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Review

Characteristics, applications and prospects of mesenchymal stem cells in cell therapy☆

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ABSTRACT

Recent advances in the field of cell therapy and regenerative medicine describe mesenchymal stem cells (MSCs) as potential biological products due to their ability to self-renew and differentiate. MSCs are multipotent adult cells with immunomodulatory and regenerative properties, and, given their therapeutic potential, they are being widely studied in order to evaluate their viability, safety and efficacy. In this review, we describe the main characteristics and cellular sources of MSCs, in addition to providing an overview of their properties and current clinical applications, as well offering updated information on the regulatory aspects that define them as somatic cell therapy products. Cell therapy based on MSCs is offered nowadays as a pharmacological alternative, although there are still challenges to be addressed in this regard.

Características, aplicaciones y perspectivas de las células madre mesenquimales en terapia celular

Palabras clave:
Células madre mesenquimales
Tratamiento celular
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RESUMEN

Los recientes avances en el área del tratamiento celular y la medicina regenerativa describen las células madre mesenquimales (*MSC*) como potenciales activos biológicos debido a su capacidad de autorrenovación y diferenciación. Las *MSC* son células multipotentes, con propiedades inmunomoduladoras y regenerativas, y debido a su potencial terapéutico están siendo ampliamente estudiadas con el objetivo de evaluar su viabilidad, seguridad y eficacia. En esta revisión, se describen las principales características y fuentes de obtención de las *MSC*, se da una visión global sobre sus propiedades y aplicaciones clínicas actuales, así como una actualización de los aspectos regulatorios que las definen como medicamentos de tratamiento celular somático. El tratamiento celular basado en *MSC* se presenta a día de hoy como una gran alternativa farmacológica aunque todavía quedan retos por abordar.

Introduction

Cell therapy is a new therapeutic approach, based on the use of cells as therapeutic agents.[1] Thus, in regenerative medicine, the study and correct determination of the type of cell to be applied to a specific treatment is essential for success. For that reason, their safety and ability to repair, replace or restore the biological function of damaged tissues and organs needs to be defined.[1]

Studies carried out to date suggest that stem cells are suitable for use in regenerative medicine. The main feature of stem

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cells is that they are unspecialized cells with the ability to self-renew for long periods of time as well as differentiate themselves (plasticity) into specialized cells with specific functions. Stem cells are classified according to their differentiation ability: totipotent, pluripotent, multipotent and unipotent, with pluripotent and multipotent stem cells being the most studied for clinical application. Pluripotent stem cells can be differentiated from any cell of the body. They are divided into embryonic and induced; whereas multipotent adult somatic or tissue-specific stem cells[2] are those whose potential for specialization is restricted to one or more cell lineages.

Stem cells currently used in cell therapy are adult stem cells. *Mesenchymal stem cells* are among the most extensively studied (*MSC*). Despite having less proliferative potential and lower plasticity compared with embryonic stem cells and induced stem cells, they are easier to obtain from tissues, their manipulation does not create ethical problems,[3] have high *in vitro* expansion capacity plus a low potential for the formation of teratomas.[4,5] All this coupled with their ability to produce cytokines and growth factors, migrate to the region where tissue damage has occurred and exert immunomodulatory actions in that site means that the study and development of *MSC* as biological assets can help provide new therapeutic alternatives with high potential in regenerative medicine and cell therapy[6] for diseases that, so far, have no effective conventional treatments such as cancer, diabetes, chronic critical limb ischaemia, myocardial infarction, Parkinson's, *etc.*

Definition and characteristics of mesenchymal stem cells

MSC also known as stromal stem cells were first described in 1968 by Friedenstein et al. as colony forming units, fibroblastoid in appearance, which adhered to plastic in culture and had the ability to regenerate bone tissue *ex vivo*.[7] In 2006, the minimum characteristics required for a cell to be considered *MSC* were redefined in the *International Society of Cellular Therapy* (*ISCT*) Congress, these *being* as follows: (1) MSC must be capable of adhering to plastic in culture, (2) express the surface antigens CD73, CD90 and CD105 in the absence of other hematopoietic antigens of type CD34, CD45 and typical B lymphocytes, monocytes and macrophages markers, (3) be multipotent and have a high plasticity to differentiate *in vitro* under standard culture conditions into osteoblasts, adipocytes and chondrocytes.[8,9]

