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Mesenchymal stem cells and their relationship to pericytes

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TABLE OF CONTENTS

1. ABSTRACT

Our body contains cells that can be propagated *in vitro* and give rise to cells with mature mesenchymal phenotypes. These cells are interesting not only because of their differentiation capability, which could be used for tissue engineering, but also because they secrete molecules which have trophic, chemoattractant, and immunomodulatory properties. Along decades of study, these cells have been referred to as fibroblastic cells, stromal cells, or mesenchymal stem cells. There is evidence that pericytes, cells that wrap endothelial cells in blood vessels, behave as stem cells in the tissues, and give rise to these progenitor cells when removed from the body and expanded in culture – a process that may reflect changes that occur *in vivo* under injury conditions. Here, we discuss the evidence that favors this thesis, and discuss culture methods, clinical and preclinical applications of mesenchymal stem cells under this perspective.

2. INTRODUCTION

When reviewing the subject “mesenchymal stem cell”, it is important to define clearly the boundaries of this term. A mesenchymal stem cell can be specified

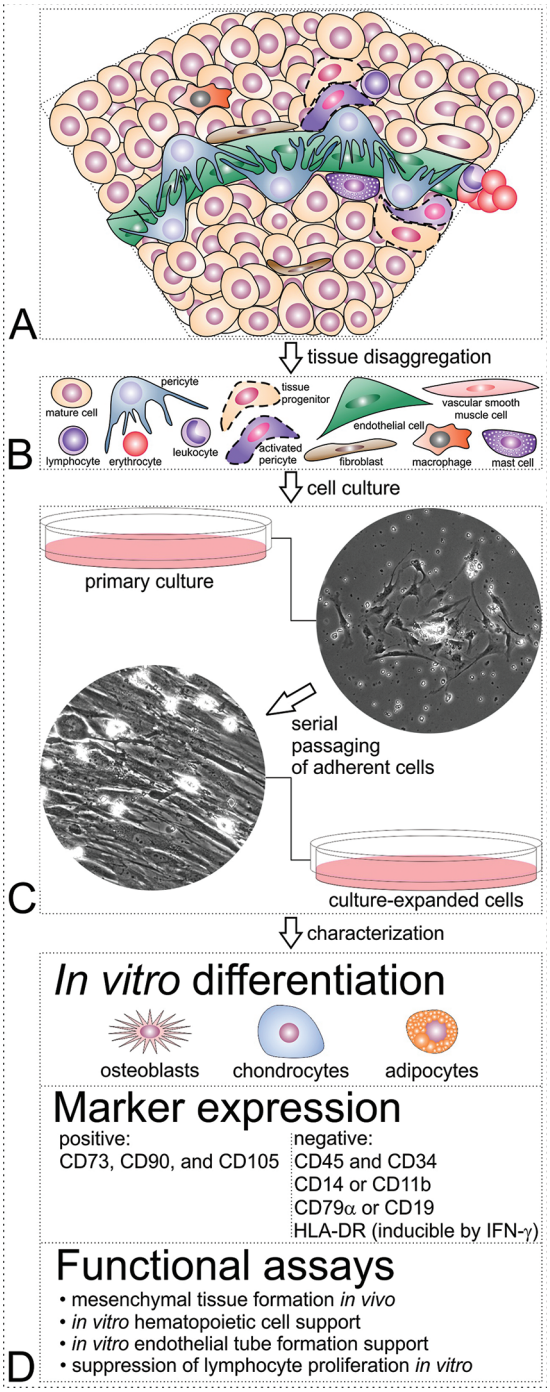

Figure 1. Schematic representation of MSC isolation, culture establishment, expansion, and characterization. (A) Structure of a tissue subjected to a disaggregation process to yield the cellular components specified in (B). The cells obtained after disaggregation are counted, resuspended in culture medium, and plated in a tissue culture-treated vessel; the adherent cells are further expanded and passaged (C). After passaging, the cells are subjected to assays to determine their ability to differentiate into cells with a mesenchymal phenotype such as osteoblasts, chondrocytes, and adipocytes, and their surface molecule profile (D). Additional assays may be performed to assess MSC capacity to form mesenchymal tissue *in vivo* (e.g. bone, cartilage), to support the survival of hematopoietic cells *in vitro*, to support the development of capillary-like structures by endothelial cells *in vitro*, and to suppress lymphocyte proliferation *in vitro*.

as being a cell able to self-renew and differentiate into cells with mature mesenchymal phenotypes (1). Various types of stem cells can be found in the body in well-defined niches; intestinal stem cells, for example, are found at the base of crypts in the intestinal epithelium (2). The precise microanatomical location of mesenchymal stem cells *in vivo*, on the other hand, is still a subject for debate. When tissue from a given organ – the bone marrow (BM), for example – is placed in culture, adherent cells that are able to proliferate and differentiate into cells with a mature mesenchymal phenotype such as adipocytes, osteoblasts, and chondroblasts (Figure 1) become established. If these adherent cell populations meet defined criteria, they may be called mesenchymal stem cells on an operational basis; however, are these cells representatives of mesenchymal stem cells *in vivo*? Cells removed from the body and subjected to culture conditions that fail to mimic those found in their original niche are likely to undergo changes. The International Society for Cell Therapy proposes that these cultured cells be called "multipotent mesenchymal stromal cells" (3), and that the term "mesenchymal stem cell" be reserved for cells that fulfil more strict criteria. In this review, we will use the term "mesenchymal stem cell" to refer to a cell type that can self-renew *in vivo* and give rise to mature mesenchymal progeny. Cultured cell populations that contain cells able to take up mature mesenchymal cell phenotypes will be referred to as mesenchymal stromal cells herein. To avoid confusion, we use the acronyms nMSCs for native mesenchymal stem cells (mesenchymal stem cells in their native state *in vivo*), and MSCs for cultured, mesenchymal stromal cells. In this review, we will describe characteristics of MSCs and discuss the evidence that indicates that pericytes correspond to nMSCs *in vivo*, and give rise to MSCs when cultured *in vitro*.

2.1. Nomenclature

Nomenclature in the MSC field has evolved since the earliest designations "fibroblastic cell" and "colony-forming unit-fibroblast" (CFU-F) which emerged from the work of A. J. Friedenstein and his colleagues on rodent BM in the 1970s (4,5). Whereas "fibroblastic cells" described cultured cells with fibroblastic characteristics, CFU-F referred to the *in vivo* cell type that would form fibroblastic colonies *in vitro*, and, by extension, give rise to fibroblastic cell cultures. Friedenstein and colleagues found that cultured, fibroblastic cells could form bony tissue when implanted under the renal capsule in animal models (6). These findings indicated that cultured, BM fibroblastic cells were osteogenic. Later, the work by T. M. Dexter and colleagues showed that survival of hematopoietic stem and progenitor cells *in vitro* was dependent on the establishment of a culture of adherent cells from BM, which comprised mainly fibroblastic cells (7). Owing to their supportive role for hematopoietic cells, these fibroblastic cells were later considered representative of the BM stroma, and were

shown to differentiate into adipocytes upon stimulation with hydrocortisone in culture (8); subsequently, the term "stromal cell" became popular. In the mid-1980s, the term "mesenchymal stem cell" was used in reference to the multipotent murine cell line 3T3 T (9). In the beginning of the 1990s, A. I. Caplan used the term "mesenchymal stem cell" mainly in the context of the developing embryo (1). Later in the 1990s, the term "mesenchymal stem cell" was used in reference to adherent, culture-expanded, multipotent cells derived from postnatal BM by A. I. Caplan's group (10-13) and others (14-16). At the end of the 1990s, the use of the term "mesenchymal stem cell" in reference to culture-expanded cells was further levered by at least two influential publications (17,18). The use of the term "mesenchymal stem cell" in reference to adherent, culture-expanded BM cells was not, however, widely accepted, and some investigators preferred to use the term "marrow stromal cell" (19) or "marrow stromal stem cell" (20) to describe these cells. These divergences regarding the nomenclature of plastic adherent, culture-expanded cells from BM (and other tissues, as discussed below) arose from the skepticism regarding the stem cell nature of these cells. In the mid-2000s, the International Society for Cell Therapy proposed that that the term "mesenchymal stem cell" be reserved to cell populations proven to be stem cells by strict criteria such as self-renewal and differentiation *in vivo* (3), and that culture-expanded cells that express determined markers and differentiate along the osteogenic, adipogenic, and chondrogenic pathways *in vitro* be called "multipotent mesenchymal stromal cells" (21). Even though this nomenclature is not necessarily precise – MSCs can be obtained from tissues that do not contain a stroma, such as the tendon, ligament, or dermis (22) – it is still widespread.

