Pharmaceutical BIOPROCESSING

Solving hydrodynamic issues in industrial animal cell culture: cellular adaptation and engineered systems

"Industrial animal cell culture for cell therapy is now roughly where it was for recombinant protein production in the 1980s, but the path going forward will be different, with a greater reliance on engineered systems."

Keywords: adaptation • animal cell culture • cell therapy • hydrodynamics

Matthew S Croughan
Keck Graduate Institute, 535 Watson Drive, Claremont, CA 91711, USA
matthew_croughan@kgi.edu

Early history of hydrodynamic issues regarding recombinant animal cells

In the early 1980s, recombinant animal cells were first employed to make glycosylated proteins for clinical trials [1]. At the same time, many academics and biotechnology firms started or expanded their research programs concerning hydrodynamic effects on animal cells. Animal cells were considered highly susceptible to lysis upon scale-up in stirred-tank bioreactors, due to their relatively large size and lack of cell wall. Many in the biotechnology community were concerned about the feasibility of large-scale production of biopharmaceutical proteins from recombinant animal cells. Although a few established firms had successfully scaled up cultures of nonrecombinant cells for other products, such as veterinary vaccines [2], it was not widely known how they had done it and whether it would translate to recombinant cells.

To move forward quickly into the clinic and also provide time for the hydrodynamic issues to be addressed, biopharmaceutical proteins from recombinant animal cells were initially manufactured through scale-out of a laboratory method, specifically growth of cells in roller bottles. As efficacious dose levels, patient numbers and initial required manufacturing capacities were determined, scale-out of roller bottles to large automated plants was adequate for some products, such as erythropoietin [3], but not others.

To address hydrodynamic issues, at least three engineered system strategies were employed, sometimes in combination: novel, low-shear bioreactor designs [4]; cell immobilization technologies wherein the cells were protected from hydrodynamic forces by, for instance, membranes or pore walls [5,6]; and modifications to stirred tank or airlift bioreactors to allow large-scale culture of animal cells attached to microcarriers or as unattached, free-floating cells in suspension (suspension culture) [2,7–9]. At the time, many did not know if recombinant Chinese Hamster Ovary (CHO cells, usually grown in an anchored format for transfection, could be readily and reproducibly adapted to suspension culture, nor did they necessarily have the luxury of delaying development timelines to attempt those adaptations. Furthermore, it was unclear if such transformed cells grown in suspension (continuous cell substrates) would be found acceptable for commercial production of human pharmaceuticals, due to concerns over residual contamination with oncogenes, host cell proteins or transforming virus [10]. Thus, work with anchored cells, including growth on microcarriers, continued to receive substantial attention through the early 1990s.

Cellular adaptation to suspension culture

From the mid-1980s to early 1990s, many firms successfully adapted their recombinant CHO cell lines from an anchored format to suspension culture. Furthermore, it became clear that downstream processing could successfully be employed to remove and/or inactivate virus and any oncogenic DNA or host cell proteins down to safe levels [11]. As such, rhu-tPA, the first clinical biopharmaceutical protein made from

FUTURE SCIENCE part of fsg

recombinant CHO cells grown in suspension culture in stirred tanks, was approved by the regulatory agencies in 1987. Since that time, a few firms still use cells grown on microcarriers for the production of recombinant proteins, while most use CHO, NS0 or other continuous cell lines grown in suspension culture. With suspension cultures, passaging of cells can be done through simple dilution or transfer and does not require removal of attached cells from a surface. Furthermore, cells in suspension are generally less susceptible to hydrodynamic death versus cells attached to microcarriers [12].

When exposed to hydrodynamic forces, especially from turbulent eddies somewhat smaller than microcarriers, cells attached to microcarriers cannot freely rotate or move in response, and thus may suffer damage or death. Hydrodynamic cell death has been noted when turbulence leads to eddies of about 130 microns or less, about two-thirds the size of a typical microcarrier [8]. Put simply, cells in suspension are much smaller than such eddies and, when exposed to them, can often just follow streamlines without damage [9,12].

Cellular adaptation to stirred-tank bioreactors

Cellular adaptation to the hydrodynamic environment in stirred-tank bioreactors is not well-documented in the literature and its significance not well-understood, particularly among those new to the field. This situation motivated this editorial, with an understanding that not all key points can be supported by a published reference.

Even though cells in suspension are usually much smaller than the smallest turbulent eddies at typical agitation rates, certain suspension cell lines have nonetheless been found to be susceptible to lysis upon scale-up in stirred tanks. This has most often been observed for cells derived from static flask cultures in molecular biology labs, and rarely seen for cells passaged for extended periods in laboratory-scale or pilot-scale stirred tanks.

