Journal of Membrane Science 478 (2015) 117–129

Contents lists available at ScienceDirect

Journal of Membrane Science

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

Filtration methodologies for the clarification and concentration of human mesenchymal stem cells

Bárbara Cunha [a,b], Cristina Peixoto [a,b], Marta M. Silva [a,b], Manuel J.T. Carrondo [b,c], Margarida Serra [a,b,*], Paula M. Alves [a,b]

[a] Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Av. da República, 2780-157 Oeiras, Portugal
[b] iBET, Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2780-901 Oeiras, Portugal
[c] Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Monte da Caparica, Portugal

ARTICLE INFO

Article history:
Received 19 August 2014
Received in revised form
9 December 2014
Accepted 23 December 2014
Available online 8 January 2015

Keywords:
Human mesenchymal stem cells
Microcarriers
Tangential flow filtration
Downstream processing
Cell concentration

ABSTRACT

Currently human mesenchymal stem cells (hMSC) are expanded using microcarrier-based stirred culture systems from one to hundreds of liters of culture volume to guarantee the required cell numbers to be delivered to the clinic. Such culture volumes need to be clarified, ensuring efficient removal of microcarriers, and concentrated without compromising the cells' characteristics. The aim of this work was to evaluate the applicability of filtration methodologies, as dead end filtration and tangential flow filtration, for the clarification and concentration of hMSC, respectively.

Different process variables and their impact on hMSC quality were evaluated, showing that polypropylene filters with pore sizes higher than 75 μm can ensure the removal of microcarriers from the cell suspension bulk, without compromising cells' recovery or viability. Furthermore, hMSC could be successfully concentrated up to a factor of ten while maintaining their identity, potency and high cell viability, allowing for the recovery of over 80% of viable cells; an initial cell concentration higher than 2×10^5 cell/mL, and polysulfone membranes with pore sizes higher than 0.45 μm were identified to be key conditions to obtain such concentration factors; shear rate and permeate flux were also shown to impact the cells' recovery yields, viability and quality.

1. Introduction

Given the particular immunomodulatory characteristics [1] and the capacity to secrete bioactive molecules with anti-inflammatory and regenerative features [2], human mesenchymal stem cells (hMSC) have become key candidates for autologous and allogeneic therapies. Currently, over 400 clinical trials are taking place using hMSC for a variety of therapies, including bone/cartilage, cardiovascular, neurodegenerative and gastrointestinal diseases as well as in diabetes, and for the treatment of graft-versus-host disease [3].

However, clinical transfer of hMSC still faces several challenges; high doses (from 10^5 to 10^9 cells per patient) [4] coupled with autologous or even allogeneic options, require suitable scales for expansion, where microcarrier-based stirred culture systems have mostly been adopted to sustain cell expansion [5]. Since hMSC are meant to be implanted, transplanted or infused into human patients, efficient purification processes are essential, as reviewed by Serra et al. [6], while compliant with FDA and EMA requirements in terms of identity, purity, potency, and viability throughout processing. To fulfill the purification requirements, cell clarification (including efficient cell-microcarrier separation), cell concentration (volume reduction) and cell washing [6,7] will be necessary. Due to the non-biodegradable nature of the majority of microcarriers, their removal becomes compulsory. Therefore, several companies and research groups have been already exploring alternatives for microcarrier separation and cell concentration based on filtration methodologies [8–10].

