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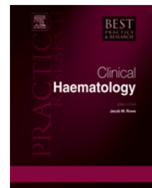

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Ex vivo expansion of mesenchymal stromal cells

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Mesenchymal stromal cells (MSCs) are adult multipotent cells that can be isolated from several human tissues. MSCs represent a novel and attractive tool in strategies of cellular therapy. For *in vivo* use, MSCs have to be *ex vivo* expanded in order to reach the numbers suitable for their clinical application. Despite being efficacious, the use of fetal calf serum for MSC *ex vivo* expansion for clinical purposes raises concerns related to immunization and transmission of zoonoses; the standardization of expansion methods, possibly devoid of animal components, such as those based on platelet lysate, are discussed in this paper. Moreover, this review focuses on the search of novel markers for the prospective identification/isolation of MSCs and on the potential risks connected with *ex vivo* expansion of MSCs, in particular that of their malignant transformation. Available tests to study the genetic stability of *ex vivo* expanded MSCs are also analyzed.
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Introduction

In addition to hematopoietic stem cells (HSCs), the bone marrow (BM) also contains mesenchymal stromal cells (MSCs). These cells were first recognized more than 40 years ago by Friedenstein et al. who described a population of adherent cells from the BM which were non-phagocytic, exhibited

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a fibroblast-like appearance and could differentiate *in vitro* into bone, cartilage, adipose tissue, tendon and muscle [1]. Moreover, after transplantation under the kidney capsule, these cells gave rise to the different connective tissue lineages [2].

In general, MSCs represent a minor fraction in BM and their exact frequency is difficult to calculate because of the different methods of harvest and separation. However, the frequency in human BM has been estimated to be in the order of 0.001–0.01% of the total nucleated cells; moreover, this frequency declines with age, from $1/10^4$ nucleated marrow cells in a newborn to about $1/2 \times 10^6$ nucleated marrow cells in a 80-year old person [3].

After their first identification in the BM [1], human MSCs (hMSCs) were isolated from a variety of other human tissues, including periosteum, muscle connective tissue, perichondrium, dental pulp, adipose tissue and fetal tissues, such as lung, BM, liver and spleen [4–7]. Amniotic fluid and placenta have been found to be rich sources of MSCs [8,9]; both fetal and maternal MSCs can be isolated from human placenta [8]. Although present at low frequency, MSCs have been also identified in umblical cord blood (UCB); in this respect, quality criteria for the selection of UCB units are considered critical for the successful isolation of MSCs from this source [10,11].

One of the hallmark of MSCs is their multipotency, defined as the ability to differentiate into several mesenchymal lineages, including bone, cartilage, tendon, muscle, marrow stroma and adipose tissue (AT) [7,12]. Usually, trilineage differentiation into bone, adipose tissue and cartilage is taken as a criterium for multipotentiality [12,13].

MSCs display unique immunological properties that modulate the responses, *in vitro* and *in vivo,* of all cells involved in the immune response, including T and B lymphocytes, dendrtic cells and natural killer (NK) cells [14–16]. However, the exact mechanisms by which MSCs exert their functions are still poorly understood. Whether MSCs mainly mediate their effect through soluble factors or cell-to-cell contact is still a matter of active investigation [14,17,18]. Moreover, the mechanisms by which MSCs display their immunosuppressive effect are largely restricted to *in vitro* studies; the *in vivo* biological relevance of the *in vitro* observations needs to be elucidated [14–16].

Thanks to their ability to home to inflamed sites and repair-injured tissues, and to their immunomodulatory properties, MSCs are today considered a promising tool in approaches of immunoregulatory and regenerative cell therapy [19]. Thus far, MSCs have been employed in phase I/II clinical trials [20–23], mainly addressing the issues of feasibility and safety of infusion; and to date no adverse effects have been registered after MSC administration. In particular, MSCs, usually harvested at passage 2 to 3 of *in vitro* culture, have been successfully employed in the clinic to enhance hematopoietic stem cell engraftment in HLA-haploidentical, T cell-depleted allografts [20] and UCB transplantation [21], as well as to treat the most severe and refractory forms of acute *graft-versus-host* disease [22]. Moreover, local injections of adipose tissue (AT)-derived MSCs have already been successfully employed in the clinical setting to treat complex perianal fistulas resistant to conventional treatments in Crohn's Disease (CD) patients [23].

Ex vivo isolation and characterization of MSCs

Most of the information available on MSC phenotypic and functional properties are derived from studies performed on cells cultured *in vitro* [13–15]. To date, MSC isolation/identification has mainly relied on their morphology and adherence to plastic, resulting in a heterogeneous population of cells, referred to as MSCs [13]. Immunephenotyping by flow cytometry is also applied to characterize *ex vivo* expanded MSCs and to define their purity. However, at present, no specific marker has been shown to specifically identify true MSCs and *ex vivo* expanded cells are currently stained with a combination of positive (CD105, CD73, CD90, CD166, CD44 CD29) and negative (CD14, CD31, CD34 and CD45) markers, at least in case of BM-derived cells (indeed, a proportion of AT-derived MSCs express CD34) [5,12,13].

Therefore, little is known about the characteristics of the primary precursor cells *in vivo,* since it has not yet been possible to isolate the most primitive mesenchymal cell from bulk cultures. One of the hurdles has been the inability to prospectively isolate MSCs because of their low frequency and the lack of specific markers. Recently, some groups have reported the identification and prospective isolation of the most primitive mesenchymal progenitors, both in murine and human adult BM, based on the expression of specific markers [24–34].

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