapinskas, E., *Scaling up research to commercial manufacturing.* Chemical Engineering Progress, 2010. **106**(11): p. 44-49.

13. Cunha, B., et al., *Filtration methodologies for the clarification and concentration of human mesenchymal stem cells.* Journal of Membrane Science, 2015. **478**: p. 117-129.

14. Cunha, B., et al., *Improving washing strategies of human mesenchymal stem cells using negative mode expanded bed chromatography.* J Chromatogr A, 2016. **1429**: p. 292-303.

15. Dawson, J.I., et al., *Enhancing the osteogenic efficacy of human bone marrow aspirate: concentrating osteoprogenitors using wave-assisted filtration.* Cytotherapy, 2013. **15**(2): p. 242-252.

16. Shields, C.W., C.D. Reyes, and G.P. López, *Microfluidic Cell Sorting: A Review of the Advances in the Separation of Cells from Debulking to Rare Cell Isolation.* Lab on a chip, 2015. **15**(5): p. 1230-1249.

17. Masri, F., *Uncovering the potential of ultra scale-down tools to enable cost-effective "Quality by Design".* Cell Gene Therapy Insights, 2016. **2**(4): p. 463-471.

18. Hutchinson, N., et al., *Shear stress analysis of mammalian cell suspensions for prediction of industrial centrifugation and its verification.* Biotechnol Bioeng, 2006. **95**(3): p. 483-91.

19. Jackson, N.B., J.M. Liddell, and G.J. Lye, *An automated microscale technique for the quantitative and parallel analysis of microfiltration operations.* Journal of Membrane Science, 2006. **276**(1–2): p. 31-41.

20. Lau, E.C., et al., *An ultra scale-down characterization of low shear stress primary recovery stages to enhance selectivity of fusion protein recovery from its molecular variants.* Biotechnol Bioeng, 2013. **110**(7): p. 1973-83.

21. Masri, M.F., et al., *An ultra scale-down methodology to characterize aspects of the response of human cells to processing by membrane separation operations.* Biotechnol Bioeng, 2017.

22. Ma, G., et al., *Mimic of a large-scale diafiltration process by using ultra scale-down rotating disc filter.* Biotechnol Prog, 2010. **26**(2): p. 466-76.

23. Acosta-Martinez, J.P., et al., *Ultra scale-down stress analysis of the bioprocessing of whole human cells as a basis for cancer vaccines.* Biotechnol Bioeng, 2010. **107**(6): p. 953-63.

24. Boychyn, M., et al., *Characterization of flow intensity in continuous centrifuges for the development of laboratory mimics.* Chemical Engineering Science, 2001. **56**(16): p. 4759-4770.

25. Delahaye, M., et al., *An ultra scale-down analysis of the recovery by dead-end centrifugation of human cells for therapy.* Biotechnology and Bioengineering, 2015. **112**(5): p. 997-1011.

26. McCoy, R., M. Hoare, and S. Ward, *Ultra scale-down studies of the effect of shear on cell quality; Processing of a human cell line for cancer vaccine therapy.* Biotechnol Prog, 2009. **25**(5): p. 1448-58.

27. Titchener-Hooker, N.J., P. Dunnill, and M. Hoare, *Micro biochemical engineering to accelerate the design of industrial-scale downstream processes for biopharmaceutical proteins.* Biotechnol Bioeng, 2008. **100**(3): p. 473-87.
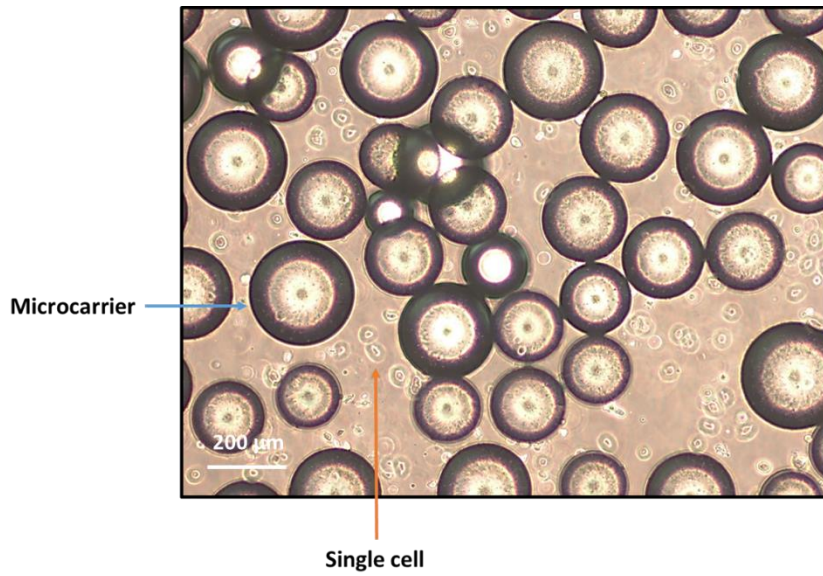
Figures/Tables:

Microcarrier

200 µm

Single cell

Figure 1: Phase-contrast image of single hMSCs in suspension following detachment from microcarriers

DSP Unit Operation	Reference
Continuous centrifugation	Bocyhyn *et al.* 2001 [24] Hutchinson *et al.* 2006 [18]
Batch centrifugation	Delahaye *et al.* 2015 [25] Hutchinson *et al.* 2006 [18] McCoy *et al.* 2009 [26]
Chromatography	Titchener-Hooker *et al.* 2008 [27] Hutchinson *et al.* 2006 [18]
Membrane separation (TFF)	Masri *et al*. 2017 [21] Ma *et al.* 2010 [22]
Depth filtration	Jackson *et al.* 2006 [19] Lau *et al.* 2013 [20]
Fill and finish (capillaries)	Acosta-Martinez *et al.* 2010 [23]

Table 1: Overview of ultra scale-down tools reported in literature as predictive tools for several different large-scale downstream processing steps and/or processing parameters