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Large-scale progenitor cell expansion for multiple donors in a monitored hollow fibre bioreactor

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Abstract

Background aims. With the increasing scale in stem cell production, a robust and controlled cell expansion process becomes essential for the clinical application of cell-based therapies. The objective of this work was the assessment of a hollow fiber bioreactor (Quantum Cell Expansion System from Terumo BCT) as a cell production unit for the clinical-scale production of human periosteum derived stem cells (hPDCs). *Methods.* We aimed to demonstrate comparability of bioreactor production to standard culture flask production based on a product characterization in line with the International Society of Cell Therapy *in vitro* benchmarks and supplemented with a compelling quantitative *in vivo* bone-forming potency assay. Multiple process read-outs were implemented to track process performance and deal with donor-to-donor-related variation in nutrient needs and harvest timing. *Results.* The data show that the hollow fiber bioreactor is capable of robustly expanding autologous hPDCs on a clinical scale (yield between 316 million and 444 million cells starting from 20 million after ± 8 days of culture) while maintaining their *in vitro* quality attributes compared with the standard flask-based culture. The *in vivo* bone-forming assay on average resulted in 10.3 ± 3.7% and 11.0 ± 3.8% newly formed bone for the bioreactor and standard culture flask respectively. The analysis showed that the Quantum system provides a reproducible cell expansion process in terms of yields and culture conditions for multiple donors.

Key Words: *bioreactor, mesenchymal stromal cell expansion, osteogenesis, quality control, process reproducibility, process monitoring and control*

Introduction

An increasing number of clinical trials [1,2] have demonstrated that cell-based therapies are becoming a reality, destined to revolutionize the health care industry. Mainly based on hematopoietic and mesenchymal stromal cells (MSCs), a large number of cell-based therapies are currently being developed for the treatment of multiple conditions ranging from skeletal defects (e.g., PREOB by Bone Therapeutics), to oncological [3,4], to cardiovascular (e.g., C-CURE by Celyad) [5] and liver disorders (e.g., HepaStem by Promethera). However, there is growing awareness of the numerous bioprocessing challenges that need to be addressed to translate initial clinical successes, which were most often based on manual laboratory-scale cell culture processes, into an industrial process that can guarantee the production of cell-based therapies with manageable cost of goods and robust and predictable *in vivo* performance [6–8].

Although clinical dose sizes vary significantly across therapeutic applications, most MSC-based therapies require between 10^7 and 10^9 cells for a single dose [9,10], while the number of cells that can be sourced from a single donor is generally much lower (e.g., approximately 1000 MSCs per milliliter of bone marrow [11,12]. Therefore, a cell expansion step is required able to produce a high number of cells with a reproducible cell quality [13,14]. With an increasing scale of culture volumes, the choice to translate from manual flask-based culture to bioreactors, be it multi-plate bioreactors, perfusion bioreactors or stirred vessels, is inevitable [15]. However, the high level of automation and precision that is essential for the desired process reproducibility often conflicts with the complexities entailed in live cell-based products. As more

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process steps are required for larger numbers of cells, not only is variability introduced by the process itself proportional to the scale of expansion [16], but with the use of large-scale automated bioreactors, the monitoring and control of biological variability also becomes more important. Because MSCs generally possess only a limited expansion capacity before critical cell characteristics start to deteriorate [17,18], cells are sourced from multiple donors, resulting in variable process input. Especially for autologous ("patient-specific" compared with "universal" or allogeneic) therapies, where donor-to-donor variation is expected to affect each batch for example in terms of nutrient requirements or optimal timing of harvest, the lack of process reproducibility poses a significant challenge to the development of a clinical production process that should ensure a minimal number of cells with a defined quality level.

