Biochemical Engineering Journal 132 (2018) 262–269

Contents lists available at ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Regular article

Bespoke cell therapy manufacturing platforms

Yonatan Levinson[a,*,1], Rajesh G. Beri[b,1], Kathryn Holderness[c,1], Inbar Friedrich Ben-Nun[a,1], Yaling Shi[a,1], Eytan Abraham[a,1]

[a] Lonza, Cell Therapy R&D, 8830 Biggs Ford Rd, Walkersville, MD, 21793-0127, United States
[b] Lonza, Biologics Inc., 101 International Drive, Portsmouth, NH, 03801, United States
[c] Lonza, Cell Therapy Process Development, 8030 El Rio Street, Houston, TX 77054, United States

ARTICLE INFO

Article history:
Received 28 July 2017
Received in revised form 18 January 2018
Accepted 20 January 2018

Keywords:
Cell therapy
Allogeneic
Autologous
MSC
iPSC
CAR-T

ABSTRACT

There is an emerging need for flexible platforms capable of manufacturing cell products at large scale for both allogeneic and autologous therapeutics. These platforms must be capable of both scaling up and scaling out, while maintaining control over the cell culture environment and process steps. We describe two platform designs: a microcarrier-based bioreactor platform capable of scaling up manufacturing of adherent cell types, such as mesenchymal stromal cells and human pluripotent stem cells (embryonic and induced), as well as a modular device capable of performing an entire cell therapy manufacturing process within a self-contained unit. These platforms are inherently adaptable to multiple cell types and process variations while simultaneously offering consistency, thereby appealing to both the developers of novel therapies as well as the manufacturers.

1. Introduction

Cell therapies, once a novel and largely academic pursuit, are fast becoming a manufacturing sector of their own within the biopharmaceutical space. Scientific advances such as chimeric antigen receptor T-cells (CAR-Ts) and other immuno-oncology products, as well as induced pluripotent stem cells (iPSCs) and their enormous regenerative and therapeutic implications, have given rise to a wave of companies seeking to alleviate disease through the administration of cellular products. Manufacturing of cells to serve as medicinal products, however, will require transitioning to technologies that meet scale-up and production challenges, similar to what has been accomplished in the fields of microbial fermentation and animal cell cultivation for human vaccine and mammalian protein manufacture.

The history and development of both human vaccine and mammalian protein manufacturing, in fact, offers a good example of a successful evolution from benchtop equipment and methods to robust, flexible industrial platforms. In its infancy, mammalian based vaccine and protein production systems were built on monolayer cultures [1], shaker flasks [2] and roller bottles [2,3]. These were good tools for academic labs to experiment with cell types, cell lines, media formulations, genetic engineering of protein production pathways, etc. However, they were not truly scalable, nor good processing tools, in that the environment could only be monitored through open handling and offline analysis, and the only environmental parameter that could truly be controlled was temperature. Roller bottle production of human erythropoietin was successfully commercialized [4] and is still being used together with monolayer systems such as Cell Factories [2] for certain legacy vaccines. All the above three manufacturing platforms have severe limitations for scaling up and for producing large quantities of recombinant proteins or vaccines.

Two major innovations in both fields were moving to closed, highly controlled and monitored stirred-tank bioreactors, as well as developing continuous cell lines with robust growth and flexible expression systems. Bioreactors are capable of controlling not only temperature, but pH, dissolved oxygen (DO), and mixing/agitation, all within a closed environment. Furthermore, they can be readily scaled up to meet market demands in a feasible fashion.

In parallel with developments in cell lines and bioreactor technology, other notable improvements have been made: moving away from serum-containing media formulations to first protein free [2] and now chemically defined media formulations [4], highly concentrated media/feed formulations and feeding strate-

Abbreviations: CAR-T, chimeric antigen receptor T-cell; DO, dissolved oxygen; iPSC, induced pluripotent stem cell; MSC, mesechymal stromal cell; PAT, process analytical technology; SS, stainless steel; SU, single-use.

* Corresponding author.
E-mail address: yonatan.levinson@lonza.com (Y. Levinson).

[1] All authors are current or previous employees of Lonza, a pharmaceutical company that develops and sells a wide range of products and services, including cell-based products for research and pharmaceutical use. Lonza may derive benefit from the sale of a product or services derived from the research and/or information provided in this publication.

https://doi.org/10.1016/j.bej.2018.01.023
1369-703X/

gies which enable higher cell density cultures, novel inline sensors to monitor cell concentration [5], nutrient/metabolites [6], development of single use bioreactors, alternative processing platforms such as perfusion [1] and concentrated fed-batch cultures [7]. Indeed, the potential improvements that can still be made for manufacturing biologics are numerous and have recently been compiled in a Technology Roadmap [8].

The improvements made over the years as described above as well as the lessons learned in both fields can and should be applied to the emerging industry of cell therapy manufacturing, while simultaneously being aware of the differences between the three cell based therapy platforms. Table 1 compares and contrasts the most common features of cell therapy manufacturing with cell based bioproduction of proteins and vaccines. From Table 1, it is clear that cell therapy manufacturing has more features in common with vaccine manufacturing than recombinant protein manufacturing. In this paper, we review some of the unique challenges surrounding cell manufacturing, and suggest platforms that balance the need for standardization and repeatability with the inherent diversity of applications needed.

