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Review

Mesenchymal stem cells: from the perivascular environment to clinical applications

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Summary. Adult stem cells represent a fundamental biological system that has fascinated scientists over the last decades, and are currently the subject of a large number of studies aimed at better defining the properties of these cells, with a prominent focus on improving their application in regenerative medicine. One of the most used adult stem cells in clinical trials are mesenchymal stem cells (MSCs), which are multipotent cells able to differentiate into mature cells of mesodermal lineages. Following the initial studies on MSCs isolated from bone marrow, similar cells were also isolated from a variety of fetal and adult human tissues. Initially considered as identical and equipotent, MSCs from tissues other than bone marrow actually display differences in terms of their plastic abilities, which can be ascribed to the tissue of origin and/or to the procedures used for their isolation. Moreover, results from additional studies suggest that cultured MSCs represent the *in vitro* version of a subset of *in vivo* resident cells localized in the perivascular environment. In this review, we will focus our attention on MSCs from tissues other than bone marrow, their *in vivo* localization and their current applications in clinics.

Key words: Mesenchymal stem cells, Perivascular environment, Pericytes, Perivascular cells, Regenerative medicine

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Historical introduction on MSCs

Cells of many tissues in the adult organism need constant replacement in order to sustain their physiological functions. This never-ending process goes from the maintenance of homeostasis (cellular turnover) within and between tissues, to tissue repair. These regenerative abilities of adult tissues are due to the presence of intrinsic progenitors or stem cells. The identification of this class of cells has attracted the interest of scientists for a long time, leading to the development of a novel branch of biomedical research, regenerative medicine, which aims to develop cell stem based therapies to cure some human diseases.

Following the pioneering studies by Till and McCulloch that provided the initial evidence on the presence of hematopoietic stem cells, bone marrow became the most studied and characterized source of postnatal stem cells (McCulloch and Till, 1960; Bianco, 2015; Gao et al., 2018). These studies resulted in the identification within the bone cavity of two different systems of adult stem cells: hematopoietic and stromal cells. However, while embryological origin, physiological role and differentiation abilities of hematopoietic stem cells have been accurately defined (Dzierzak and Speck, 2008; Medvinsky et al., 2011), less is known about stromal cells.

Bone marrow stromal cells were first described by Owen and Friedestein as plastic adherent cells with the ability to differentiate into skeletal tissues *in vitro*. These cells represent the progenitors that give rise to a self-renewing population of stromal cells following *in vitro* culture. When transplanted into a competent

environment, these cells are able to regenerate a bone niche (stroma and bone) that is capable of creating a permissive environment (niche) for hematopoietic cells (Owen and Friedenstein, 1988). In 1991, adult bone marrow stromal cells were named Mesenchymal Stem Cells (MSCs), by analogy with the embryonic mesenchymal cells that are able to give rise to all skeletal elements of the body (Caplan, 1991). Bone marrow MSCs (bm-MSCs) are currently considered as postnatal skeletal stem cells due to their localization (skeleton), their differentiation commitment (skeletogenesis) and their *in vivo* ability to recreate the bone niche (Bianco et al., 2006; Robey and Bianco, 2006). Bm-MSCs thus play a fundamental role in the maintenance of bone marrow environment homeostasis, being responsible for bone niche growth, turnover and regeneration (Bianco, 2014).

Starting from the initial studies in bone marrow, several laboratories have provided evidence that multipotent cells, nearly undistinguishable from bm-MSCs in terms of morphology, immunophenotype and *in vitro* multipotent abilities, can be isolated from several adult and fetal human tissues (Murray et al., 2014). A common property of these cells is their ability to differentiate, under appropriate cell culture conditions, into cells of the mesodermal lineage, such as osteocytes, adipocytes and chondrocytes (Zuk et al., 2001; Sabatini et al., 2005; Dominici et al., 2006). With the factual possibility to isolate MSCs from virtually all human organs, the International Society for Cellular Therapy stated the minimum requirements to consider primary isolated cells as MSCs. MSCs are currently defined as plastic adherent cells, expressing CD73, CD90, CD105, and negative for CD45, CD34, CD14, CD11b, CD79α, CD19 and HLA-DR. In addition, MSCs must be able to differentiate into osteocytes, adipocytes and chondrocytes after *in vitro* differentiation induction (Dominici et al., 2006).

However, in spite of fulfilling the same identification criteria, bm-MSCs and MSCs isolated from other adult or fetal tissues are two separate entities. Indeed, bm-MSC, beyond behaving as skeletal stem cells, can also preserve the hematopoietic stem cell niche and are able to sustain hematopoiesis by recapitulating and regulating the hematopoietic environment (Bianco, 2014; Crane et al., 2017). In agreement with these findings, further studies aimed to address bm-MSCs origin and localization *in vivo* , revealed that these cells represent the *in vitro* counterpart of adventitial reticular cells (ARCs), thus establishing a strong connection between the properties of physiological resident cells and cultured cells. ARCs, in fact, are the bone marrow stromal supportive cells, and are localized around the sinusoidal network in direct contact with the endothelial cells that surround it (Bianco and Gehron Robey, 2000; Short et al., 2003; Jones and McGonagle, 2008). In marked contrast, MSCs isolated from other tissues have no impact on the regulation of hematopoietic environment, although they may be involved in the regulation of additional tissue-specific mechanisms, as discussed later. Moreover, despite being defined by specific criteria, it is accepted that MSCs represent a heterogeneous cell population containing different cell types, which may vary depending on the tissue of origin and on the isolation procedures (Pevsner-Fischer et al., 2011; Chen et al., 2012, 2013). Accordingly, MSC subpopulations expressing specific markers and endowed with distinctive differentiation potentials and proliferation rates, can be obtained from most tissues (De Ugarte et al., 2003; Vogel et al., 2003; Mihu et al., 2008; Battula et al., 2009; Murray et al., 2014). In this review, we will discuss available data on MSCs isolated from the stroma of adult organs and their relation with perivascular progenitors/stem cells. We will discuss current knowledge about the localization of these cells *in vivo* , their physiological role and their effective and/or conceivable translational applications. Namely, we will use the term MSCs to refer to mesenchymal stem cells isolated from the stroma of tissues other than bone marrow according to the reported criteria (Dominici et al., 2006), while we will refer to the latter as bm-MSC.