Other characteristics, in addition to those proposed by the *ISCT*, which can help to define and classify a cell as *MSC* is having a mesodermal origin, sharing some fibroblast characteristics, being able to self-renew, for which, during cell division, only one of the two resulting cells will start cell differentiation programs, exhibiting a relatively low immunogenicity and having the ability to differentiate under certain conditions into different lineage cells, "differentiation plasticity".[10,11]

Sources and cultures of mesenchymal stem cells

MSC can be obtained from bone marrow, adipose tissue, cord blood, dental pulp, smooth, skeletal and cardiac muscle, liver, spleen, testes, menstrual blood, pancreas, periosteum, synovial membrane, dermis, pericytes, trabecular bone, lung, placenta, peripheral blood, periodontal ligament and amniotic fluid aspirates.[12–16] Of these, the most important are bone marrow, adipose tissue and cord blood[17] (Fig. 1). Once *MSC* are isolated by plastic adherence and are cultured *in vitro*, may, under certain conditions, differentiate into mesodermal lineages such as osteocytes (bone cells), chondrocytes (cartilage cells), adipocytes (fat cells), myoblasts (precursors of muscle cells) and cardiomyocytes (heart cells),[8,18–20] or they can even differentiate into endodermal (hepatocytes, pancreatic cells) and ectodermal (keratinocytes, astrocytes and neurons)[21–23] lineage cells. Even though *MSC* have a broad differentiation capacity, they are considered multipotent and not pluripotent cells because their tetraploid complementation could not be proven. This consists on a test whereby, after injecting *MSC* in a mouse blastocyst, chimeric mice are expected to be obtained, in which, after the development of the embryo, the injected cells lead to cells belonging to the embryonal layers (endoderm, mesoderm and ectoderm).

MSC are easy to isolate, expand *in vitro* and handle throughout the cell culture process to which they must be subjected in order to obtain the number of cells needed to define the cell dose for patient administration. They can also be cryopreserved without experiencing phenotypic alterations, losing their proliferative capacity or their differentiation ability after the thawing process.[24] However, it has been described that when these undifferentiated cells are subjected to culture division processes for more than 7 passages, their cytogenetic characteristics and telomerase activity begin to be affected.[25] This leads to culture ageing and the emergence of chromosomal alterations[26] while causing the loss of cell multipotentiality and replicative senescence.[25,27] All this results in an accumulation of genetic and epigenetic alterations in these cells[28] inducing the transformation of *MSC* into immortalized cells with ability to form tumors.[26] In fact, some studies defend that cellular senescence acts as a tumour suppressor mechanism, able to stop cell growth, thereby reducing the risk of transformation of *MSC* into cancer cells,[29,30] while other studies have shown that the performance and differentiation potential may vary depending on age, donor condition, tissue collection conditions as well as media and conditions employed during cell culture.[31]

Therefore, when designing the study and development of a therapeutic treatment based on the administration of *MSC*, the characteristics and availability of donor tissue (autologous and allogeneic), the number of cell doublings during *in vitro* expansion and specificity and differentiation capacity of these cells should be taken into account.[32]

Characterization of mesenchymal stem cells

Because no *MSC* can be identified by a unique specific marker, these cell types must be defined based on a combination of phenotypic markers and functional properties. Thus, following the guidelines set by *ISCT*, one of the minimum criteria required today for a cell to be considered a *MSC* is the expression of certain surface antigens: CD73, CD90, CD105, CD166 and varying levels of stromal markers STRO-1, CD29, CD44, CD71, CD271 and GD2 ganglioside[14]; and the absence of hematopoietic antigens or other antigens typical of other cell populations present in the same tissues as the *MSC* such as CD45, CD11b, CD34, CD14, CD19, CD79a and major histocompatibility complex class II.[8,9,14,33] In addition, some authors have reported that *MSC* can express ligands essential for interaction with T cells, such as VCAM-1, ICAM-2, LFA-3[14,33] but they never express costimulatory molecules like CD80, CD86, CD40, CD40L and Fas ligand[14,34] (Table 1).