3. CONDITIONS EMPLOYED FOR MSC ISOLATION, CULTURE EXPANSION AND DIFFERENTIATION

3.1. MSCs can be obtained from several organs

Although nMSCs and MSCs were initially derived from BM, many studies have found evidence that they also exist in other postnatal tissues. As early as 1972, Friedenstein and Lalykina (23) demonstrated that the thymus contains adherent cells that could form bone when expanded in culture and placed in diffusion chambers together with transitional epithelium cells or decalcified bone matrix. MSCs have been derived from skin (24), adipose tissue (25), periosteum (26), tendon (27), periodontal ligament (28), skeletal muscle (29), synovial membrane (30), bronchia (31), placenta (32), umbilical cord, including the umbilical vein (33,34) and the Wharton's jelly (35), saphenous vein (36), pancreatic islets (37), and endometrium (38). We have derived MSCs from the aorta, vena cava, brain, spleen, liver, kidney, lungs, BM, skeletal muscle, thymus, and pancreas of mice (39). In our experiments, MSCs could be established even when the animals had their blood removed by perfusion with culture medium under anesthesia, and no MSC cultures could be established from circulating blood. Establishment of MSC cultures from umbilical cord blood has been reported (40), but reproducibility of this procedure is low (41). MSCs have been detected in the blood of rats subjected to hypoxia (42), which suggests that mesenchymal progenitors may be mobilized to peripheral blood under specific conditions. MSCs have been found in menstrual blood, possibly as a consequence of shedding of the decidua (43).

The establishment of MSCs from various tissues as shown above indicates that nMSCs reside in multiple tissues. However, it is always important to keep in mind that this assumption may be skewed by cellular plasticity. For example, epithelial cells may undergo epithelial-to-mesenchymal transition, and give rise to cultures with MSC characteristics (44). Epithelial-to-mesenchymal transition from urinary tract epithelial cells could explain the establishment of MSCs from urine (45). Endothelial cells may undergo endothelial-to-mesenchymal transition, and become MSC-like cells (46). Dedifferentiation of mature cells such as chondrocytes (47) and adipocytes (48) in culture also results in MSCs. Clearly, mature cell types like epithelial cells, endothelial cells, chondrocytes and adipocytes most likely do not behave as stem or progenitor cells *in vivo*.

3.2. Methods for MSC culture establishment, maintenance, and characterization

3.2.1. MSC culture establishment

As shown above, MSCs can be derived from a wide range of organs and tissues. The methodology used to process these tissues vary according to their nature. BM cells, for example, can be mechanically dispersed using a syringe coupled to a needle by aspiration/flushing cycles. Removal of excess red blood cells can be performed by centrifugation of the sample on a density gradient (e.g., Fycoll-Hypaque), or by incubation in red blood cell lysis solution, which normally contains ammonium chloride. Removal of red blood cells is not mandatory, but it may make cell counting difficult and hinder adhesion of nucleated cells to culture vessels. Counting is usually performed with a hemocytometer or Neubauer chamber. If red blood cells are present, they may be eliminated by incubation of a fraction of the sample to be counted with an acetic acid solution (4 – 6% v/v in water) at a proportion of 1:2 to 1:20 (sample: solution). Türk's solution (3% acetic acid containing some drops of either Gentian Violet, Methylene Blue, or Giemsa stain) can be used instead of acetic acid solution alone to highlight nuclei and facilitate cell counting. Whenever acetic acid is used, nucleated cells die, and viability cannot be assessed concomitantly with cell counting. Cell viability can be assessed by diluting a fraction of the cell suspension with a 0.3.% solution of Trypan Blue at

a proportion of 1:1; non-viable cells will incorporate the stain and turn blue.

For solid tissues, there are at least three possible ways to process tissue samples in order to establish MSCs, namely explant culture, mechanical disaggregation, and enzymatic disaggregation, the latter being the most widely used. In the explant technique, fragments of the tissue are distributed through the bottom of a culture vessel, usually a tissue culture-treated Petri dish, and allowed to stand in the tissue culture incubator for some time (usually an hour or so). After this initial incubation, medium is added to the culture vessel, and adherent cells grow from the tissue fragments. Mechanical disaggregation involves any process that uses mechanical procedures to reduce the tissue to very fine fragments. One example is to force the tissue through a series of needles of decreasing size using a syringe. The third way to process a tissue sample to establish MSC cultures is enzymatic disaggregation. Various enzymes are commercially available to release cells from tissues. Some examples include collagenase, dispase, pronase, papain, hyaluronidase, elastase, and trypsin. Enzyme choice depends on the tissue and target cell type. Connective tissues may be digested with collagenase, or a combination of collagenase and dispase. Mixing different types of enzymes in the same disaggregation solution does not always work, as enzymes may have different ion requirements. Collagenase, for example, requires bivalent ions; digestion using trypsin, on the other hand, usually works best in the absence of bivalent ions. Additionally, since enzymes used for tissue disaggregation are proteases, it is possible that mixed enzymes will degrade each other and reduce disaggregation efficiency. That does not seem to be the case for a combination of collagenase and dispase, which are used simultaneously in some protocols (28,49).

At the end of tissue processing, gross tissue remnants are usually removed manually or by passing the cell suspension through a cell strainer. After cell counting, the cells are resuspended in culture medium and seeded at a specified density, which may range from 4,000 to 2,000,000 cells per cm^2, depending on the tissue, species, and protocol used. Various basal culture media can used for MSCs, including minimal essential medium (MEM), the alpha modification of MEM (α-MEM), Dulbecco's modified Eagle's medium (DMEM), Iscove's modified Dulbecco's Medium (IMDM), and even Roswell Park Memorial Institute 1640 (RPMI) medium. Proprietary formulations are also available. For research only use, the basal medium used is usually supplemented with fetal bovine serum at concentrations that vary from 10% (most frequently used) to 20% (v/v), even though serum-free media are also available. Antibiotics (e.g., penicillin and streptomycin, gentamycin) and antimycotics (e.g., amphotericin B) may be added to prevent microbiological contamination of the cultures, although they may be dispensable depending on the conditions under which the cultures are established. Although medium supplementation with fetal bovine serum has been used for experimental clinical applications of MSCs (17), it is always desirable to suppress animal-derived components from the medium when the cells are to be applied to humans. In this regard, platelet lysate from pooled platelet-rich plasma (50), and pooled human AB serum or thrombin-activated platelet-rich plasma (51) have proved to be able to support the proliferation of human MSCs *in vitro*. Addition of epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), and platelet-derived growth factor (PDGF)-BB to platelet-poor plasma has been shown to promote MSC proliferation *in vitro* as well (52).

3.2.2. MSC expansion in culture

After MSC primary cultures are established, the adherent cells are further selected while being expanded by subcultivation. Once primary cultures are confluent (i.e. when the bottom of the culture vessel is fully covered by the cells) or subconfluent, the cells are released by some treatment that, in most instances, consists of incubation with a Ca^{2+}, Mg^{2+}-free solution that contains trypsin (in the range of 0.0.25% to 0.5.%) and EDTA (at a concentration in the neighborhood of 0.5. mM). By repeating this process, called passage, every time the subcultures are confluent, there is selective pressure that favors proliferative, adherent cells. Human bone barrow-derived MSCs may be considered free of significant contamination by other cell types at the end of the second passage (18). That is not the case for murine BM-derived MSCs, which require a number of additional passages before hematopoietic cells are not detectable (53). Murine and human MSCs also differ regarding their expansion potential *in vitro*: while murine MSCs can proliferate for more than 100 population doublings (39), human MSCs gradually lose their proliferative potential in culture as a consequence of telomere shortening (54).