In the first phase of this work, the Quantum system was evaluated as a tool to scale-up a standard tissue culture flask-based expansion step for an autologous bone tissue engineering therapy under development in our lab. The expansion process should ensure the maintenance of a set of MSC quality characteristics, such as their pluripotency and differentiation potential [19,20]. More important, these cells should maintain an unimpaired regenerative potential when implanted in an *in vivo* potency assay. Second, monitoring tools were implemented to track changes in bioreactor process conditions, either induced by process variations or biological variation. Finally, a case study on the clinical-scale expansion of three donors is presented allowing to compare bioreactor process reproducibility.

Methods

General experimental workflow

The evaluation of the large-scale bioreactor-based expansion step fits in the development of an autologous bone tissue engineering therapy, based on human periosteum derived stem cells (hPDCs, section 2.2 for details). Only a small number of cells (between 1×10^4 and 1×10^5) can be obtained via a biopsy of the periosteum of the patient, whereas it is hypothesized that for a tibia defect 3 cm wide, approximately 300 million cells are required [21,22]. The general experimental workflow (Figure 1) consists of an initial pre-culture phase (section 2.2 for details) in which the cells from a periost biopsy are expanded by standard flask-based culture to be able to seed the bioreactor surface with a minimum initial seeding density. The second phase is the bioreactor-based expansion process (for details, see "Standard flask-based hPDC culture" later in the article). Three flask-based control conditions were included in parallel with the bioreactor culture

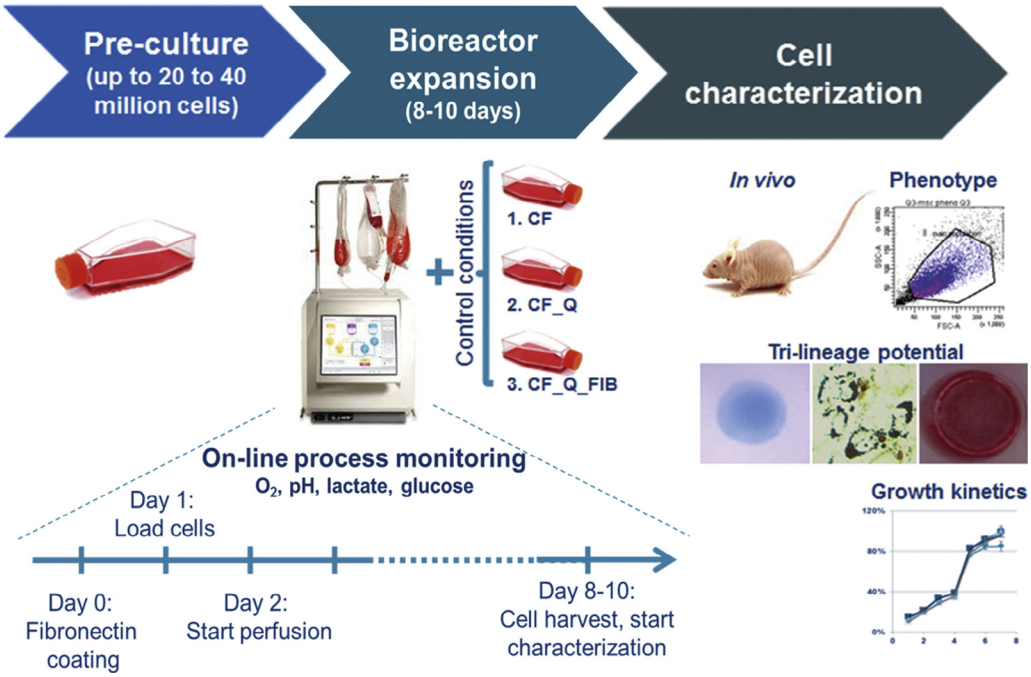

Figure 1. General experimental work flow with an initial flask-based pre-culture phase, followed by the Quantum system bioreactor culture. During bioreactor culture, the cell growth was monitored on the basis of daily lactate measurements, and three tissue culture flask–based control conditions were taken along to investigate the effect of flow rate, lowered seeding density and fibronectin coating. Immediately after cell harvest, the cell characterization was initiated, including, among others, an *in vivo* bone-forming potency assay. The different assays and analysis presented here are discussed in more detail in the text.