Depending on the origin of *MSC*, expression of surface markers varies; for example, adipose tissue *MSC* express higher surface antigens levels for: CD34, CD49d, CD54; bone marrow *MSC* express higher levels of CD106 surface antigen and *MSC* obtained from cord blood rarely express CD90 surface antigen.[17]

Similarly, although the current guidelines established by the *ISCT* still apply when defining a cell as *MSC*, one must resort to other available cell identification tools to distinguish *MSC* from other cell types, like in the case of fibroblasts. *MSC* have a fibroblastic

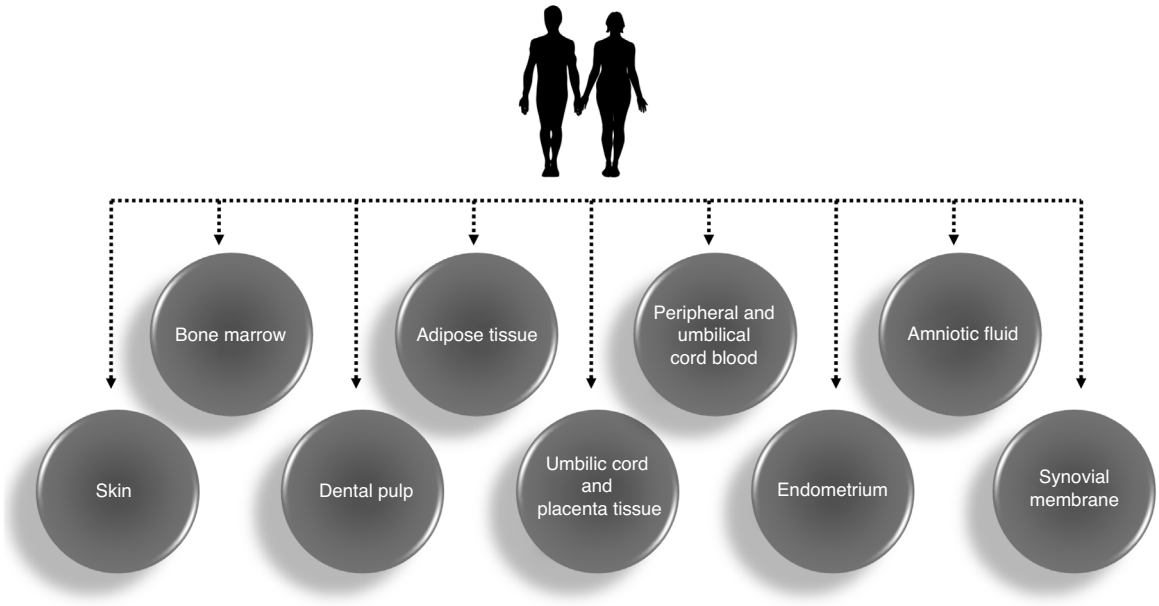

Fig. 1. Main sources for obtaining human mesenchymal stem cells.

Table 1
Function of mesenchymal stem cell key markers.

Markers present in mesenchymal stem cells	Function
CD29 or *integrin β 1* (ITGB1)	Cell adhesion and signal recognition
CD44 or ECM-III or HCAM or HUTCH-1 or Pgp-1 or hermes antigen	Migration, cell adhesion, proliferation and cell-cell interaction
CD51 or *integrin α V* (ITAGVα)	Adhesion and signal transduction
CD58 or LFA-3	Adhesion and T cell activation
CD71 or *transferring receptor 1* (TfR1)	Iron transport into the cell in proliferative state
CD73 or ecto-5′-nucleotidase	Lineage marker and cell adhesion mediator
CD90 or Thy-1	Marker of early mesenchymal precursors
CD105 or endoglin	Regulates extracellular matrix components such as fibronectin and collagen
CD106 or VCAM-1 or INCAM-110	Adhesion molecule, plays a key role in the process of immunosuppression
CD146 or MCAM or MELCAM or cell surface glycoprotein MUC18	Cell adhesion and multipotent cell state regulation
CD166 or ALCAM or DMEM or SC-1/DM-GRASP/BEN (in chicken) or KG-CAM (in rat)	Cell adhesion and is involved in maintaining the undifferentiated state
CD271 or LNGFR or p75 NTR	Seems to be involved in the development processes, cell survival and differentiation. It has been described as one of the most specific markers of *MSC* characterization and purification
Ganglioside GD2	Essential in cell-cell communication and cell recognition
STRO-1	Specific antigen for undifferentiated state
Integrin alpha 11 (ITGA11, “α11 integrin”)	Characterizes populations derived from bone marrow with increased osteogenic differentiation potential and lower adipogenic differentiation potential. It is one of the main molecules being used to increase knowledge about lineage relationships within *MSC* and facilitate the study of more homogeneous populations