3.2.3. MSC culture characterization

As soon as MSC cultures are established, they are subjected to characterization assays in order to validate their mesenchymal, stromal nature (Figure 1D). Firstly, MSC cultures must contain cells able to differentiate into mesenchymal cell types when cultured under appropriate conditions. Osteoblastic differentiation may be induced by culture in a medium that contains dexamethasone, ascorbic acid or its stable form, ascorbic acid 2-phosphate, and a source of phosphate such as beta glycerol phosphate (10). The cells are kept in osteogenic medium for three weeks; at the end of this time, mineralized extracellular matrix can be highlighted with a calcium-binding stain such as Alizarin Red S, or with a silver nitrate staining technique (von Kossa stain). The expression of genes that code for proteins characteristic of osteoblastic cells such as bone morphogenetic

protein 2, bone sialoprotein II, and osteopontin can be confirmed by reverse-transcription polymerase chain reaction (RT-PCR) or its real-time version (quantitative RT-PCR, qRT-PCR) (55). Adipogenic differentiation can be induced by a number of protocols that usually include addition of dexamethasone and insulin to the basal medium (56). Adipogenic differentiation is detected by visualization of lipid vacuoles in the cells, and these vacuoles can be stained with stains such as Oil Red O or Sudan Black IV. The expression of genes characteristic of adipocytes such as the transcription factors PPARγ and C/EBPα may be confirmed by RT-PCR (57). Chondrogenic differentiation is induced by culturing the cells at a high density in a serum-free or low serum culture medium that contains transforming growth factor β (TGF-β). High cell density may be achieved by dispensing a drop of a highly concentrated cell suspension in a culture vessel, allowing the cells to settle in the bottom of the drop, and then completing the vessel with chondrogenic medium (25). Another way to achieve high cell density is by allowing cells to form a pellet at the bottom of polypropylene containers (58). Chondrogenic pellets can be included in paraffin and sectioned. Histological sections may be stained with histological stains that reveal sulfated proteoglycans (e.g. Toluidine Blue, Safranin O, Alcian Blue), or with general-purpose stains such as Hematoxylin and Eosin to observe cell morphology. Additionally, histological sections can be deparaffinized and stained with appropriate antibodies to reveal the presence of collagen type II (58). Detection of transcripts of chondrocyte-associated genes such as collagens type II or X can also be performed (58).

Flow cytometry is another tool widely used to characterize MSCs. Using this technique, high cell numbers can be immunophenotyped to determine the frequency of cells that express given markers at the expense of a relatively low amount of antibodies. ISCT proposes that MSCs must contain more than 90% of cells positive for CD73, CD90, and CD105, but no more than 5% of cells positive for CD45 or CD34, CD14 or CD11b, CD79α or CD19, and HLA-DR (21). A number of other molecules are known to be expressed or not expressed by MSCs (18).

In addition to *in vitro* differentiation and immunophenotyping, MSCs may be further characterized by functional assays *in vitro* and *in vivo*. When loaded into porous ceramic cubes and implanted subcutaneously *in vivo*, MSCs form bone, cartilage, and some fibrous tissue (59); when treated with dexamethasone, loaded into diffusion chambers and implanted intraperitoneally *in vivo*, these cells also exhibit osteochondrogenic differentiation (60). *In vitro*, MSCs support the growth and differentiation of hematopoietic stem and progenitor cells (61). MSCs also provide support for the formation of blood capillary-like structures by endothelial cells (ECs) *in vitro* (62). Another functional characteristic of MSCs is to suppress the proliferation of T lymphocytes *in vitro* (63).

3.2.4. Different sources or methods imply different cells or names

With so many possibilities of tissue of origin, isolation procedures, and culture methods used in different laboratories to isolate, culture, and characterize MSCs, it is likely that not all MSCs are identical. We have found differences between MSC populations according to their tissue of origin (39,64). Additionally, some methodologies have provided cells that have MSC characteristics, but are different from MSCs isolated and cultured using traditional methods. For example, MSC-like cell populations established under hypoxic conditions in low-serum medium have been termed marrow-isolated adult multilineage inducible (MIAMI) cells (65). MSCs derived from dental pulp are generally referred to as “dental pulp stem cells” (DPSCs) (49). MSCs derived from periodontal ligament are called “periodontal ligament stem cells” (PLSCs) (28). MSCs derived from adipose tissue have been suggested to be termed “adipose stem cells” (ASCs) owing to their differences as compared to BM-derived MSCs (66).

4. MSC DIFFERENTIATION POTENTIAL AND PLASTICITY

As shown above, MSC cultures contain cells able to differentiate into osteoblasts, chondrocytes and adipocytes. Some reports indicate that MSCs can give rise to other mesenchymal cell types such as smooth muscle cells (67), and striated myocytes (13,68) *in vitro*. Apparently, site of origin is a determinant of whether or not murine MSCs can give rise to contractile, striated cells. When MSC were established from various organs of mice, only those from aorta and skeletal muscle gave rise to myofibers in primary cultures without addition of specific inducers (39). Although contractile, striated cells that originated in primary skeletal muscle cultures could derive from intrinsically myogenic muscle progenitor cells, emergence of myofibers in aorta primary cultures might represent bona fide myogenic differentiation. MSC differentiation into myofibers also seems to be species-dependent. Even though incorporation of human MSC nuclei into murine muscle fibers has been documented (69), acquisition of a striated muscle phenotype by MSCs *in vitro* by exposure to specific molecules seems to be restricted to rodents, as convincing evidence of this phenomenon in human cells is lacking. Emergence of striated, contractile cells in cultures of MSCs derived from liposuction material (25), for example, may reflect differentiation of intrinsic muscle myogenic cells scraped off from muscle underlying subcutaneous fat during liposuction procedures.

While differentiation of MSCs into mesenchymal cells is expected, cells in MSC cultures have been shown to acquire non-mesenchymal characteristics too. Early reports described MSC differentiation into neural cells (70,71) after exposure to antioxidants or

retinoic acid. Differentiation of MSCs into hepatocyte-like cells (72) and insulin-secreting cells (73,74) have been reported as well. Additionally, MSCs differentiation into ECs has also been suggested (75).

The broad differentiation potential of MSCs indicates that they may be used for a number of approaches aimed at replacing, by tissue engineering or cell therapy, cells or tissues lost after diseases or accidents. In view of this, highlighting some points is necessary. First, such a broad differentiation capability *in vitro* or *ex vivo* does not necessarily reflect MSC or nMSC differentiation ability in their original location *in vivo*: for example, MSCs, or nMSCs, that reside in adipose tissue are not supposed to differentiate into osteoblasts. Second, since most studies on MSCs have not analyzed differentiation in a number of cell clones, MSCs may represent heterogeneous populations that contain cells with various differentiation abilities. Third, it is possible that, in some cases, the broad differentiation ability of MSCs as observed *in vitro* may reflect plasticity that does not necessarily translate *in vivo*.