In 1989, Petersen [13] found that hybridomas could be gradually adapted, over several months, to approximately 25% increase in impeller rotation speed, up to 235 rpm. In 1991, Kramer *et al.* [14] found that recombinant CHO cells were initially killed (down to approximately 20% viability) in a bioreactor run at 793 rpm with a Rushton impeller, but after several weeks of such exposure, eventually grew as well as control cultures at low revolutions per minute (rpm). In 1992, Schmid *et al.* [15] found that hybridoma cells could be gradually adapted to high agitation, but that immediate exposure of unadapted cells to the same conditions (720 rpm) resulted in rapid cell death.

By the early to mid-1990s, it became apparent that prior adaptation of production cells to the hydrodynamic environment in stirred tanks was a common feature of every successful scale-up story, whether or not it was understood or had been done intentionally. Accordingly, prior adaptation of all production lines, as well as even some host cell lines, to the hydrodynamic environment in stirred tanks was soon employed by industry leaders to partly enable subsequent, successful scale-up of each line.

Hydrodynamic death in stirred tank bioreactors can occur not only through turbulence from the impeller, but also from forces generated by sparging air/oxygen bubbles [7,9,12]. To avoid cell death from sparging, Pluronic F-68 or other protective agents are added as supplements to the culture medium [12]. A few industrial processes are run without such protective agents. This puts greater demands on bioreactor engineering design and/or cellular adaptation.

Current hydrodynamic issues for recombinant animal cells

As discussed above for the production of proteins from recombinant animal cells, the impact of hydrodynamic forces upon scale-up was partly resolved through cellular adaptation, first to suspension culture, then to the hydrodynamic environment in stirred tanks. This was done in parallel with engineered system approaches, such as research and design of stirred tanks for suspension culture, as well as engineering design of other processing equipment, such as cross-flow filtration units and centrifuges for medium exchange or harvest. For the last 20 years or so, there have still been occasional issues with hydrodynamics, such as cell lysis in harvest units leading to antibody reduction [16].

Recently, there is a drive to greater process intensification, to provide for clinical and eventual commercial production in single-use bioreactors, at scales of 2000 L or less. Maximum cell densities are being pushed to levels of 100 million cells per ml or greater. Mixing and mass transfer requirements are increasing while cultures are getting thicker. Cross-flow filtration and/or centrifuge systems are used more frequently, for longer periods, to enable perfusion and higher cell densities. Thus, there is greater demand for design of engineering systems [12]. More cellular adaptation is also likely coming.

Hydrodynamic issues regarding therapeutic cells

When used for recombinant protein production, cells may undergo changes upon adaptation to, for example, serum-free culture medium, suspension culture, a high-shear growth environment, exposure to high levels of waste products and other commercial production

conditions. Nonetheless, the adapted cells often still make the same quality biopharmaceutical protein, as readily assessed through methods developed over the last 30+ years.

When the cells themselves are the product, adaptation of the cells is not necessarily desirable and may in fact be problematic, with impacts more difficult to assess. Cells are more difficult to characterize than proteins. Immortal, transformed cells are now well accepted as hosts for recombinant protein production but would rarely if ever be acceptable for cell therapy. There were likely unknown changes to such cells as they were adapted to commercial conditions. The changes often had little or no impact regarding recombinant protein production, but may well impact cells destined for therapy. If normal primary cells are moved from a static flask to a stirred vessel, changes may occur to the population in general as well as to individual cells. Fluid dynamics is known to impact cellular differentiation [17] and could possibly impact transformation to a tumorigenic state.

In summary, for cell therapy applications, it is unlikely that cellular adaptation will play a major role to resolve the impact of hydrodynamic forces upon scale-up. Engineered system strategies will need to make up the difference and play a larger role than for recombinant protein production. If protective agents such as Pluronic F-68 cannot be employed, the challenges regarding engineered systems will be even greater. To date, most stem cell based therapies are using 2D planar culture systems. For commercial production of allogeneic therapies with high dose requirements, novel bioreactor designs and cell processing equipment are being developed and brought to market. Many groups are now working again on the design and testing of novel single-use bioreactors [17–19], including extensive use of computational fluid dynamics [17,18]. Bioreactors may need to provide a wide range of hydrodynamic environments, such as uniform low shear for the growth phase [17] and high shear for harvest [20]. Industrial animal cell culture for cell therapy is now roughly where it was for recombinant protein production in the 1980s, but the path going forward will be different, with a greater reliance on engineered systems.

Financial & competing interests disclosure

The author has no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

References

1 Jayapal KP, Wlaschin KF, Yap MGS, Hu W-S. Recombinant proteins from CHO cells – 20 years and counting. *Chem. Eng. Prog.* 103(10), 40–47 (2007).

2 Birch JR, Arathoon R. Suspension culture of mammalian cells. In: *Large-Scale Mammalian Cell Culture Technology.* Lubiniecki AS (Ed.). Marcel Dekker, NY, USA, 251–270 (1990).