MSC: mesenchymal stem cells.

morphology, also adhere to plastic and have high homology with fibroblasts regarding the presence or absence of surface antigens described to characterize *MSC* [CD73 (+) CD90 (+), CD105 (+), CD14 (−) CD34 (−) CD45 (−)]. Therefore, for this case, it was necessary to select new specific markers that differentiate between *MSC* (CD106, CD146, ITGA11) and fibroblasts (CD10, CD26).

Therapeutic potential of mesenchymal stem cells

Despite being one of the main tools used today in cell therapy, the mechanism of action of *MSC* has not yet been deciphered. However, the results obtained from preclinical and clinical studies conducted in recent decades show that *MSC* have a high immunomodulating, regenerative and healing capacity in addition to providing trophic support to the damaged cells. *MSC* regulate the paracrine secretion of growth factors, cytokines, antifibrotic factors and angiogenic mediators. It has been shown that the conditioned media generated from *MSC* cultures have a repair capacity similar that of cell-implantation itself, being able to exercise its immunomodulatory paracrine, anti-apoptotic, angiogenic, anti-scarring and chemoattractant capacity directly into the damaged tissue without injecting *MSC*.[35]

Two of the main reasons why *MSC* have been approved for therapeutic applications by the competent authorities have to do with their inherent ability not to express the major histocompatibility complex class II[36] on their surface and being able to go specifically to areas of hypoxia. It has been found that the intravenous administration of *MSC* in the body after injury causes these cells to go to the inflammation tissues. This *homing* or nesting process by *MSC* in the body's damaged area provides both functional and protective effects within the same. *MSC* interact with the immune system in the damaged host tissue by interfering with

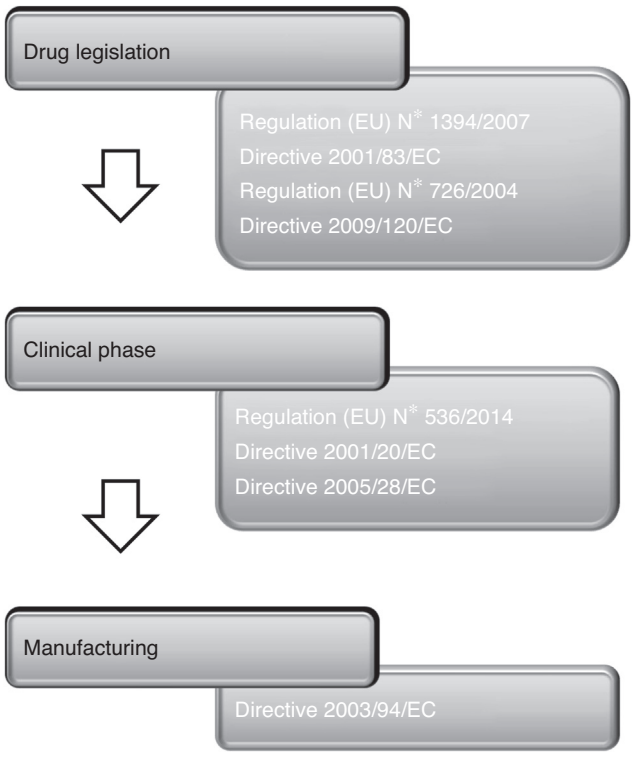

Fig. 2. Summary of the legislative framework of the European Union applicable to human mesenchymal stem cells.

