Biochemical Engineering Journal 120 (2017) 49–62

Contents lists available at ScienceDirect

Biochemical Engineering Journal

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

Process development for expansion of human mesenchymal stromal cells in a 50 L single-use stirred tank bioreactor

Tristan Lawson*, Daniel E. Kehoe[1], Aletta C. Schnitzler, Peter J. Rapiejko, Kara A. Der, Kathryn Philbrick[2], Sandhya Punreddy, Susan Rigby, Robert Smith, Qiang Feng[3], Julie R. Murrell, Martha S. Rook

EMD Millipore Corporation, 80 Ashby Road, Bedford, MA 01730, United States

ARTICLE INFO

Article history:
Received 4 May 2016
Received in revised form
21 November 2016
Accepted 22 November 2016
Available online 23 November 2016

Keywords:
Bioreactors
Mesenchymal stem cells
Microcarriers
Scale-up
Bioprocess design
Large scale cultivation

ABSTRACT

The application of cell-based therapeutics requires development of refined and scalable culture processes. Stirred tank bioreactors facilitate growth of human mesenchymal stromal cells (hMSC) while meeting these needs. A process for expansion of hMSCs in a 50 L bioreactor was developed. Parameters evaluated include agitation rate, pH and dissolved oxygen (DO) control set-points, and media formulation. The pH and DO levels were determined empirically in 3 L bioreactors prior to implementation at the 50 L scale. The agitation operating range for microcarrier cultures in the 50 L bioreactor was calculated based on theoretical and empirical analyses of solid suspension and shear limitations. Additionally, small-scale experiments demonstrated that hMSC growth was improved in αMEM supplemented with human platelet lysate in comparison to DMEM supplemented with FBS. A 43-fold expansion of harvested hMSCs was achieved in 11 days in a 50 L bioreactor incorporating these process improvements. Cells expanded in the bioreactor exhibited the expected surface marker expression, trilineage differentiation potential, T cell growth inhibition and indoleamine 2,3-dioxygenase inducibility. The results highlight that identification of optimal pH, DO, agitation rates and culture medium for microcarrier-based bioreactor expansion of adherent cells is paramount to developing a platform to support cell-based therapies.

1. Introduction

Mesenchymal stromal cells are a subset of adult multipotent cells that have a number of immunomodulatory characteristics and are identified by a defined surface marker expression profile [1–4]. Although hMSCs can be derived from a variety of adult tissues, bone marrow, adipose tissue, umbilical cord and placenta are most commonly used [5–8]. It has been reported that hMSCs have a potential role in the treatment of a wide range of conditions including acute myocardial infarction, graft versus host disease and diabetes, and are currently under investigation in over 300 clinical trials [9]. Human MSCs can be utilized for both autologous therapy (*i.e.*, patient-specific cells) as well as allogeneic therapy (*i.e.*, cells from a universal donor). Since hMSCs are rare in adult tissues, the harvested cells must be expanded *in vitro* to generate sufficient numbers for clinical administration [10]. The number of hMSCs required for administration to a single patient has ranged from 1 to 8×10^6 hMSCs per kilogram of body mass, depending on the particular indication [11,12]. Although the amount of cells required for a single 70 kg patient dose could be generated in either planar culture or in a bench scale bioreactor [13], the need for multiple doses supporting multiple patients render planar expansion platforms impractical [14,15]. Therefore, the implementation of autologous hMSC therapies requiring multiple doses of hMSCs or allogeneic therapies necessitates the development of cell culture processes in larger scale (*e.g.*, 10 L–200 L) bioreactor platforms [13,16]. Inherent to larger systems are improved economies of scale, increased

* Corresponding author.
E-mail addresses: tristan.lawson@emdmillipore.com (T. Lawson), daniel.kehoe@genzyme.com (D.E. Kehoe), aletta.schnitzler@emdmillipore.com (A.C. Schnitzler), peter.rapiejko@gmail.com (P.J. Rapiejko), kara.der@emdmillipore.com (K.A. Der), philbrick.ka@husky.neu.edu (K. Philbrick), sandhya.punreddy@emdmillipore.com (S. Punreddy), susan.rigby@emdmillipore.com (S. Rigby), robert.smith@emdmillipore.com (R. Smith), afeng@vcanbio-ctb.com (Q. Feng), julie.murrell@emdmillipore.com (J.R. Murrell), martha.rook@emdmillipore.com (M.S. Rook).
[1] Present address: Genzyme Corporation, 68 New York Ave, Framingham, 01701 MA, United States.
[2] Present address: Department of Physical Therapy, Movement and Rehabilitation Sciences, Northeastern University, 360 Huntington Avenue, 301 Robinson Hall, Boston, 02115 MA, United States.
[3] Present address: Vcanbio Center for Translational Biotechnology Corporation, 21 Strathmore Road, Natick, 01760 MA, United States.

http://dx.doi.org/10.1016/j.bej.2016.11.020

lot sizes, and greater lot-to-lot consistency, all while facilitating compliance with developing regulatory requirements for cellular therapies [14,15].

Since hMSCs generally require a solid support on which to grow, it has been suggested that microcarrier-based culture is the ideal manufacturing process currently available that is capable of meeting projected lot size requirements [17]. Small-scale cultures of hMSCs on microcarriers in static culture provide a simple platform for media and microcarrier selection. Spinner flasks are a basic model for stirred culture, however these vessels lack the ability to control additional critical process parameters such as pH and DO. Attempts to expand hMSCs in fully controlled bioreactors have mostly been limited to rocking and stirred tank systems which range from 250 mL to 5 L in volume [8,18–25], with one additional study at a 35 L working volume [26]. Alternative approaches to MSC culture, for instance hollow fiber bioreactors, have been utilized [27]. However, the maximum surface area for cell growth can be limited and there may be issues with nutrient gradients [15]. Studies at pilot scales (10 L–200 L) are therefore required given the substantial number of cells necessary for therapeutic doses and the process robustness necessary to reliably achieve these objectives [15]. Additionally, the scalability of stirred tank bioreactors, coupled with the simplicity of sampling and cell harvesting that they offer, identify stirred tank systems as ideal candidates for this application.