5. MSCS SECRETE IMPORTANT BIOACTIVE MOLECULES

In the mid-2000s, after a number of reports on the use of MSCs to treat various conditions in experimental animal models, some reports indicated the beneficial effects of MSC treatment were not attributable to differentiation, but to soluble molecules through paracrine action (76-79). Consequently, the rationale for the use of MSCs as therapeutic agents shifted from differentiation to paracrine action (80). The molecules secreted by MSCs can be classified into six groups: supportive, antiapoptotic, angiogenic, chemoattractant, antiscarring, and immunomodulatory (81). Molecules produced by MSCs and that provide physiological support to other cell types include stem cell factor (SCF), leukemia inhibitory factor (LIF), macrophage colony-stimulating factor (M-CSF), stromal-derived factor 1 (SDF-1, also known as CXCL12), and angiopoietin 1. Antiapoptotic molecules comprehend vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), insulin-like growth factor 1 (IGF1), stanniocalcin 1, TGF-β, basic fibroblast growth factor (bFGF), and granulocyte-monocyte colony-stimulating factor (GF-CSF). Angiogenic molecules secreted by MSCs include VEGF, IGF-1, placental growth factor (PlGF), monocyte chemotactic protein 1 (MCP-1, also known as CCL2), bFGF, and interleukin 6 (IL-6), in addition to extracellular matrix components. Chemoattractant molecules secreted by MSCs include CCL2, CCL3, CCL4, CCL5, CCL6, CCL20, CCL26, CX3CL1, CXCL1, CXCL2, CXCL5, CXCL8, CXCL10, CXCL11, and CXCL12. Anti-scarring factors produced by MSCs include HGF and bFGF. Molecules with immunomodulatory effects expressed by MSCs include prostaglandin E2 (PGE-2), human leukocyte antigen G (HLA-G), HGF, inducible nitric oxide synthase (iNOS), TGF-β, indoleamine 2,3-dioxygenase (IDO), and LIF. Figure 2 summarizes paracrine bioactive molecules synthesized by MSCs.

6. IMMUNOREGULATORY FUNCTION OF MSCS

MSCs express low levels of MHC class I and no MHC class II or costimulatory molecules (82), so that they can escape immune recognition. Although this represents an interesting opportunity for allogeneic stem cell therapy, the presence of class I antigens and the fact that INF-γ can induce the expression of class II molecules may explain the findings of allogeneic MSC rejection reported (83,84).

In addition to their plasticity and regenerative capacity due to the secretion of bioactive molecules, MSCs display also important immunomodulatory properties. Their ability to suppress the proliferation of T lymphocytes was first described in 2002 (63,85), and they are currently known to interact with all elements of the innate and adaptive immune systems. The mechanisms mediating the immunomodulatory effects involve cell contact and, mainly, the production of bioactive soluble factors, as summarized below (recently reviewed in (86-88)).

6.1. Interaction with the innate and adaptive immune systems

MSCs stimulate the production of monocytes, as shown by an increase in the emigration of these cells from the BM niche after systemic administration of a toll-like receptor (TLR) ligand as LPS (89), and can also recruit macrophages into injured tissues, stimulating their tissue repair activities, mainly by the secretion of prostaglandin E2 (90). *In vitro* studies have also shown that microbial-stimulated MSCs can activate neutrophils and inhibit mast cell and natural killer cell function, resulting in a decrease of inflammatory/allergic reactions (reviewed in (86,91)). Other mediators involved in these functions are HLA-G, indoleamine 2,3-dioxygenase (IDO) and nitric oxide (NO) (92).

Upon activation by inflammatory molecules, MSCs have been shown to be able to prevent the proliferation and/or activation of all cells of the adaptive immune system (reviewed in (87)). The main immunomodulatory molecules include leukaemia inhibitory factor, IL-10, TGF-β, galectin 1 and 3, IDO, NO, carbon monoxide and prostaglandin E2. T cells are affected in multiple ways: MSCs inhibit the differentiation of naive T cells into Th17 cells, the secretion of proinflammatory cytokines by these cells, and induce their differentiation into T-regulatory cells; furthermore, the activation and proliferation of T cells is suppressed by shifting their phenotype from a T helper (Th) 1 to a Th2 immune phenotype (reviewed in (93)).

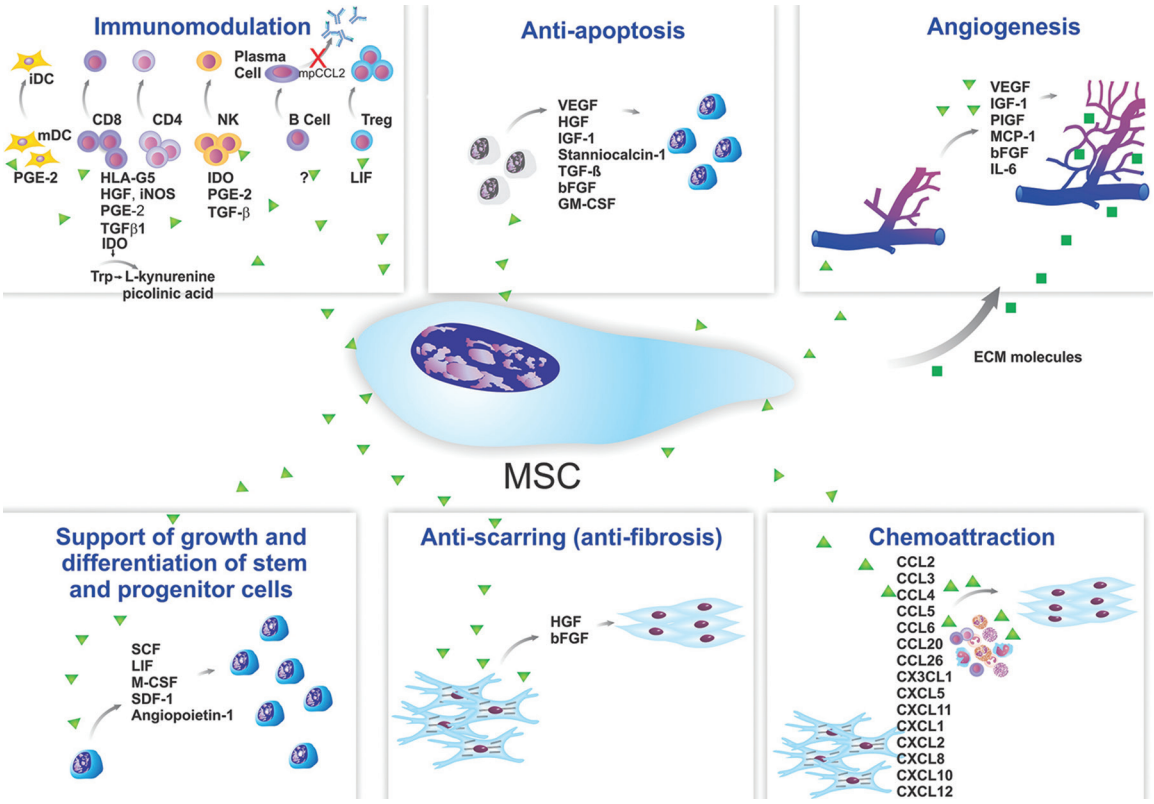

Figure 2. Bioactive molecules secreted by MSCs. Secreted molecules were classified into six functional groups according to their paracrine effects: immunomodulation, anti-apoptosis, angiogenesis, support of growth and differentiation of stem and progenitor cells, anti-scarring, and chemoattraction. mDC, myeloid dendritic cell; iDC, induced dentritic cell; PGE-2, prostagladin E2; CD8, $CD8^+$ T cell; CD4, $CD4^+$ T cell; HLA-G5, human leukocyte antigen G5; HGF, hepatocyte growth factor; iNOS, inducible nitric oxide synthase; TGF-β, transforming growth factor β; IDO, indoleamine-pyrrole 2,3-dioxygenase; Trp, tryptophan; NK, natural killer cell; mpCCL2, metalloproteinase-modified CCL2; Treg, regulatory T cell; LIF, leukemia inhibitory factor; VEGF, vascular endothelial growth factor; IGF-1, insulin-like growth factor 1; bFGF, basic fibroblast growth factor; GM-CSF, granulocyte-monocyte colony-stimulating factor; PlGF, placental growth factor; MCP-1, monocyte chemotactic protein 1; IL-6, interleukin 6; SCF, stem cell factor; M-CSF, monocyte colony-stimulating factor; SDF-1, stromal-derived factor 1. Reproduced from (81) with permission from Elsevier.

