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Serum-free process development: improving the yield and consistency of human mesenchymal stromal cell production

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Abstract

Background aims. The cost-effective production of human mesenchymal stromal cells (hMSCs) for off-the-shelf and patient specific therapies will require an increasing focus on improving product yield and driving manufacturing consistency. *Methods.* Bone marrow–derived hMSCs (BM-hMSCs) from two donors were expanded for 36 days in monolayer with medium supplemented with either fetal bovine serum (FBS) or PRIME-XV serum-free medium (SFM). Cells were assessed throughout culture for proliferation, mean cell diameter, colony-forming potential, osteogenic potential, gene expression and metabolites. *Results.* Expansion of BM-hMSCs in PRIME-XV SFM resulted in a significantly higher growth rate ($P < 0.001$) and increased consistency between donors compared with FBS-based culture. FBS-based culture showed an inter-batch production range of 0.9 and 5 days per dose compared with 0.5 and 0.6 days in SFM for each BM-hMSC donor line. The consistency between donors was also improved by the use of PRIME-XV SFM, with a production range of 0.9 days compared with 19.4 days in FBS-based culture. Mean cell diameter has also been demonstrated as a process metric for BM-hMSC growth rate and senescence through a correlation ($R^2 = 0.8705$) across all conditions. PRIME-XV SFM has also shown increased consistency in BM-hMSC characteristics such as per cell metabolite utilization, *in vitro* colony-forming potential and osteogenic potential despite the higher number of population doublings. *Conclusions.* We have increased the yield and consistency of BM-hMSC expansion between donors, demonstrating a level of control over the product, which has the potential to increase the cost-effectiveness and reduce the risk in these manufacturing processes.

Key Words: *cell-based therapy, comparability, consistency, human mesenchymal stromal cell, manufacturing, regenerative medicine, serum-free, yield*

Introduction

The successful development of cell-based therapies has the potential to address a number of currently unmet clinical indications and to improve patient care across the world. Growing interest in this emerging field is evident by the large number of recent acquisitions of cell-based therapy companies by larger biopharmaceutical multinationals; for example, FUJIFILM Holdings Corporation (TSE: 4901) recently acquired Cellular Dynamics International (NASDAQ: ICEL), a developer and manufacturer of induced pluripotent stem cells. However, despite the progress, there are a number of challenges that remain before cell-based therapies can be incorporated into routine clinical practice and their full potential realized.

Human mesenchymal stromal cells (hMSCs) have demonstrated the potential to target a number of these currently unmet conditions, with clinical trials currently underway for indications such as acute myocardial infarction, stroke and a host of inflammatory and immune disorders [1]. For the majority of these clinical indications, however, the *in vitro* expansion of cells is required to deliver an effective therapeutic dose. The intention of this expansion step is to manufacture a sufficient number

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of cells to deliver therapeutic benefit without having a detrimental impact on the quality of the cell at decreasing production costs. Understanding and defining the quality attributes of hMSC therapies will be critical for their successful manufacture. This is proving difficult, however, owing to their complex, multifaceted and poorly understood *in vivo* mechanism of action [2].

Cell-based therapies can be broadly divided into two categories: patient-specific therapies (autologous) and off-the-shelf therapies (allogeneic). Traditional biopharmaceutical manufacture is predominantly focused on universal treatments in which multiple patients can be treated from a single batch. The manufacture of patient-specific therapies, however, will require the careful consideration of regulatory challenges as well as the distribution and delivery of a safe, effective and affordable cell-based therapy [3]. This also introduces a range of additional challenges, not least of all how a cell therapy manufacturing process can be developed to consistently manufacture products from multiple donors [4]. This will also be necessary for off-the-shelf products because cellular senescence will limit their expansion potential [5]. The key difference between off-the-shelf and patient-specific therapies, however, is that a donor selection process can be used for off-the-shelf products to select donor cell lines that are similar on the basis of expansion potential and desired quality attributes.

A crucial factor determining the economic success of off-the-shelf cell-based therapies in terms of affordability probably will hinge on whether the patient receiving the hMSC therapy will require immunosuppressive medication, increasing the overall lifetime cost of the treatment, although most clinical trials do not currently use them [6]. It has been demonstrated previously [7,8] that the use of serum during cell culture processes can lead to an undesired increase in immune response *in vivo*, and therefore the use of serum-free alternatives has the potential to reduce the requirement for post-infusion immunosuppressive medication. The development of defined medium formulations for specific cell-based therapies can also use this type of clinical output as a basis for their development. These long-term considerations for hMSC product manufacture and delivery will be important to drive the development of cost-effective and reimbursable therapies, which has proved difficult to date.

Achieving the consistent manufacture of medicinal products is a key requirement for regulatory approval and begins with assessing and reducing process variation when possible [9]. Driving a consistent process will demonstrate a state of control over the product and provides a foundation for comparability, whereby process changes during clinical development can be validated and allows for the product to be manufactured at multiple sites. A key aspect of reducing variation in the process will be reducing and eventually eliminating the use of fetal bovine serum (FBS) from the cell culture medium [10]. In addition to lot-to-lot variability, there are further process constraints on the use of FBS such as limited supply [11], spiraling cost, potential for pathogen transmission, increased risk of recipient immune reaction [12] and reduced scope for process optimization. All of these considerations mean that moving toward a serum-free process would be beneficial in achieving scalable, tunable and consistent hMSC manufacturing processes. In addition, serum-free culture has been shown to be amenable to scalable expansion technology such as micro-carriers and stirred bioreactors, producing higher hMSC yields per time unit than serum-based processes, which will be important for driving down the production cost of hMSC therapies [13,14] and is a key focus for our group. That said, the current cost of serum-free medium (SFM) for research is generally higher than serum-based medium; however, as the demand increases and higher yield processes can be developed, these costs probably will be reduced over time.

Considering the innate biological variability that exists between donors and the importance of ensuring a consistent manufacturing process, driving this philosophy into process development at an early stage is critical. Therefore, the aim of this study was to demonstrate how the development of a serum-free expansion process can drive increased consistency and yield of hMSC manufacture between donors and the benefits that this can bring as the process scale increases.

Methods

Monolayer culture

Human MSCs were isolated from bone marrow aspirate purchased from Lonza obtained from two healthy donors with informed consent: BM-hMSC 1 (lot: 071313B) and BM-hMSC 2 (lot: 071281D). The local ethics committee approved the use of the sample for research. Cells from passage 1 were cryopreserved at a density of $1-2 \times 10^6$ cells/mL in a freeze medium containing 90% (vol/vol) FBS (Hyclone) and 10% (vol/vol) dimethyl sulfoxide (Sigma-Aldrich). For serum-free experiments, hMSCs cryopreserved in serum underwent one adaptation passage in SFM. Cells were grown in T-flasks seeded at 5000 cells/cm^2 at 37°C in humidified air containing 5% CO_2. For serum-based culture,