The implementation of stirred tank bioreactors for large-scale, industrial hMSC production is challenged by a lack of significant process knowledge for microcarrier-based hMSC cultivation and harvest. One critical requirement for success is the achievement of a well-mixed state that ensures a uniform growth environment throughout the vessel. Within the context of any given bioreactor design, uniform mixing is achieved via optimization of the impeller agitation rate (*i.e.*, power input per unit volume). Characterization of mixing times and their associated k_La values in response to specific power inputs is typically sufficient to achieve optimal mixing for suspension-cell cultures. In contrast, cultivation of cells adhering to solid supports such as microcarriers, presents additional challenges requiring users to perform more extensive characterization. Theoretically, higher impeller agitation speeds are required in order to achieve and maintain microcarrier suspension as compared to suspension cells. However, the use of power inputs that are higher than necessary can result in damaging levels of hydrodynamic shear since cells attached to microcarriers are more sensitive to fluid-mechanical forces than freely-suspended single cells [13,28–31]. It is therefore imperative to develop an operating window that balances these limitations. This can be achieved by leveraging the mathematical model developed for the suspension of solid particles (*i.e.* microcarriers) [32] to define the lower agitation limit, and the Kolmogorov microscale to define the upper agitation limit [33].

Cell culture medium formulation is another factor that can greatly impact cell growth when transitioning to stirred tank reactors. There is an increased trend to move away from animal-derived materials in order to eliminate zoonotic agents and the associated risks for cell therapies. Fetal bovine serum (FBS) in particular is associated with regulatory, supply, and lot-variation challenges [34]. Eliminating this commonly-used reagent will require thorough evaluation of animal origin-free materials for compatibility with cell therapy applications. Human platelet lysate (PL) represents a serum alternative that is a step toward this goal. Inherently rich in cytokines [35], PL-containing media has been shown to support hMSCs expansion without the need for additional growth factor supplementation [36], and was herein investigated as an alternative to serum for a large-scale process.

The feasibility of microcarrier-based cell expansion was demonstrated previously using 3 L single-use bioreactors to expand hMSCs that remained multipotent and continued to display both the genotypic and phenotypic characteristics of cells produced using traditional planar culture [37]. This study describes the expansion of hMSCs in a 50 L stirred tank bioreactor with refined control parameters and media after process development at 3 L scale. The agitation operating range was calculated based on the Zwietering correlation and Kolmogorov's theory of turbulent eddy lengths. αMEM and PL supported better hMSC growth in comparison to DMEM and FBS. Incorporating these process changes resulted in more than a doubling of the cell yield compared to the initial run. The cells expanded in the bioreactor retained the expected surface marker expression, trilineage differentiation potential, and abilities to inhibit T cell proliferation, undergo indoleamine 2,3-dioxygenase (IDO) induction and upregulate putative immunomodulatory surface proteins. In short, it is important to identify optimal control set-points and media for microcarrier-based bioreactor expansion of adherent cells in order to improve the economics and efficiency of cell therapy manufacturing.

2. Materials and methods

2.1. Stirred-tank bioreactor systems

A process for expansion of hMSCs was developed using the commercially available Mobius® 3 L and 50 L Single-use Bioreactors (MilliporeSigma). The bench-scale Mobius® 3 L Bioreactor is a rigid stirred-tank bioreactor while the Mobius® 50 L Bioreactor is an inflatable stirred-tank Flexware® assembly used in a stainless steel vessel. The Mobius® 50 L Bioreactor utilizes the Mobius® SensorReady Technology for process monitoring and control. The SensorReady assembly is an external loop that is connected to the bioreactor Flexware® assembly which allows for a configurable number of probes to be used. Supplementary Table S1 (See in the online version at DOI: http://dx.doi.org/10.1016/j.bej.2016.11.020) outlines the features for these systems.

2.2. Microcarrier mixing study

It is practically impossible to quantify the mixing and suspension of microcarriers while maintaining a closed, sterile environment during a cell culture process. Therefore, a model system was employed which allowed for open sampling and observation of microcarrier distribution within the vessel. A Mobius® 50 L Single-use Bioreactor (MilliporeSigma) was loaded with 7 g/L or 15 g/L of collagen-coated microcarriers (Pall, Port Washington, NY) in PBS (MilliporeSigma) supplemented with 0.2% Pluronic® F-68 (MilliporeSigma). Preliminary studies showed the attachment, growth, and detachment of MSCs was best supported by collagen-coated microcarriers in comparison to a number of other commercially available microcarriers. The bioprocessing container was then opened from the top and samples were obtained at various heights and at different impeller agitation rates. The microcarriers in each sample were then isolated via cell strainers, dried and weighed to determine microcarrier concentrations.

2.3. Cell banks, cell culture media and small scale culture

Primary human MSCs were derived from donor bone marrow obtained from Lonza (Walkersville, MD). All studies were performed with the same isolate of cells (single donor) and the cumulative population doublings were no more than 35 at harvest. The hMSCs were expanded from frozen stocks in multi-layered tissue culture flasks (Corning, Corning, NY) coated with gelatin (MilliporeSigma) and maintained at 37 °C and 5% CO_2 prior to transfer to bioreactors. The media used in these studies was composed