Tangential flow filtration is a well established technology, commonly used for the initial clarification of therapeutic proteins from mammalian, yeast, and bacterial cell cultures [11], as well as for the purification and concentration of virus particles [12]. In the majority of biopharmaceutical clarification processes, cells are merely a by-product of the process, where the main goal is to

Abbreviations: *A*, Area; cGMP, Current Good Manufacturing Practices; *d*, Diameter; CIP, Cleaning in Place; DPBS, Dulbecco's Phosphate-Buffered Saline; DSP, Downstream Processing; FBS, Fetal Bovine Serum; HF, Hollow Fiber; hFF, Human Foreskin Fibroblast; hMSC, Human Mesenchymal Stem Cell; *J*, Inlet Flux; J_p, Permeate Flux; NMWC, Nominal Molecular Weight Cut-off; P_f, Inlet/Feed Pressure; P_r, Retentate Pressure; P_p, Permeate Pressure; PES, Polyethersulfone; PS, Polysulfone; *Q*, Flow Rate; *r*, Radius; TFF, Tangential Flow Filtration; TMP, Transmembrane Pressure; *u*, Cross-flow velocity; *V*, Volume; γ, Shear rate; τ, Shear stress

* Corresponding author at: iBET, Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2780-901 Oeiras, Portugal.
Tel.: +351 21 446 94 31; fax: +351 21 442 11 61.
E-mail address: mserra@itqb.unl.pt (M. Serra).

http://dx.doi.org/10.1016/j.memsci.2014.12.041
0376-7388/

clear them from the system. Conversely, for cell therapy utilizations, TFF processes have to be redesigned to meet the new demands, where cells are intended as the final product. Nonetheless, the impact of the TFF process parameters on the cells' characteristics needs assessment. TFF poses several advantages making it an attractive solution for cell therapy DSP; since this technology is widely used in protein and virus processing, there is a large offer of fully automated, disposable and integrated (concentration and washing) TFF systems, with a wide range of processing capabilities. Besides flexibility, TFF's advantages also include linear scale-up behavior and fairly low shear forces and pressures [13,14], justifying its growing use in perfusion systems [15].

The main goal of this study was to assess several process parameters of well-established and compatible with current good manufacturing practices (cGMP) filtration techniques, dead end filtration and TFF, on the efficiency in removing microcarriers from hMSC culture bulk, and on the concentration of hMSC (up to a concentration factor of ten), respectively. In particular, for the clarification step, the influence of different filter materials and pore sizes on cell recovery yield and viability was evaluated. For the cell concentration step, the impact of cell concentration, membrane material and pore size, as well as operating conditions of shear rate and permeate flux on cell recovery yields and quality was evaluated.

2. Materials and methods

The followed workflow is presented in Fig. 1A.

2.1. Cell expansion

In this work, human bone marrow-derived mesenchymal stem cells (hMSC), obtained from STEMCELL™ Technologies (Grenoble, France), were used. Cells were expanded in static or stirred culture conditions. All reagents used to perform the cell culture were purchased from Gibco® Life Technologies™ (Carlsbad, USA), unless otherwise stated.

2.1.1. Static culture

hMSC were routinely cultured in MesenCult®–XF Medium (STEMCELL™ Technologies) supplemented with 2 mM L-Glutamine and propagated in tissue culture flasks (Thermo Scientific™ Nunc™, Massachusetts USA), previously coated with MesenCult™–SF Attachment Substrate (STEMCELL™ Technologies), at 37 °C in a humidified atmosphere of 5% CO_2 in air, as previously described [16]. At 70–80% cell confluency, the medium was removed and cells were washed with Dulbecco's phosphate-buffered saline (DPBS) and incubated with TrypLE Select (1X) dissociation reagent for 5 min at 37 °C. After cell detachment, hMSC were resuspended in MesenCult®–XF medium, and centrifuged at 300 *g* for 5 min at room temperature. The cell pellet was resuspended in MesenCult®–XF medium and transferred to new pre-coated culture flasks, at an inoculum cell concentration of 4×10^3 cell/cm^2. 50% of the culture medium was exchanged at day 5.