In vitro and *in vivo* studies have shown that MSCs can also suppress B-cell terminal differentiation and B cell responses (reviewed in (94)). Furthermore, MSCs can suppress the differentiation, maturation and function of dendritic cells, inducing them into a regulatory phenotype.

6.2. Immunosuppression vs. immunostimulation by MSCs

MSCs can respond in different ways to the inflammatory microenvironment, reflecting a plasticity in their immunomodulation capacity. This plasticity has been associated to the stimulation of TLRs expressed by MSCs, polarizing the cells into two phenotypes: priming with TLR4 agonist induces what has been called MSC1 cells, secreting mostly pro-inflammatory mediators such as IL-6, IL-8 and TGF-β, whereas TLR3 priming induces anti-inflammatory MSC2 cells, that express mostly COX2, IL-4 and IDO (95). This would also explain the need for a "licensing" of MSCs by inflammatory cytokines such as TNF-α, INF-γ and IL-1 to exert their immunosuppressive actions (86).

We have recently suggested a physiological role for this immunomodulatory activity of MSCs (96). Although much has been learned on this unique function of MSCs in the past decade, there is an urgent need to investigate tissue-resident cell populations so that the meaning of this unique feature can be understood and used in the clinical setting.

7. nMSC IDENTITY TRACED TO PERICYTES *IN VIVO*

7.1. Pericytes

Pericytes, also known as Rouget cells or periendothelial cells, are cells that possess many processes that wrap ECs in blood vessels (97) (Figure 1A). In restrictive definitions, pericytes are present only in pre-capillary arterioles, capillaries, and post-capillary venules, and are embedded in a basement membrane (98). However, cells with pericyte characteristics are distributed throughout the vasculature at a subendothelial location (99). In tissues with a high fluid traffic across small-caliber blood vessels, e.g. liver

Table 1. Molecular markers used to identify pericytes

Marker	Reference (s)	Comments
alkaline phosphatase	(181,182)	
alpha smooth muscle actin (α-SMA)	(183)	
aminopeptidase A	(184)	
aminopeptidase N	(184)	
angiopoietin 1	(185)	
annexin A5	(186)	
antigen defined by the 3G5 antibody	(187)	Also expressed by a subpopulaiton of T cells (188), and corneal keratocytes (102).
CD34	(189)	
class III β-tubulin	(190)	A marker for activated pericytes as described in rats.
delta-like homolog 1 (DLK1)	(191)	
Kir6.1. potassium channel	(191)	
leptin receptor (LEPR; CD295)	(192)	
low-affinity nerve growth factor receptor (p75 NGFR; CD271)	(193,194)	This marker has been used to select human BM (195) and adipose tissue (196) cells that can give rise to MSCs.
melanoma cell adhesion molecule (MCAM; CD146)	(132,197)	MCAM is also a marker for endothelial cells and smooth muscle cells (198).
nerve/glial antigen 2 (NG2), a.k.a. high molecular weight, melanoma-associated antigen (HMW-MAA),	(199)	Initially described as a marker of activated pericytes
neuroepithelial stem cell intermediate filament (NESTIN)	(184,200)	
platelet-derived growth factor receptor α (PDGFRα; CD140a)	(201)	
platelet-derived growth factor receptor β (PDGFRβ; CD140b)	(202)	
regulator of G protein signaling 5 (RGS5)	(203)	
stromal-derived factor (SDF; CXCL12)	(204)	
sulfonylurea receptor 2 (SUR2)	(191)	

and BM, the basement membrane is so thin that it will not completely surround periendothelial cells, which are formally called perisinusoidal cells, although some basement membrane remains present between them and ECs. In this review, we use a less restrictive definition of pericyte, i.e. periendothelial cells that are distributed throughout the vasculature but not necessarily embedded in a basement membrane, and that directly contact ECs. Direct contact with ECs through holes in the basement membrane is an important criterion to distinguish pericytes from vascular smooth muscle cells, as the latter do not contact ECs directly (100). These contacts between pericytes and ECs have a characteristic peg-and-socked appearance as seen by electron microscopy (101). In this review, we use the designation “pericyte” in reference to the various forms of periendothelial cells that directly contact ECs, which include classical pericytes and their tissue-specific forms such as Ito or stellate cells in the liver, adventitial reticular cells in the BM, and mesangial cells in the kidney.

7.1.1. Pericyte markers

To date, pericytes are distinguishable mainly by their microanatomical position relative to ECs, and by the identifiably physical connections between these two cell types, which include peg-and-socket contacts that contain tight and gap junctions (100). There is no consensus regarding a single marker for pericytes; some of these markers are listed in Table 1. One of the problems concerning the identification of pericyte markers is that a number of papers refer to cultured cells as being pericytes in spite of the phenotypical changes that occur from the instant pericytes are displaced from their microanatomical location *in vivo* to the moment they are analyzed, after expansion *in vitro*. During this process, markers expressed by pericytes *in situ* may be lost and, additionally, markers not present in pericytes *in vivo* may become expressed. For this reason, we refer to cultured cells derived from pericytes as “cultured pericytes”. Another factor that adds to the difficulty in defining pericyte markers is the fact that distinguishing pericytes

from other perivascular cells such as vascular smooth muscle cells or perivascular fibroblasts is not always easy or possible. Finally, in some cases a given molecule may be a somehow specific marker for pericytes in one species, but its expression is absent in another species. The antigen recognized by the 3G5 antibody, a pericyte marker that is also expressed in corneal keratocytes, is detectable in humans, rats, rabbits, bovines, and pigs, but is not detectable in the mouse (102). In other cases, the expression of a given pericyte marker is rather restricted to pericytes in one species but not in another: alkaline phosphatase is expressed by pericytes but not ECs in humans, but is present in both cell types in mice (103).

7.1.2. Pericyte functions

The functions attributed to pericytes include a) mechanical and physiological control of blood vessel stability and permeability (100); b) regulation of blood flow in small-caliber blood vessels (104); and c) secrete extracellular matrix components, some of which are constituents of the basement membrane, as inferred from *in vitro* studies (105). Furthermore, evidence indicates that pericytes interact with immune system cells, behave as stem cells in the body, and contribute to tissue repair. These three latter functions are of special interest in this review, and will be discussed in separate sections (7.1.3., 7.1.4., and 7.2., respectively).

7.1.3. Pericyte effects on immune system cells

In addition to the functions listed above, evidence indicates that pericytes directly interact with immune system cells. In the thymus, pericytes in the corticomedullar junction vessels control the exit of naïve T cells from the thymus (106). Pericytes also help neutrophil movement across and under the endothelium (107). When exposed to the inflammatory mediators tumor necrosis factor (TNF) or lipopolysaccharide (LPS), pericytes stimulate neutrophils to enter the tissues by secreting MIF-1 (CCL2) and upregulating the expression of intercellular adhesion molecule 1 (ICAM-1; CD54) (108). Additionally, direct interaction of pericytes with extravasating neutrophils has been shown to provide the latter with stimuli that render them more efficient and resistant to death (108), which led the authors of that study to propose that pericytes are an “active component of innate immune responses”.

Further evidence indicates that influence of pericytes is not restricted to innate immune cells. A work by Balabanov *et al*. (109) suggests cultured rat brain pericytes can activate antigen-primed T cells *in vitro*. In that work, brain microvessels were isolated, ECs were removed by fluorescence-activated cell sorting, and the remaining cells were cultured. Those cells, which were positive for alpha smooth muscle actin (α-SMA) and CD11b but were negative for class II MHC, displayed surface class II MHC molecules after exposure to interferon γ (IFN-γ). Class II MHC-expressing cells pulsed with ovalbumin were able to induce proliferation of T lymphocytes obtained from the spleen of rats previously immunized with ovalbumin, but cultured perivascular cells not exposed to IFN-γ, or cultured perivascular cells exposed to IFN-γ but not pulsed with ovalbumin, did not induce T cell proliferation, suggesting class II MHC-dependent T helper cell activation.