2. Complexities in cell therapy manufacturing

2.1. Cell therapy processes are highly variable

The term 'cell therapy' encompasses a wide variety of cell types, cell sources, and applications for the final cell product. In short, cell therapy includes any treatment in which human cells are administered as a drug product. Historically, bone marrow transplantation is the oldest example of an established cell therapy, based on the regenerative potential of bone marrow stem cells. Current regenerative cell therapy directions include restoring heart tissue for cardiac repair [9,10], retinal tissue for macular degeneration [11], bone and cartilage for orthopedic injuries [12,13], or insulin-secreting cells for diabetes [14]. Other cells are developed for their ability to perform a therapeutic function, such as cancer-targeting CAR-T cells [15] and natural killer cells [16,17], or mesenchymal stromal cells (MSCs), which home to sites of disease and respond in a variety of ways, including paracrine activity, organelle transfer, and exosome/microvesicle transfer [18].

In addition to the specific biology of each cell type, there are more process-oriented variables. Some cells, such as MSCs, are adherent and therefore require a substrate such as microcarrier suspensions or hollow fibers to provide large amounts of surface area in a compact space, whereas other cells, such as T-cells, and adherence independent. Some cells, such as iPSCs can be grown in suspension, as aggregates [19], or as substrate-adherent cultures [20]. Sensitivity to shear in bioreactor systems also has variable effects across cell types [21,22]. Some expansion processes can take place in a matter of days, while others can last for weeks. Almost all cell processes require feeding, but some benefit from continuous perfusion, others can be maintained in batch mode as small feeds are spiked into the growth environment, while still others require periodic total media replacements, especially when directing the transformation from more naïve stem cells into differentiated cells (as is the case with PSCs). There is also considerable variation in the number of cells in a dose required for a given differentiated cell type and/or indication, ranging from hundreds of thousands to billions of cells per dose [23].

Although every cell therapy process has its unique components, it is not practical or cost-effective to design equipment that is optimized for only one product. Instead, it is useful to group cell therapy products based on shared process characteristics, and define strategies and technologies that work best for manufacturing and scaling up each group as a whole.

2.2. Product-centric versus patient-centric processes

While individual cell therapy products and processes themselves are quite varied, when classified according to manufacturing and scale-up strategy, they fall into two categories: product-centric (generally allogeneic) processes, which much be scaled up in the traditional sense, and patient-centric (generally autologous) processes which much be "scaled out" Fig. 1.

Product-centric therapies, in which the treated patients are all receiving the same product, can be scaled up in a manner much like mammalian cultures for protein and vaccine production. These are generally allogeneic therapies, in which the donor and recipient are different individuals. For example, many MSC therapy processes create Master Cell Banks from one or a small number of donors, whose cells can be further expanded into product for thousands of patients. This is possible due to MSCs' observed "immunoprivileged" status in vivo; though, it should be noted that the universality and nature of this status is still an open question [24,25]. In order to scale up production for such products, it is possible to grow large batches of cells in bioreactors, as will be described below, each of which can contain hundreds, or potentially thousands of doses. There are some allogeneic cell therapies which require donor matching, such as some forms of bone marrow transplantation [26], and thus cannot be scaled-up batch wise in this way.

Patient-centric therapies, generally autologous, are mostly those in which cells are extracted from an individual and administered back into that individual, often following a manipulation or expansion stage. Examples include CAR-T therapies, as well as generation of patient-specific iPSCs which can then be used to generate patient-specific differentiated tissue [27]. In patient-centric processes, each dose is unique, and thus cannot be produced together in one large batch. Thus, they must be "scaled out"; that is, technology must be developed to repeatedly perform thousands of individual processes. An additional complication specific to autologous cell therapies is the fact that the patients themselves are the source of the cell therapy starting material. Since the patients themselves are sick and likely have undergone a number of previous non cell therapy treatments of varying success, the starting material for autologous therapies may be of varying quality and therefore challenging.

2.3. Critical quality attributes

One of the biggest challenges in cell therapy manufacturing is the myriad of ways in which even slight changes to the process can have unknown effects on cell biology, thereby altering the therapeutic efficacy of the final product. It is therefore of paramount importance, for any cell therapy, to have a good understanding of the product's mechanism of action, and to further understand the critical quality attributes that are indicators of good quality and efficacy (cell surface markers, secreted factors, expressed genes, viability, etc.). These parameters vary significantly from product to product, even within the same cell type. However, as we will describe below, manufacturing platforms that are able to measure the cells and their environment in-process will offer the tightest control over critical quality attributes. On the other hand, closed manual systems with limited measuring ability pose a greater risk to the product final quality.

3. Allogeneic/product-centric scale-up challenges

Currently, many allogeneic/product-centric cell therapy processes are for adherent cells, such as MSCs. Although there is significant literature demonstrating the ability to expand MSCs in