Table 2
Diseases studied in phase III clinical trials with mesenchymal stem cells.[a]

Diseases	No. of clinical trials
Haematological disorders	
Graft *versus* host disease	4
Hematopoietic recovery	1
Hematologic malignancy	1
Neuromuscular disorders	
Spinal cord injury	3
Cartilage and bone injury	
Articular cartilage defects	1
Bone fracture	1
Brain injury	
Stroke	2
Cerebral palsy	1
Gastrointestinal disorders	
Perianal fistula	1
Ischaemic-type biliary lesions	1
Cardiovascular diseases	
Chronic heart failure	2
Acute myocardial infarction	2
Ischaemic cardiomyopathies	2
Autoimmune diseases	
Diabetes type 1	1
Ankylosing spondylitis	1

[a] Data have been obtained on the ClinicalTrials.gov website (http://www.clinicaltrials.gov) on 12th November 2016. The search criteria included the following keywords: "Mesenchymal", "open studies" and "phase 3".

the function of dendritic cells and T cells, thus allowing to create a local immunosuppressant microenvironment due to their ability to secrete cytokines.[37] This immunosuppression can be potentiated in the presence of interferon γ.[38] Cytokine activation in the damage zone also allows adhesion molecules and integrins to be activated, which are essential for *MSC* to migrate and be anchored in the damaged tissues. *MSC* modulate immune reactions in collagenopathies or bone marrow transplants; besides, they also play an essential role in the development and differentiation of the lymphohematopoietic system, as it secretes growth factors and regulatory cytokines that not only are not detected by the immune system of the host but also exert an immunomodulatory capacity on it.[39]

It has been shown that *MSC* perform a regenerative function in damaged or injured tissues such as skin, bone, cartilage, liver, cornea, due to their ability to differentiate into specialized cells, such as chondrocytes, osteocytes, epithelial cells, kidney cells and retinal pigment epithelial cells, among other.[40] It has also proved effective in tissue regeneration of the periodontium, myocardium, nervous system, *etc.*

Regulatory aspects of mesenchymal stem cells in Europe

Cell therapy products are considered drugs in the European Union since 2003; they were introduced in the legislation through Directive 2003/63/EC, along with gene therapy products.[41] Later, in 2007, cell therapy, gene therapy and tissue engineering products were defined as advanced therapy medicinal products in the Regulation (EC) No. 1394/2007 (Fig. 2). It regulates the use of *MSC*, defining it as *somatic cell therapy medicinal product* (sCTMP), which must contain viable cells, which can be subjected to substantial manipulation (*in vitro* expansion and culture) during their production,[5] and whose essential biological function may not be the same in donor and recipient.[41]

Therefore, *MSC* production should be subject to *Good Manufacturing Practice* (GMP) guidelines, where the quality and safety of cells to be produced must be guaranteed, the same as any other medication of non-cellular origin.[42] Fulfilling GMP standards includes quality control of the biological starting material, reagents and consumables used throughout the entire *MSC in vitro* expansion process until the required doses are obtained, besides the final product excipients. Similarly, *MSC*'s manufacturing process as sCTMP must be completely sterile, so the facilities (clean rooms or GMP rooms) must be certified, the processes must be validated and staff should be trained[43] to perform such work. Finally, the cell drug finally obtained from *MSC* must satisfy predefined specifications based on the identity, purity, potency, dose, genetic stability and cell viability.[44]

When performing clinical trials, the origin of the sample (bone marrow, adipose tissue, umbilical cord), *MSC* isolation method (enzymatic or non-enzymatic), selection (adherence and phenotype), *in vitro* expansion process (culture media, *xenogeneic-free*, oxygen concentration, maximum number of passages, *etc.*)[45] and dose determination should be taken into account, as they are not yet fully standardized. Therefore, future strategies for *MSC* use in cell therapy should address these aspects to reach more specific and unified use criteria.

Clinical applications

Cell therapy involves the transplantation of autologous cells (cells from the same patient) or allogeneic (cells from a donor), either through a local or systemic administration.