Even though it is possible that cultured pericytes activate T helper cells when exposed to IFN-γ, evidence on the opposite effect is also available. Maier and Pober (110) found that cultured pericytes isolated from human placenta through a classical method (culture of adherent cells derived from microvessels) do not effectively stimulate allogeneic T cell proliferation even after induced expression of class II MHC molecules by IFN-γ, while allogeneic T cell activation was observed after coculture with ECs that expressed class II MHC molecules induced by this cytokine. When allogeneic $CD4^+$ T cells were cocultured with MHC II^+ ECs and MHC II^+ cultured pericytes, T cell alloactivation was significantly inhibited (110). Tu *et al*. (111) also provided evidence for an immunosuppressive function of cultured pericytes. They demonstrated that cultured retinal pericytes obtained through a classical method, which were positive for α-SMA and NG2, can suppress activated T cell proliferation *in vitro*. In that study, the authors found this effect to be dependent on the T cell: pericyte ratio, which varied from 1:80 to 1:10, with the highest T cell antiproliferative effect at 1:10 (111). The authors also found that the amount of T cell-derived cytokines IFN-γ and tumor necrosis factor α (TNF-α) in the cocultures was reduced accordingly.

Evidence that pericytes favor tumor growth by suppressing the action of the immune system is also available. Bose *et al*. (112) isolated pericytes from murine B16 or CT26 subcutaneous tumors, or from healthy kidneys, based on NG2 and PDGFRβ expression, and lack of expression of CD31 and CD45. The majority of tumor-derived cultured pericytes was found to express class II MHC molecules and the costimulatory molecule CD80 on their surface, while kidney-derived cultured pericytes were negative for these molecules. When cultured pericytes derived from healthy kidneys or tumors were pulsed with ovalbumin and cocultured with $CD4^+$ T cells obtained from the spleens of mice immunized with ovalbumin, the expression of anergy-related genes was detected only in the cocultures that contained tumor-derived cultured pericytes. Consequently, the authors concluded that pericytes induce anergy in antigen-primed $CD4^+$ T cells (112). Ochs *et al*. (113) isolated $CD90^+/CD31^-$ cells from human gliomas and confirmed that the cells isolated by them shared expression of some molecules with commercially available cultured human brain pericytes. Cultured cells derived from $CD90^+/CD31^-$ cells were able to inhibit T cell proliferation *in vitro*. After confirming that perivascular $CD90^+$ cells

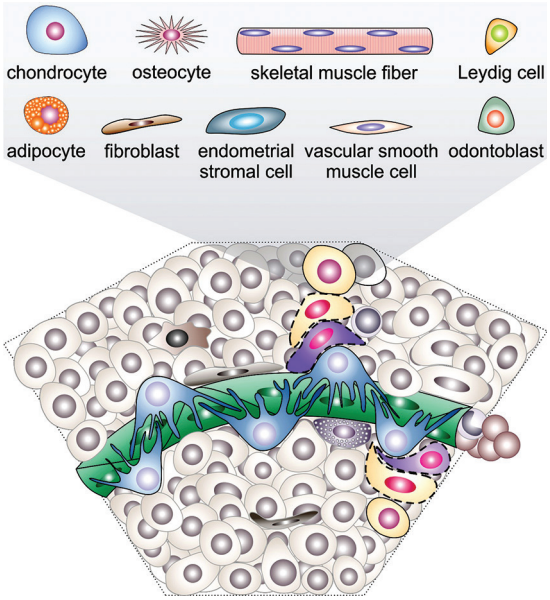

Figure 3. Mature cell types that arise from pericytes *in vivo*. This schematic representation of a tissue shows pericytes (in blue) giving rise to activated pericytes (purple cells with a dotted contour), which transition to a tissue progenitor cell phenotype (yellow cell with dotted contour) that gives rise to a tissue-specific cell. Experiments in animal models indicate that pericytes can give rise to mature cell types under physiological or injury conditions. To date, there is evidence that pericytes give rise to chondrocytes, adipocytes, osteocytes, fibroblasts, skeletal muscle fibers, endometrial stromal cells, vascular smooth muscle cells, Leydig cells, and odontoblasts *in situ*. For more information, see section 7.1.4.

coexpress PDGFRβ, the authors found a positive correlation between frequency of perivascular $CD90^+$ cells with glioma grade, and a negative correlation between frequency of perivascular $CD90^+$ cells and frequency of $CD45^+$ (hematopoietic) and $CD8^+$ (cytotoxic T lymphocytes) cells, which suggests that increased pericyte coverage in glioma vasculature may favor tumor progression by reducing the influx of immune cells.

7.1.4. Pericytes as stem cells in the body

In addition to contributing to blood vessel homeostasis as described above, another function of pericytes include their role as stem cells in the body. Evidence that pericytes give rise to mature cells *in situ* comes from tracing their fate through various approaches. Differentiation of pericytes into adipocytes after tissue injury in rats has been suggested by Richardson (114); not too long ago, genetic fate tracing of pericytes allowed for the conclusion that they give rise to adipocytes under physiological conditions in adipose tissue (115). Pericytes have been suggested to give rise to fibroblasts *in vivo* based on circumstantial evidence (116-118) and, more recently, on genetic fate tracing experiments in mice (119). Circumstantial evidence also indicates that pericytes give rise to chondrocytes (120,121) and osteocytes (122) in experimental injury models. Genetic fate tracing allowed for the confirmation that pericytes give rise to osteocytes *in vivo* during development and after tissue injury (123). Pericytes were pointed out as the source of new Leydig cells in a rat model of Leydig cell injury (124). Label-retaining cell experiments indicated that pericytes give rise to endometrial stromal cells through the monthly regenerative cycles of the endometrium (125). In the mouse incisor, which displays continuous growth, pericytes were shown to give rise to odontoblasts in a genetic lineage tracing model under physiological conditions and also after tissue injury (126); in that study, another non-pericytic endogenous tooth cell type was found to give rise do odontoblasts along with pericytes. Another study that used genetic lineate tracing indicates that pericytes can give rise to skeletal muscle fibers during a narrow time window during mouse development (103). More recently, circumstantial evidence indicated that pericytes give rise to smooth muscle cells in the lungs (127).

The results of the studies described above indicate that differentiation of cultured pericytes into mesenchymal cell types as observed *in vitro* reflects their intrinsic ability to give rise to mature cell types *in situ* as previously postulated by us (39). Data from these *in vivo* experiments indicate that pericytes that leave their perivascular niche become proliferative, i.e. become activated, and increase in number, a characteristic of progenitor cells. Progenitor cells, on their turn, may take up mature phenotypes according to the context of their surroundings. Figure 3 summarizes pericyte differentiation *in situ* as discussed in this section. Evidently, the model shown in Figure 3 is a generalization that has the purpose of drawing attention to similarities, rather than the differences, in this process in the organism. Given the microenvironmental differences between tissues, it seems unlikely that pericytes from various locations of the body have exactly the same differentiation potential (22). That said, these pericytes correspond to cells able to self-renew *in vivo* and give rise to mesenchymal progeny (i.e. they are nMSCs), even though their differentiation potentials may be distinct.

7.2. Relationship between pericytes and MSCs

Similarities between cultured pericytes and MSCs come from at least five lines of evidence. First, $3G5^+$, cultured pericytes obtained through classical methods exhibit the ability to differentiate into osteoblasts, adipocytes and chondrocytes when loaded in diffusion chambers and implanted *in vivo* (128,129). Second, cultured pericytes share expression of a number of molecules with MSCs (reviewed in (96,130,131)). Third, perivascular cells share expression of some molecules such as NG2, α-SMA, CD44, CD73, CD90, and 105 with MSCs (132). Fourth, the number of fibroblastic colonies observed *in vitro*, an estimate of the number of nMSCs/MSCs in a given tissue, positively correlates with vascular density (133). Fifth – and more recently established – cultured pericytes influence immune system cells, and express class II MHC molecules in response to IFN-γ as do MSCs (see section 7.1.3.).

