Biochemical Engineering Journal xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Biochemical Engineering Journal

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

Regular article

Evaluation of a monitored multiplate bioreactor for large-scale expansion of human periosteum derived stem cells for bone tissue engineering applications

T. Lambrechts[a,b], I. Papantoniou[a,c], S. Viazzi[a,b], T. Bovy[d], J. Schrooten[a,e], F.P. Luyten[a,c], J.-M. Aerts[a,b,*]

[a] Prometheus, Division of Skeletal Tissue Engineering, KU Leuven, Belgium
[b] M3-BIORES, KU Leuven, Kasteelpark Arenberg 30, 3001 Heverlee, Belgium
[c] Skeletal Biology and Engineering Research Center, KU Leuven, Herestraat 49, 3000 Leuven, Belgium
[d] Pall Life Sciences, Rue de Ransbeek 310, 1120 Brussels, Belgium
[e] Department of Materials Engineering, KU Leuven, Kasteelpark Arenberg 44 PB2450, 3001 Heverlee, Belgium

ARTICLE INFO

Article history:
Received 1 April 2015
Received in revised form 27 July 2015
Accepted 29 July 2015
Available online xxx

Keywords:
Bioreactors
Scale-up
Bioprocess monitoring
Stem cell characterization
Tissue cell culture
In vivo bone formation

ABSTRACT

In light of the large-scale expansion of human periosteum derived stem cells for the treatment of large bone defects, a multiplate bioreactor system (Pall Integrity Xpansion) in combination with an integrated holographic imaging platform (Ovizio iLine S microscope) was evaluated. The culture process was quantitatively characterized by imaging data, metabolite concentrations and a breakdown of the cell recovery fractions. The resulting cell quality was assessed based on the minimal criteria for mesenchymal stem cells, including viability on cell culture plastic, identity markers and tri-lineage differentiation potential. Additionally, an *in vivo* bone forming potency assay was used in an ectopic mice model that resulted in compelling bone formation ($11.6 \pm 3.1\%$ and $12.8 \pm 3.3\%$ for the bioreactor and control tissue culture flask condition, respectively). Therefore, it was shown that the bioreactor is able to produce large quantities of cells, while maintaining satisfactory cell quality.

1. Introduction

Currently, more than 1300 active clinical trials are reported using cell-based therapies to treat a wide variety of indications ranging from cardiovascular to neurological disorders, as well as skeletal disorders [1]. Around 380 of these trials use mesenchymal stem cells (MSCs) as the therapeutic cell source. While most applications require between 10^7 and 10^9 MSCs for a single dose [2,3], cell expansion is traditionally carried out in 2D (monolayer) static culture set ups (*e.g.*, tissue culture flasks or cell factories) that require extensive manual open-process interventions for media exchange and cell harvest. The high labor and infrastructure costs associated with these techniques hamper the socio-economic viability of the therapy after clinical translation. In combination with a lack of suitable potency assays related to the *in vivo* biological response of the cells [4], the relatively low number of successful clinical translations of cell-based therapies are mainly attributed to the challenges associated with the production of the required cell numbers, while at the same time assure high and reproducible cell quality [3,5–7]. This highlights the rising need to develop and incorporate automated bioreactor systems for large-scale production of progenitor cells for clinical applications.

Additionally, due to the inherent complexity of biologic processes, traditional cell culture processes that were designed by a rule-of-thumb approach are difficult to adapt to an efficient and robust clinical process that is able to deliver an efficacious product to every single patient. The full potential of cell-based therapies will therefore only be able to be harnessed by a cell culture process that is standardized, scalable and able to deliver clinically relevant cell numbers, while at the same time assure potent biological functionality *in vivo* [8–10]. Recently, considerable effort is placed in developing such processes based on bioreactor systems, for example in the form of hollow fiber bioreactors [11,12] and wave-rocking bioreactors for microcarrier-based expansion [13].

In this work, a monitored multiplate bioreactor (Pall Integrity® Xpansion™ equipped with Ovizio iLine S microscope) was evaluated as a platform for the clinical-scale expansion of human

* Corresponding author at: M3-BIORES, KU Leuven, Kasteelpark Arenberg 30, 3001 Heverlee, Belgium. Fax: +32 16 321480.
E-mail address: Jean-Marie.Aerts@biw.kuleuven.be (J.-M. Aerts).

