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Review

Scalable microcarrier-based manufacturing of mesenchymal stem/stromal cells

António M. de Soure, Ana Fernandes-Platzgummer, Cláudia L. da Silva, Joaquim M.S. Cabral*

Department of Bioengineering and iBB—Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, Lisboa, Portugal

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ABSTRACT

Due to their unique features, mesenchymal stem/stromal cells (MSC) have been exploited in clinical settings as therapeutic candidates for the treatment of a variety of diseases. However, the success in obtaining clinically-relevant MSC numbers for cell-based therapies is dependent on efficient isolation and *ex vivo* expansion protocols, able to comply with good manufacturing practices (GMP). In this context, the 2-dimensional static culture systems typically used for the expansion of these cells present several limitations that may lead to reduced cell numbers and compromise cell functions. Furthermore, many studies in the literature report the expansion of MSC using fetal bovine serum (FBS)-supplemented medium, which has been critically rated by regulatory agencies. Alternative platforms for the scalable manufacturing of MSC have been developed, namely using microcarriers in bioreactors, with also a considerable number of studies now reporting the production of MSC using xenogeneic/serum-free medium formulations. In this review we provide a comprehensive overview on the scalable manufacturing of human mesenchymal stem/stromal cells, depicting the various steps involved in the process from cell isolation to *ex vivo* expansion, using different cell tissue sources and culture medium formulations and exploiting bioprocess engineering tools namely microcarrier technology and bioreactors.

Contents

* Corresponding author.
E-mail address: joaquim.cabral@tecnico.ulisboa.pt (J.M.S. Cabral).

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1. Introduction

In the 1960s-1970s, Friedenstein et al. isolated and characterized a sub-population of adherent spindle-shaped cells from the murine bone marrow (BM) capable of generating colony-forming unit-fibroblasts (CFU-F) (Friedenstein, 1990). These cells, derived from the mesoderm, were later designated as "mesenchymal stem cells" and shown to have multilineage differentiation potential (Caplan, 1991; Pittenger et al., 1999). Given that this unfractionated plastic-adherent cell population is highly heterogeneous and there was no convincing evidence to support the "stemness" of these cells, it was suggested that these cells should be named multipotent mesenchymal stromal cells or rather designated using the acronym MSC, which in this review stands for mesenchymal stem/stromal cells (Horwitz et al., 2005). While no specific cell surface marker has been identified for MSC, minimal criteria was established in a position paper by the International Society for Cellular Therapy (ISCT) to define these cells: these must adhere to plastic; express CD73, CD90, CD105 and lack the expression of CD11, CD14, CD79α or CD19, CD34, CD45 and HLA-DR; and have osteogenic, adipogenic and chondrogenic differentiation potential under standard culture conditions (Dominici et al., 2006). More recently, Samsonraj et al. proposed additional phenotypic criteria to be used in combination with the existing ISCT minimum standards that will allow an improved assessment of the potency of MSC and potentially provide a basis for establishing the quality of these cells prior to their clinical use (Samsonraj et al., 2015). Although originally identified in the BM (Castro-Malaspina et al., 1980; Friedenstein et al., 1970), MSC can also be isolated from other adult sources such as the adipose tissue (AT) (Kern et al., 2006), dental pulp (Pierdomenico et al., 2005) and synovial membrane (SM) (De Bari et al., 2001a; Santhagunam et al., 2014), as well as from perinatal sources such as the umbilical cord matrix (UCM) (Simões et al., 2013), umbilical cord blood (Kern et al., 2006), amniotic fluid (Scherjon et al., 2003) and the placenta (Igura et al., 2004).

Owing to their availability from a wide range of sources, high proliferative potential *in vitro*, multilineage differentiation ability, trophic features and immunomodulatory properties, these cells are seen as promising candidates in cell based-therapies for the treatment of a range of diseases (Bernardo and Fibbe, 2013; Caplan and Dennis, 2006; Pittenger et al., 1999; Wu et al., 2007). Although there is still no consensus on the ideal target therapeutic cell doses to be administered into patients, MSC infusions in previous and ongoing clinical trials have typically required considerable high cell numbers, generally in the order of several million cells per kilogram of body weight (Lublin et al., 2014; Molendijk et al., 2015; te Boome et al., 2015). However, only a very limited amount of MSC can be isolated from the aforementioned sources. In the BM, for instance, these cells represent only 0.01% of the total mononuclear cell fraction (Miao et al., 2006). Thus, it is crucial to set-up strategies to address this issue, such as the establishment of culture systems capable of promoting the efficient isolation and expansion of MSC without compromising their therapeutic features.

Most studies have focused on the expansion of MSC on static 2- dimensional (2D) polystyrene culture flasks testing different culture parameters (*e.g.* medium formulations) with the aim of maximizing cell proliferation, maintaining intrinsic cell features and therapeutic potential, as well as the their safety for human use (dos Santos et al., 2013). Overall, the main focus is to reach the highest yield possible of therapeutic cells, in a cost-efficient way, while ensuring high product quality and adequate storability conditions. Furthermore, the cell products generated must comply with good manufacturing practices (GMP) (reviewed in (Sensebé et al., 2013)) in terms of regulatory framework, monitoring/control, production procedures, quality assurance and product delivery (Fekete et al., 2012; Lange et al., 2007; Simões et al., 2013).

In the last decade, innovative 3-dimensional (3D) scalable systems have been developed for MSC manufacturing, namely microcarrier-based culture platforms, which have shown very promising results regarding the above mentioned goals (dos Santos et al., 2014; Heathman et al., 2015a; Rafiq et al., 2013). In this review we give a comprehensive overview on the different steps involved in the manufacturing of MSC from different human sources, from isolation to *ex vivo* expansion, using different culture formats (monolayer, 3-D spheroids or microcarriers) and culture medium formulations, with a main focus on the exploitation of bioprocess engineering approaches through the use of microcarrier-based bioreactors for cell production.

2. *Ex vivo* expansion of MSC

2.1. Source and isolation of MSC

The success in obtaining clinically-relevant MSC numbers for cell-based therapies is dependent not only on an optimized expansion protocol but, as a first step, on the establishment of an efficient isolation method. For instance, MSC obtained from different tissues present different features (*e.g.* proliferative potential in vitro) (Simões et al., 2013), immunomodulation (Ribeiro et al., 2013) which may help determining the most appropriate source for a specific clinical application. In this section we will review the different methods used for the isolation of MSC from different sources, specifically BM, AT, SM and UCM.

2.1.1. Bone marrow (BM)

BM was the original site from where MSC were firstly derived and most clinical trials performed to date have used MSC from this source (Friedenstein et al., 1970; Heathman et al., 2015b). Typically, BM samples are first subjected to a density gradient centrifugation step using a polymeric solution (*e.g.* Ficoll-Paque PLUS (1.078 g/mL), Percoll (1.130 g/mL)) in order to separate the mononuclear cell (MNC) fraction from other marrow constituents such as erythrocytes and plasma (Gottipamula et al., 2014). This MNC fraction contains, among other cell populations, a small percentage of MSC that can be isolated in two ways: (i) adherence to plastic (polystyrene) tissue culture flasks or (ii) immunobased cell-sorting methods. The latter method has the advantage of allowing the collection of a more homogeneous cell population, at least in what concerns the specific cell marker(s) used for isolation. As such, different epitopes such as Stro-1, CD271, SUSD2, MSCA-1,