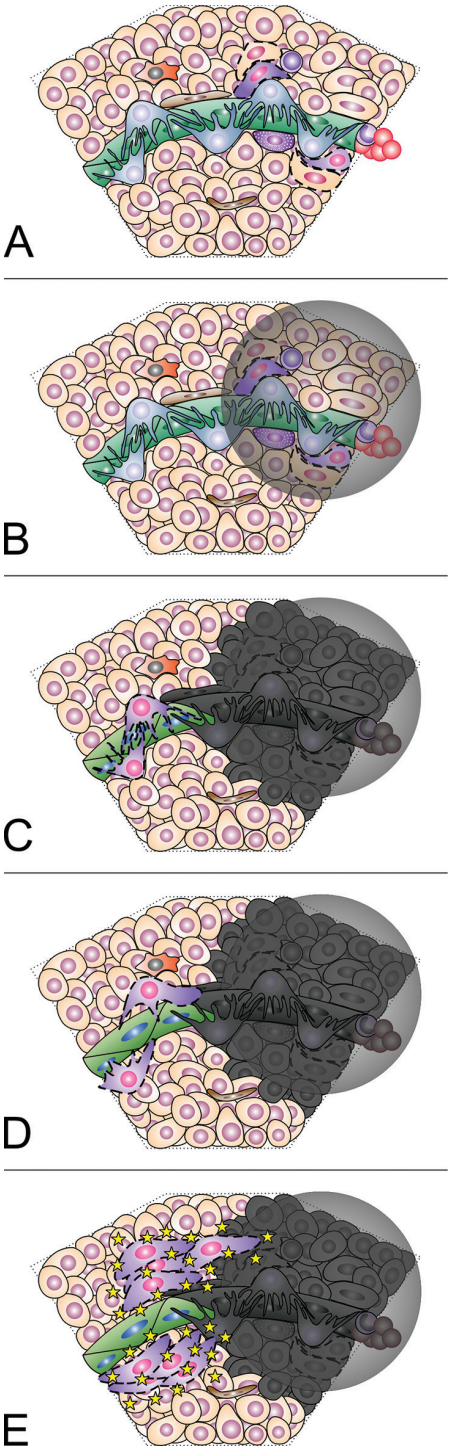

Figure 4. Schematic representation of a proposed mechanism of pericyte participation in tissue injury. When a healthy tissue (A) suffers a focal lesion, represented by a gray circle in (B), a number of cells die (as represented by a transition to a gray color) (C). The local changes signal for phenotypical changes of nearby pericytes (blue cells), which become activated (pink cells with a dotted contour). Activated pericytes loosen their physical association with endothelial cells (represented in green), leave their original perivascular location (D), proliferate, and migrate toward the injured area (E), where they can contribute to tissue repair by means of the secretion of a number of biomolecules (yellow stars) as MSCs (Figure 2).

From the evidence above, it is conceivable that MSCs correspond to an *in vitro* cell population comparable to activated pericytes. The context in which MSC cultures are established, where a number of signals present in the pericyte niche are lost, and a number of danger signals are provided by lack of integer ECM and dying cells, can favor pericyte activation as they mimic tissue injury. If this process is envisioned in a situation of tissue injury *in vivo*, and in light of the evidence that pericytes leave their periendothelial location to give rise to paravascular proliferative cells, it is conceivable that, after tissue injury, pericytes give rise to progenitor cells that are similar to MSCs and, consequently, secrete a number of molecules that contribute to tissue repair (87,96). In this model, depicted in Figure 4, activated pericytes migrate short distances toward the surroundings of the lesion, with no need for recruitment of mesenchymal progenitors from distant sites through the blood circulation. In line with this, Hoogduijn *et al*. (134) reported absence of nMSCs in the blood of patients with various organ diseases, except in one case of trauma, in which pericytes from physically disrupted sites could have been mobilized to the bloodstream.

8. CLINICAL AND PRECLINICAL APPLICATIONS OF MSCS

8.1. Cell therapy

Cell therapy using MSCs has always been a very attractive treatment option. As previously mentioned, in addition to being a secretory source of biomolecules, the potential of MSCs for differentiation is well known, and both their plasticity and the production of regulatory molecules may influence the outcome of diseases. Applications of MSCs are mostly encountered for diseases based on some kind of injury and/or inflammation process. Some examples are shown in Table 2, whereby the diseases mentioned are scenarios that have been studied clinically. Graft-versus-myocardial infarction, Chron's disease, multiple sclerosis, and co-transplation with pancreatic islets are situations, where the function of MSCs will direct the imunomodulating signaling of the disease. In cases such as Heart disease, cancer therapy, as well as skeletal, liver, and cartilage repair, the key roles for these cells is focused on plasticity and the induction of neovascularization. The following sections will describe some results of using MSCs in some of the pathologies mentioned above.

8.1.1. Myocardial infarction

Globally, heart diseases remain one of the major causes inducing death and morbidity, and for some time, cell therapy has been used at pre-clinical and clinical levels to tackle this pathology. The paracrine factors released by stem cells render them an outstanding therapeutic option and they have already been successfully applied in the treatment of heart diseases. Recently published articles on treatments using mononuclear bone marrow

Table 2. Clinical trials involving MSCs (February 2015)

Condition	Number of entries
Graft-versus-host-disease	40
Skeletal repair	7
Myocardial infarction	21
Liver repair	2
Multiple sclerosis	25
Co-transplantation with pancreatic islets	1
Cartilage repair	20
Cancer therapy	48
Tissue engineering	7
The Clinical Trials database (www.clinicaltrials.gov) was mined using the term “mesenchymal stem cell” (quotation marks included).	

cells (BMCs) have confirmed results already found in previous clinical studies (reviewed in (81)). Clinical trials increasingly become more exhaustive and divide participants into treatment and control groups, which are subsequently evaluated by double blind analysis (135,136). For example, injection of BMCs in the myocardium of patients with chronic myocardial ischemia resulted in a significant improvement of symptoms related to angina and myocardial perfusion, relative to a group of patients who received merely placebos (135). The same group of researchers moreover tested injections of BMCs in patients with refractory angina. Interestingly, both cardiac functionality as well as the general quality of life were found to be improved, whereby the magnitude of improvement was greater for diabetic relative to other patients, which was considered a predictive factor for this type of treatment and pathology (136). Diabetic patients showed, regardless of presenting cardiac dysfunction, an improvement in sympathetic innervation in the area that received cells, suggesting a growing demand for more specific and controlled studies for each type of patient (137). MSCs have also been used in clinical protocols for the treatment of myocardial infarction. Clinical trials such as POSEIDON and C-CURE are currently finalized and present results indicative of a safe way to improve cardiac functionality in treated patients, similar to treatments with BMCs (138,139). Apart from being safe and effective in such treatments, MSCs can also be easily maintained *ex vivo* for subsequent clinical applications. However, a universally common feature of the results of these trials is that the improvement is not permanent, and the survival rate of transferred cells in the treated tissue remains to be improved.

In order to overcome this obstacle, many research groups aim to develop more modern and effective protocols. New therapy protocols, although still at pre-clinical stages, try to associate these cells with proteins that stimulate therapeutic effects. For example, Mao *et al*. (140) have shown that genetically transformed MSCs, expressing signalling proteins, participate in and stimulate the ventricular remodelling and improve cardiac functionality. As a model, this pre-clinical study used pigs with acute myocardial infarction, and the animals were treated with MSCs or modified MSCs, overexpressing kinase-binding integrin (integrin linked kinase - ILK), which participates in the cardiac regeneration process, while maintaining the functionality of the cardiac cells. In a similar pig model, de Jong *et al*. (141) used MSCs encapsulated in alginate and transfected with a lentiviral vector that carried the gene glucagon-like peptide-1, which is a cardioprotective hormone (141).

