Cytotherapy, 2017; 19: 9–18

International Society for Cellular Therapy
ISCT®

CrossMark

Bioreactors for cell therapies: Current status and future advances

SHANNON EAKER[1,*], EYTAN ABRAHAM[2,*], JULIE ALLICKSON[3,*], THOMAS A. BRIEVA[4,*], DOLORES BAKSH[1], THOMAS R.J. HEATHMAN[5], BIREN MISTRY[4] & NAN ZHANG[6]

[1]GE Cell Therapy Technologies, Marlborough, MA, USA, [2]Lonza Walkersville, Inc., Walkersville, MD, USA, [3]Wake Forest University School of Medicine, Winston-Salem, NC, USA, [4]Celgene Cellular Therapeutics, Warren, NJ, USA, [5]PCT, a Caladrius Company, Allendale, NJ, USA, and [6]National Heart, Lung and Blood Institute, NIH, Bethesda, MD, USA

Abstract

The use of bioreactors in cell therapy applications is on the rise, as clinical trials and commercialization of cell therapy–based products are moving to the forefront of treatment opportunities. This review focuses on the considerations and benefits of using bioreactors for cell therapy manufacturing, with an emphasis on autologous versus allogenic, scalability, tissue engineering, automation and comparability and consistency in the final product. Evaluation and choice of the right bioreactor for any given process and indication is paramount when moving into scalable platforms and processes that can support the cell therapy industry's needs.

Key Words: *allogeneic, autologous, bioreactor, cell culture, cell therapy, scale-out, scale-up, stem cell, tissue engineering*

Overview of bioreactors

Cell culture expansion is an important unit operation of most cell therapy manufacturing processes. Cell culture typically contributes the largest component of manufacturing time and profoundly affects cell product characteristics for both simple expansion processes and complex processes such as cell differentiation. Accordingly, manufacturing tools such as bioreactors, which maintain a culture environment for cells that produce (or are) a product of interest, are a valuable platform for cost-effective and consistent production of high-quality cell therapies. Bioreactors have been central in cell-based generation of products such as alcoholic beverages, chemicals, antibiotics, monoclonal antibodies and vaccines, and have recently been receiving increasing attention for cell therapies [1–3]. The advantages of using bioreactors in cell therapy include reduced manufacturing costs, improved in-process control, allowing economies of scale as well as product quality and consistency [4,5].

Bioreactor types

A wide variety of bioreactor designs originating from the production of cell-derived compounds are available to accommodate nearly any scale and culture requirement. This variety is particularly useful for cell therapies, which often require complexities such as feeder cells, three-dimensional cultures, patient-specific manufacturing, controlled cell-cell contact, low oxygen concentration and undisturbed local microenvironments. Further, most cell therapies require cells to be harvested from the bioreactor, whereas this requirement is not always needed for many cultures used to produce cell-derived products.

The main categories of bioreactors include stirred tanks, fixed and packed beds, rocking platforms and hollow fiber systems [6]. These categories differ most dramatically in geometry and fluid agitation methods, which include stirring by an impeller or pendulum wand, rocking or bulk media flow. Most bioreactors are capable of real-time monitoring and automated control of culture parameters such as control of dissolved carbon dioxide and oxygen, temperature and pH by CO_2 or addition of acid or base. In addition, most bioreactors use closed-system manipulations and gas filtration to prevent microbial contamination and cross-contamination. These closed-system manipulations also minimize the safety risks of operator exposure to the product stream.

The wide variety of bioreactor designs introduces an equally wide variety of key attributes such as cost and scalability, development needs and labor

*International Society for Cellular Therapy (ISCT) Process and Product Development Subcommittee.
Correspondence: Shannon Eaker, Ph.D., GE Cell Therapy Technologies, 100 Results Way, Marlborough, MA 01752. E-mail: shannon.eaker@ge.com

(Received 18 August 2016; accepted 22 September 2016)

ISSN 1465-3249
http://dx.doi.org/10.1016/j.jcyt.2016.09.011

requirements. Criteria to select bioreactors include culture conditions required for the cells of interest, simplicity, development investment needed to adapt from static culture to a candidate bioreactor, scalability, material demand and cost. Ultimately, the simplest bioreactor that can provide process needs as well as cell quality is often the best choice. This is a trend that mirrors that of the biotechnology industry where stirred tank bioreactors have become the industry standard platform for large-scale production [7]. Importantly, choice of culture scale should include considerations of downstream processes to accommodate increasing cell outputs.