http://dx.doi.org/10.1016/j.bej.2015.07.015
1369-703X/

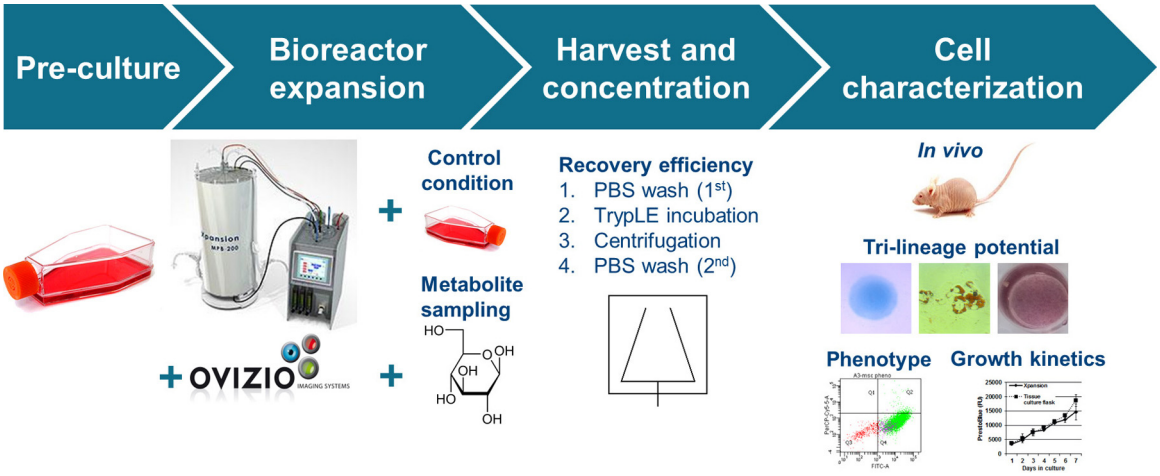

Fig. 1. Overview of the general experimental outline with an initial tissue culture flask-based pre-culture phase, followed by the Xpansion bioreactor culture. During the bioreactor culture the cell growth was monitored based on daily images from the Ovizio iLine S microscope and samples of the medium. Three standard tissue culture flasks were cultured in parallel as a positive control. Immediately after cell harvest and cell concentration the cell characterisation was initiated, including among others, an *in vivo* bone forming potency assay.

periosteum derived stem cells (hPDCs) and its ability to monitor the cell expansion process was evaluated. hPDCs are a promising source of progenitor cells for the treatment of skeletal defects. During the natural bone healing process they have been shown to be the main contributors to tissue regeneration [14,15], while after *ex vivo* expansion, they have been recently shown to possess improved bone forming capabilities compared to other MSC sources (*e.g.*, bone marrow and synovium) when seeded on calcium phosphate carriers [16].

Using this multilayered bioreactor, limited changes to the classical planar cell culture process are required as opposed to, for example, microcarrier-based cell expansion where complex process variables need to be optimized (*e.g.*, material/surface properties, hydrodynamics) [17]. This is due to the fact that cells are seeded and cultured on a 2D cell culture surface similar to the conditions found in standard tissue culture flasks. In addition, the extensive quantitative bioreactor read-outs that can be obtained at-line, such as dissolved oxygen concentrations, pH and microscopic images, can be used to improve the control over the expansion process, ultimately leading to a more robust *in vivo* outcome [18].

The objective of this work is the development of a clinical scale bioreactor process for the expansion of hPDCs in the Xpansion bioreactor. The advantages of thorough process monitoring are illustrated and the synergy between the multiplate bioreactor and the mounted microscope is highlighted. In addition to the standard post-harvest *in vitro* cell characterization assays that were performed, an *in vivo* potency assay was implemented which is often lacking in bioprocess studies relevant to cell therapy applications.

2. Materials and methods

The experimental outline consists of 4 consecutive phases: (1) a pre-culture phase in tissue culture flasks in order to reach the amount of cells required for bioreactor seeding, (2) 7 days of bioreactor expansion with continuous monitoring of multiple process parameters, (3) cell harvest and concentration, and (4) post-harvest cell characterization in which the cells from the bioreactor are compared to a target quality profile of cells cultured in parallel in standard tissue culture flasks (Fig. 1). The *in vitro* cell characterization is inspired on the International Society for Cellular Therapy (ISCT) minimal criteria for MSCs [19], supplemented with a bone forming assay in order to assess the *in vivo* potency of the cells [20]. The bioreactor process was first evaluated and adapted to the needs of hPDCs in three small scale process development runs in the Xpansion-10. In a following phase the process was translated to a larger scale bioreactor (Xpansion-50) that resembles the clinical-scale production.

2.1. Flask-based hPDC culture

hPDCs were isolated from 4 different donors by means of enzymatic digestion of a periost biopsy as described by Eyckmans et al. [21]. Procedures were approved by the ethical committee for Human Medical Research (KU Leuven) and patient informed consent forms were obtained. The isolated cells were cultured in T25 flasks for the first passage in standard culture medium consisting of high glucose GlutaMAX™ Dulbecco's modified Eagle's medium (Life Technologies, Merelbeke, Belgium) supplemented with 10% irradiated fetal bovine serum (FBS; HyClone, Cramlington, UK), 1% sodium pyruvate (Invitrogen) and 1% antibiotic–antimycotic (100 units/mL penicillin, 100 mg/mL streptomycin, and 0.25 mg/mL amphotericin B; Invitrogen). Cells were further cultured in T175 flasks with a seeding density of 5700 cells/cm^2 and sub-cultured at ±80% confluence. At passage 3, the cells from the 4 different donors were pooled all together and further expanded in T175 flasks up to passage 7 (approximately 12 total population doublings). hPDCs generally maintain linear growth curves for over 30 population doublings [22]. At all passages, cells were harvested by trypsinization for 10 min with TrypLE Express (Invitrogen).

2.2. Bioreactor based hPDC culture

The Pall Life Sciences Xpansion™® (Pall Life Sciences, Brussels, Belgium) is a multiplate bioreactor that houses from 10 to 200 hydrophilized polystyrene plates of ±612 cm^2 each (Fig. 2). The plates are tightly packed around a central aeration column, that provides gas exchange controlled by an active gas flow controller. Based on the integrated temperature, dissolved oxygen and pH sensors, the culture conditions can be monitored and controlled [23,24]. Additionally, by making use of the holographic Ovizio iLine S microscope (Ovizio Imaging Systems, Brussels, Belgium), the cells inside the bioreactor can be visualized and critical process parameters such as cell density and morphological features can be non-invasively extracted in a quantitative way.

Please cite this article in press as: T. Lambrechts, et al., Evaluation of a monitored multiplate bioreactor for large-scale expansion of human periosteum derived stem cells for bone tissue engineering applications, Biochem. Eng. J. (2015), http://dx.doi.org/10.1016/j.bej.2015.07.015