8.1.2. Fibrosis

Even though formation of fibrous tissue is an integral part of the recovery stages of damaged tissue, it may form scar tissue when uncontrolled, leading to tissue malfunction or necrosis. During the repair process, MSCs express and release cytokines, growth factors, and other bioactive molecules, which contribute to the formation of new tissue (142). MSCs were, for example, successfully used in models of liver injury, where they induced a reversal of the fibrosis, and exerted a regulatory potential on the expression of metalloproteinases and collagen (143). In an animal model, the injection of MSCs led to a protection by preventing obstruction-induced EMT and chronic renal fibrosis. Furthermore, decreased expression of proteins such as α-SMA and fibroblast specific protein was observed (144). Clinical application of MSCs to patients with cirrhosis corroborated the safety of this type of therapy, as well as improvements in liver function and biochemical disease markers, concomitant with a decrease of fibrosis markers, such as collagen and TGF. The main result of this study was the safe application of MSCs for liver diseases, especially for the control of fibrosis (145). In a recent clinical protocol, patients received MSC injections into unneovascularized myocardial regions, and after 18 months of treatment, significantly improved ejection fractions as well as decreased scar masses were observed (146).

8.1.3. Stroke

MSCs also represent an attractive treatment option for lesions in the central nervous system, and their neuroprotective potential has already been described (147). Local administration of MSCs in pre-clinical models for patients with neurological damage is pivotal for cell therapy. In previous clinical trials involving stroke patients, it was evident that the course of treatment is a key element in the therapeutic approach. For example, intravenous injections containing MSCs are safe, but lead only to minor improvements of parameters implemented in order to monitor the disease (148,149). Compared with intravenous injections, intracerebral injections of BM-derived MSCs (BMSCs) exhibit significantly improved results, especially with respect to the improvement and

functional recuperation of the tissue and to the presence of the BMSCs in the tissue. In some cases, it was even possible to demonstrate migration of the BMSCs to the site of the injury (150). Currently, five clinical trials, using MSCs intracerebrally in the context of stroke incidents, are in the experimental phase, but results have not yet been released (ClinicalTrials.gov Identifiers: NCT01714167, NCT01716481, NCT01468064, NCT01962233, and NCT01297413).

8.2. Tissue engineering

Tissue engineering is a biomedical technology that aims to assist and accelerate the regeneration of damaged tissues based on the natural healing potential of the patient by enhancing, replacing, or rebuilding damaged or diseased tissues. Tissue engineering involves the transplantation of cells and the development of biocompatible biomaterials (whether inert or not) to induce tissue regeneration (reviewed in (151)).

One of the major breakthroughs in this area was the use of biomaterials as a support for cellular integration, maintenance, and differentiation. The cells that constitute organs and tissue are located in a complex molecular network known as the extracellular matrix (ECM). Its molecules are responsible for promoting the means as well as the signalling pathways that guide cell growth and differentiation. Structural aspects of the ECM are highly important, as it provides e.g. the appropriate architecture for cell development. Biomaterials, in the form of natural or synthetic polymer molecules, can thus act as artificial substitutes for the ECM (152). As they, by themselves, do not possess a viable cell component, which would be responsible for tissue remodelling, suitable biomaterials always act in conjunction with growth factors and specific culture media to form functional and biologically active tissue (153).

Hence, the ultimate objective of tissue engineering is to establish triads of cells, biomaterials, and bioactive molecules with the aim of restoring the natural process of the physiological system. Currently, we have a large variety of these three components at our disposition. For the treatment of bone lesions, for example, the best option is biomaterials containing calcium and hydroxyapatite, as they have the potential to induce osteogenesis and as their structures are similar to the structure of the tissue. Many biomolecules, such as e.g. the bone morphogenetic protein (BMP) family, have been characterized as regulators in the bone formation process (154). Among the potential options for cells to be employed in these constructs, MSCs are the most frequently used.

Applications of MSCs in tests for tissue engineering are mainly based on the recovery of cartilage and bone. Pre-clinical protocols associate cells with various biomaterials and biomolecules essential for a good differentiation performance. Gene transfer is used as a tool to stimulate and accelerate the process of tissue specialization (155,156). Clinical trials, on the other hand, are still in early stages and focus on cells with safer and less bold associations such as fibrin glue.

8.2.1. MSCs for the vascularization of engineered tissues

One of the most challenging issues in tissue engineering is vascular integration of an engineered construct with the host (157). Therefore, inclusion of ECs in engineered tissues with the aim of forming a rudimentary vascular network is desirable. In this scenario, MSCs, as descendants from pericytes, could be used both as a source of secreted factors that contribute to EC cell survival and angiogenesis, and as constituents of vessel walls.

A number of studies have demonstrated MSC ability to support the formation of capillary-like structures *in vitro* by ECs. In the mid-2000's, Gruber *et al*. (158) showed that culture medium conditioned by BMMSCs is able to induce migration and tube formation by human umbilical vein ECs (HUVECs) and human uterus microvascular ECs (HUMECs). Shortly after that, Ghajar *et al*. (159) found that addition of MSCs to prevascularized tissue leads to increased stability of vascular structures *in vitro*. Later, Sorrell *et al*. (62) described the ability of BMMSCs to co-align with capillary-like structures formed by ECs, and to contribute to vascular network formation and stabilization. Subsequent reports have described similar findings, and further detailed mechanisms involved in the angiogenic potential of MSCs derived from various sources (160-171).

The angiogenic properties of MSCs is mediated not only by pro-angiogenic signaling molecules as described earlier in this review: production of proteases that degrade ECM by MSCs (172,173) also favors EC sprouting. Additionally, soluble molecules and physical interactions between MSCs and ECs help stabilize capillary-like structures in coculture experiments. Physical interactions between MSCs and ECs include those mediated by $\alpha 6\beta 1$ integrin present on the surface of MSCs and laminin deposited by ECs (174). Establishment of gap junctions between MSCs and ECs is also likely, as demonstrated in a coculture system in which the murine mesenchymal cell line 10T1/2 formed gap junctions with ECs (175); in this system, physical connection between both cell types activated TGF-β, which specifies a mural cell phenotype. Physiological integration between MSCs and ECs is possibly mediated by molecules shown to be involved in the crosstalk between pericytes and ECs, which include angiopoietin-1 secreted by pericytes (176), and PDGF-BB and heparin-binding, epidermal growth factor-like factor (HB-EGF) secreted by ECs (177,178). From the tissue engineering standpoint, knowledge on the mechanisms that mediate the bidirectional

communication between MSCs and ECs may prove beneficial for manufacturing prevascularized tissues. Other factors that may interfere in this process include timing and MSC: EC proportions during coculture. Direct contact between MSCs and ECs prior to ECs cultivation on Matrigel® inhibit ECs proliferation, migration and tube formation in a mechanism dependent of Wnt/VE-Cadherin/β-catenin signaling pathway (179). Addition of high numbers of MSCs onto ECs capillary-like structures formed on Matrigel® induces tube degeneration and EC apoptosis via gap junctions (180).

9. CONCLUSION

The concept of mesenchymal stem cells (MSCs) has evolved considerably owing to the study of cultured cells able to differentiate into mature mesenchymal cells *in vitro* (MSCs). Currently, evidence indicates that pericytes are nMSCs in the body, and that they can give rise to MSC cultures – even though plasticity of other cell types may contribute to MSC culture establishment. The similarities between cultured pericytes and MSCs leave room for the assumption that activated pericytes that develop during tissue injury *in vivo* significantly contribute to tissue repair by paracrine mechanisms. Consequently, the role of MSCs as sources of paracrine biomolecules in cell therapy protocols most likely mimics the intrinsic behavior of activated pericytes. Finally, differentiation of MSCs for tissue engineering may be as important as their supportive activity on ECs, which can be exploited to generate prevascularized engineered tissues.

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