Since 1995, when the first clinical trial was conducted, where 15 patients were treated with autologous bone marrow *MSC*[46] to date, the clinical use of these cells has been extensively studied. Currently, 2076 stem cell clinical trials are being conducted worldwide, a total of up to 405 of these clinical trials focus on the study of the efficacy and safety of *MSC* in therapeutic applications (Fig. 3). Most of these clinical trials are in phase I, phase II

Fig. 3. Distribution of clinical trials with mesenchymal stem cells that are taking place around the world in November 2016. Source: clinicaltrials.gov. Available in: https://clinicaltrials.gov/ct2/results/map?term=mesenchymal&recr=Open.

Table 3
Cell therapy drugs with mesenchymal stem cells authorized in the world.

Name	Company	*MSC* origin	Dose	Diseases
HeartiCellgram-AMI®	FCB-Pharmicell Co Ltd.	*Autogenous bone marrow MSC*	10^6 *MSC*/patient kg	Acute myocardial infarction
Cartistem®	Medipost Co Ltd.	*Allogeneic umbilical cord blood MSC*	500 μl of hyaluronic acid hydrogel/cm^2 of cartilage defect	Knee cartilage defects caused by a traumatic and degenerative osteoarthritis
Cuspitem®	Anterogen Co Ltd.	*Autologous adipose tissue MSC*	3×10^7 *MSC*/cm of fistula diameter	Crohn's fistulas
Prochymal®	Osiris Therapeutics Inc.	*Allogeneic bone marrow MSC*	8 doses of 2×10^6 *MSC*/kg during 4 consecutive weeks	Graft *versus* host disease in paediatric patients (aged 2–17 years)
TEMCELL HS®	JCR Pharmaceuticals Co. Ltd.	*Allogeneic bone marrow MSC*	8 doses of 2×10^6 *MSC*/patient kg during 4 consecutive weeks	Graft *versus* host disease Myocardial infarction

MSC: mesenchymal stem cells.

or combined phases I–II; only about 24 of these trials are in phase III (Table 2).

So far, the results obtained from both *in vitro* and *in vivo* studies conducted with *MSC* recommend these cells as a promising therapeutic alternative for the treatment of various diseases such as cartilage and bone lesions,[47] diabetes[48] multiple sclerosis[49] and myocardial infarction,[50] among other; having proved, to date, that *in vivo* administration of *MSC* is safe and feasible. However, some challenges remain, like demonstrating its efficacy for many diseases currently under research, determining its long-term effects when administered to the patient, dose selection, route of administration, *etc.* Hence, the limited number of *MSC*-based sCTMP currently authorized. In 2011, AMI HeartiCellgram® (South Korea) was the first *MSC-formulated drug approved globally*. Since then other drugs have been approved: Cartistem® and Cuspitem® (South Korea), Prochymal®(Canada and New Zealand) and Temcell® (Japan, equivalent to Prochymal).[51–53] However, no sCTMP have been marketed yet in the EU (Table 3).

In addition to the drugs previously presented for use in somatic cell therapy, there are different products in the USA with allogeneic *MSC* authorized as medicinal products: OsteoCel® (NuVasive), Trinity (Orthofix), Allostem® (AlloSource) and LiquidGen® (Skye Orthbiologics). All based on a matrix (allograft) in which the cells are deposited with the objective of promoting osteogenesis and reduce inflammation in bone lesions.

Conclusions and future prospects

The results obtained in both *in vitro* and *in vivo* trials have made possible to establish *MSC* as a medicine for cell therapy. At the same time, the criteria for its manufacturing as a therapeutic product has

been established, ensuring its quality during the same. This comprehensive control is important to maintain the repair potential of *MSC* unchanged, whose efficacy lies in the paracrine, immunomodulatory and regenerative capacity on damaged tissue.

For this reason, *MSC* are considered today a therapeutic alternative for treating multiple disorders such as diseases of immune, degenerative or traumatic origin. However, despite its proven safety, there are still many obstacles to overcome when using these cell types as medicines in cell therapy. Their nature, applicability and regulation through niches or microenvironments should be studied more deeply, since their functionality may vary depending on the direct interaction with other cells or the release of soluble factors specific for each microenvironment. For this reason, it is important to study the behaviour of different *MSC* populations in relation to specific niches, rather than generalizing its therapeutic use.

Conflict of interests

The authors declare no conflict of interest.

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