One of the primary factors influencing the choice of bioreactors for cell therapies is the anchorage dependence of the cells. Suspension cultures of anchorage-independent cells can be readily applied to stirred-tank and rocking platform bioreactors, in which cells are suspended via agitation in a highly scalable manner that has been proven in the production of cell-based products. These systems are only effective for non-adherent cell types that do not require motionless culture conditions. All bioreactor types, when run in perfusion mode with non-anchorage-independent cells require cell retention provisions. For these reason, batch stirred-tank and rocking platform bioreactors are most frequently chosen for suspension cells.

Adherent cell bioreactors require a substrate for cell attachment. Hollow fibers and fixed or packed beds with appropriate chemistry are effective cell attachment substrates; however, uniformly seeding and harvesting cells from such bioreactors is challenging, especially at larger scales. In stirred-tank and rocking platform bioreactors, solid substrates called microcarriers are suspended and provide a scaffold for cells to attach. To maintain suspension and homogeneity of the microcarriers (typically 100–300 μm), especially at high densities of microcarriers, requires increased agitation to maintain suspension as compared with anchorage-independent cells. In addition, larger particles are more susceptible to shear damage from turbulent fluid motion [8].

Bioreactor engineering

The rich history of bioreactor engineering provides a vast body of knowledge that can be leveraged for cell therapy manufacturing use. In particular, engineering methods are applied to challenges related to design and scale-up that arise to different degrees for each bioreactor type. Approaches to characterization and fundamental understanding are well-developed for common bioreactors. The design of agitation and its scale-up impact based on fundamental understanding of fluid dynamics is particularly well-developed for stirred-tank bioreactors [7,9,10].

Importantly, agitation must be designed to manage not only damaging shear exposure of cells, but also the efficiency of mass transfer, suspension of cells and avoidance of inhomogeneities that cause cell inconsistencies. Similar to agitation, mass transfer of nutrients, waste and gases is well-understood for multiple bioreactor types [11]. In addition, there is strong experience with uniform cell seeding onto solid substrates such as microcarriers with anchorage-dependent cells. In contrast, few studies have addressed the harvest of healthy, functional adherent cells from growth substrates. Recently, great progress has been made in the harvest of adherent cells from microcarrier cultures [12].

Adapting cells to process conditions is an important strategy for bioreactor engineering in the field of cell-derived products. Adaptation of the cells involves engineering cells that impact bioreactor design as a supplement to engineering the bioreactor itself. For example, cell lines used to produce cell-derived products have been adapted over many passages to achieve anchorage independence and greater shear tolerance to improve bioreactor scalability [13–16]. This approach is not available to most cell therapies, which can grow for a finite number of population doublings before becoming senescent [17–19]. Because many of the population doublings are leveraged to manufacture the product, growth potential is typically insufficient to adapt the cells to new conditions. One potential solution to this challenge might be to establish cell stocks under the desired conditions so that the desired properties become inherent to the cells of interest.

Considerations for allogeneic cell products

Demand for allogeneic cell products can vary greatly from cell type to cell type and thus requires a large range of culture scales. As a result, a variety of bioreactors fulfill the needs of cell therapy cultures. In the limiting case in which a single patient is dosed from a batch of cell products derived from an unrelated donor, the patient-specific manufacturing needs are identical to those for autologous therapies (see Considerations for Patient-Specific Manufacturing of Autologous or Allogeneic Cell Products). At the opposite extreme are allogeneic products that treat multiple patients and demand larger-scale bioreactors as the number of patients treated increases. The largest bioreactor demands arise from a universal donor platform, where highly expandable cell types such as mesenchymal stromal cells (MSCs) might be used. These cell products approach similar scale requirements to those that might be used to make cell-derived proteins/drugs.

For adherent cell types, microcarrier, packed bed, fixed bed and hollow fiber bioreactors have been most