2.1.2. Stirred cultures

hMSC were inoculated at 2.5×10^4 cell/mL in 125 mL spinner vessels (Corning Inc., New York, USA) containing Synthemax II microcarriers (at 16 or 48 g/L; Corning) and half of final the working volume of culture medium (MesenCult®–XF). The spinner vessels were placed inside an incubator (37 °C, humidified atmosphere of 5% CO_2 in air) under intermittent stirring (On: 30 rpm, 1 min; Off: 0 rpm, 20 min). After 5 h, fresh MesenCult®–XF Medium was added up to 100% of final working volume to the cultures, and stirring (at 30 rpm) was turned on. Five days after expansion, 50% of the culture medium was exchanged. At 70–80% cell confluency (day 7), cells were detached from the microcarriers and the culture bulk was harvested from the spinner vessel: Briefly, after washing with DPBS, TrypLE Select (1X) was added to the cells and the cultures were incubated at 37 °C for approximately 15 min under continuous stirring (60 rpm) to promote cell detachment.

hMSC were also cultured in Biostat Qplus stirred tank bioreactors (Sartorius Stedim Biotech, Göttingen, Germany) with 0.25 L working volume. Data acquisition and process control were performed using MFCS/Win (Sartorius Stedim Biotech). Cell culture in the bioreactor was performed using dissolved oxygen of 20% oxygen tension, pH of 7.2 and temperature of 37 °C.

2.2. Downstream processing

2.2.1. Clarification (microcarriers' removal)

After hMSC detachment and harvesting, microcarriers were removed from the cell bulk suspension using dead end filtration. Nylon and polypropylene net disk filters (both from EMD Millipore, Massachusetts, USA) with different pore sizes (100, 80 and 30 μm) were tested to process up to 0.2 L of cell culture bulk. Briefly, filters were previously sterilized with 70% ethanol for at least 3 h, assembled into a Sterifil® Aseptic System and Holder (EMD Millipore) and preconditioned with DPBS, following the manufacturer's instructions. The cell culture bulk was filtered coupling a vacuum system to the Sterifil® Aseptic System.

Sterile OptiCap® XL 1 Capsules (EMD Millipore) with 75 and 100 μm pore size were used to filter up to 2 L of cell bulk suspension. Tandem Model 1082 peristaltic pump from Sartoflow® Slice 200 benchtop crossflow system (Sartorius Stedim Biotech) was used and a flow rate of 300 mL/min was applied to perform the filtration process.

2.2.2. Cell concentration (volume reduction)

The clarified cell suspension was concentrated using tangential flow filtration (TFF), as represented in Fig. 1B.

To start the TFF process, initially the air from the device was removed by filling the recirculation loop with cell suspension using a peristaltic pump (Tandem Model 1081 peristaltic pumps from Sartoflow® Slice 200 benchtop crossflow system, Sartorius Stedim Biotech) on the feed side set up to a fixed flow rate, in order to obtain fluxes of 175, 375 and 750 L m^{-2} h^{-1} (Table 2); on the permeate stream was either i) a valve restricting the tubing at a fixed value or ii) a recirculation pump (Watson Marlow Model 120S/DV 200 rpm pump, Watson-Marlow Pumps Group, Massachusetts, USA) set to a fixed flow rate, in order to obtain fluxes of 60, 120 and 250 L m^{-2} h^{-1} (Table 3). In both cases, the control of the permeate stream is fixed and constant throughout the process.

After achieving the desired concentration factor, the TFF loop is completely drained and the cell suspension is recovered. Pressure was monitored using SciPres luer pressure sensors (SciLog, Wisconsin, USA) on the inlet, outlet and permeate stream. Weight and pressure data were monitored and acquired every 15 s using the Sartoflow® Slice 200 benchtop crossflow system. The experiments were performed inside of a laminar flow hood chamber in order to ensure a sterile environment.

A water flux test was performed before and after each experiment to determine the membrane's permeability. To ensure sterility, hollow fiber devices were sanitized with 0.5 M NaOH (Sigma-Aldrich, Steinheim, Germany) at 50 °C for 45 min. The membranes were preconditioned with five membrane volumes of sterile DPBS before the concentration step.

Six different hollow fiber devices were tested (Table 1); hollow fiber devices A and B were kindly provided by Asahi Kasei, Tokyo,