3 Chuck A, Deshpande R, Distler A, Sander S, Seely J. Commercial production of recombinant erythropoietins. In: *Erythropoietins and Erythropoiesis.* Molineux G, Foote MA, Elliot SG (Eds). Birkhauser Verlag, Switzerland, 133–150 (2003).

4 Goodwin TJ, Prewett TL, Wolf DA, Spaulding GF. Reduced shear stress: a major component in the ability of mammalian tissues to form three-dimensional assemblies in simulated microgravity. *J. Cell. Biochem.* 51, 301–311 (1993).

5 Tyler J. Microencapsulation of mammalian cells. In: *Large-Scale Mammalian Cell Culture Technology.* Lubiniecki AS (Ed.). Marcel Dekker, NY, USA, 343–361 (1990).

6 Vournakis NJ, Rundstadler PW. Optimization of the microenvironment for mammalian cell cultures in flexible collagen microspheres in a fluidized-bed bioreactor. In: *Animal Cell Bioreactors.* Ho CS, Wang DIC (Eds). Butterworth-Heinemann, Boston, USA, 305–325 (1991).

7 Aunins JG, Goldstein JM, Croughan MS, Wang DIC. Engineering developments in homogeneous culture of animal cells; oxygenation of reactors and scaleup. *Biotech. Bioeng. Symp.* 17, 699–723 (1986).

8 Croughan MS, Hamel J-F, Wang DIC. Hydrodynamic effects on animal cells grown in microcarrier cultures. *Biotech. Bioeng.* 29: 130–141 (1987).

9 Kunas KT, Papoutsakis ET. Damage mechanisms of suspended animal cells in agitated bioreactors with and without bubble entrainment. *Biotech. Bioeng.* 36, 476–483 (1990).

10 Lubiniecki AS. Continuous cell substrate considerations. In: *Large-Scale Mammalian Cell Culture Technology.* Lubiniecki AS (Ed.). Marcel Dekker, NY, USA, 483–513 (1990).

11 Lubiniecki AS, Wiebe ME, Builder SE. Process validation for cell culture-derived pharmaceutical proteins. In: *Large-Scale Mammalian Cell Culture Technology* Lubiniecki AS (Ed.). Marcel Dekker, NY, USA, 515–541 (1990).

12 Hu W, Berdugo C, Chalmers J. The potential of hydrodynamic damage to animal cells of industrial relevance: current understanding. *Cytotechnology* 63, 445–460 (2011).

13 Petersen JF. Shear stress effects on cultured hybridoma cells in a rotational couette viscometer. *Doctoral Dissertation* Rice University, TX, USA (1989).

14 Kramer PJ, Jewett JR, Croughan MS. Adaptation of CHO cells to high agitation. Presented at: *AIChE Annual Meeting.* Los Angeles, CA, USA, November 1991.

15 Schmid G, Huber F, Kerschbaumer R. Adaptation of hybridoma cells to hydrodyanmic stress under continuous culture conditions. In: *Animal Cell Technology: Development, Processes, and Products.* Spier RE, Griffiths JB, Macdonald C (Eds). Butterworth Heinemann, Oxford, UK, 203–205 (1992).

16 Trexler-Schmidt M, Sargis S, Chiu J, Sze-Khoo S, Mun M, Kao YH, Laird MW. Identification and prevention of antibody sisulfide bond eeduction during cell culture manufacture. *Biotech. Bioeng.* 106, 452–461 (2010).

17 Jossen V, Pörtner R, Kaiser SC, Kraume M, Eibl D, Eibl R. Mass production of mesenchymal stem cells — impact of bioreactor design and flow conditions on proliferation and differentiation. In:*Cells and Biomaterials in Regenerative Medicine.* InTech, Rijeka, Croatia Eberli D (Ed.).119–174 doi:10.5772/59385 (2014). www.intechopen.com/books/cells-and-biomaterials-in-regenerative-medicine/mass-production-of-mesenchymal-stem-cells-impact-of-bioreactor-design-and-flow-conditions-on-prolife

18 Loffelholz C, Kaiser SC, Werner S, Dieter E. CFD as a tool to characterize single-use bioreactors. In: *Single-Use Technology in Biopharmaceutical Manufacturing* Eibl R, Eibl D (Eds). John Wiley & Sons, NY, USA, 263–279 (2011).

19 Sousa MFQ, Silva MM, Giroux D, Hashimura Y *et al.* Production of oncolytic adenovirus and human mesenchymal stem cells in a single-use, vertical-wheel bioreactor system: impact of bioreactor design on performance of microcarrier-based cell culture processes. *Biotech. Progress* (2015) (In Press).

20 Nienow AW, Rafiq QA, Coopman K, Hewitt CJ. A potentially scalable method for the harvesting of hMSCs from microcarriers. *Biochem. Eng. J.* 85, 79–88 (2014).