Perivascular origin of MSCs

The reported association between bm-MSCs and the perivascular compartment, which led to define bm-MSCs as the *in vitro* counterpart of the ARCs (Bianco, 2014), and the evidence that blood vessels are present in all adult and fetal tissues from which MSCs could be isolated, suggested a close relation between MSCs and *in vivo* perivascular cells. In agreement with this hypothesis Shi and Gronthos showed that MSCs isolated from dental pulp originate from the perivascular compartment (Shi and Gronthos, 2003).

First reports on perivascular cells were published at the end of the 19th century, when they were described by the French physiologist C. Rouget (Rouget, 1873). The name pericytes was introduced fifty years later by K.W. Zimmermann, who observed the presence of three different subtypes of pericytes along the vasculature, including a transitional form of smooth muscle cells, pointing to the identification of new perivascular cell types in addition to endothelial and smooth muscle cells (Zimmermann, 1923; Krueger and Bechmann, 2010). Nonetheless, only in the mid 80s electron microscopy imaging revealed that pericytes establish intimate contacts with endothelial cells, surrounding all the blood vessels with their cytoplasmic elongations (Weibel, 1974; Sims, 1986). Further studies showed that pericytes are enclosed within the basal membrane of small vessels, like capillaries, venules or small arterioles (Sims, 1991; Allt and Lawrenson, 2001). *In situ* analysis allowed the identification of different surface markers shared by pericytes that reside in different tissues. Pericytes express at least one of the following markers: CD146, Neural/glial antigen 2 (NG2), Beta-type platelet-derived growth factor receptor (PDGFR-β) and Alkaline phosphatase (AP), while they do not express endothelial

markers such as CD31, CD34, Von Willebrand factor and CD144, and are also negative for the expression of the pan-hematopoietic marker CD45 (Dellavalle et al., 2007; Tonlorenzi et al., 2007; Crisan et al., 2008; Quattrocelli et al., 2012). Based on these studies, *in vivo* pericytes have been defined according to their anatomical localization and to the expression of specific markers (Crisan et al., 2008; Souza et al., 2016).

At the end of the 90s, a study on cultured pericytes isolated from bovine retinal capillaries showed that these cells were able to differentiate into different mesodermal lineages *in vitro* (Doherty et al., 1998). Further studies on cultured pericytes isolated from different adult and fetal tissues demonstrated that these cells were able to differentiate into osteocytes, adipocytes, chondrocytes and muscle cells, confirming their mesodermal multipotential properties (De Angelis et al., 1999; Dellavalle et al., 2007; Tonlorenzi et al., 2007; Crisan et al., 2008; Quattrocelli et al., 2012). The initial idea that pericytes exclusively surround microvessels was later challenged by the observation of pericyte-related cells also encircling large vessels (Andreeva et al., 1998). Accordingly, studies on perivascular cells surrounding arteries revealed the presence of another class of perivascular cells, named adventitial cells. Indeed, adventitial cells are present in the tunica adventitia of large blood vessels such as arteries and veins (Hu et al., 2004; Campagnolo et al., 2010; Hu and Xu, 2011; Souza et al., 2016). The ability of these cells to differentiate into smooth muscle cells suggested a direct role as progenitors in vascular remodeling (Zengin, 2006; Hu and Xu, 2011; Majesky et al., 2011; Souza et al., 2016). Additional studies indicated that human adventitial cells, similarly to pericytes, were able to differentiate into adipocytes, osteoblasts and myofibroblasts after *in vitro* expansion (Hoshino et al., 2008; Corselli et al., 2012). A study by Pèault and collaborators showed that adventitial cells can be typified as CD34+ CD31- CD146- CD45- resident cells, which differs from the immunophenotype of pericytes from small vessels (Corselli et al., 2012). Interestingly, these authors also showed that, under appropriate *in vitro* conditions, adventitial cells could acquire a pericyte-like immunophenotype suggesting the presence of a CD34+ CD31- perivascular cell population that could behave as progenitors of pericytes (Yoshimura et al., 2006; Corselli et al., 2012).

Studies over the past years have provided evidence that pericytes and adventitial cells isolated using distinct preparative methods, and following *in vitro* expansion, express a common range of surface markers and present a collective ability to differentiate into at least three basic connective tissue lineages (i.e. adipocytes, chondrocytes and osteocytes). These findings, together with the evidence that perivascular cells natively express MSCs markers *in vivo*, strongly indicated that MSCs can be associated with cultured perivascular cells (Crisan et al., 2008). Nevertheless, depending on the isolation procedures applied and the tissue of origin, additional distinguishing properties can be observed in isolated MSCs or perivascular cells, which is likely to reflect the original presence of heterogeneous populations of stem/progenitor cells in the stroma of most adult organs (Muraglia et al., 2000; Guilak et al., 2006; Russell et al., 2010; Manini et al., 2011). Moreover, the use of specific markers to isolate perivascular cells, such as CD146 or AP to identify pericytes, and CD34 to identify adventitial cells, suggests that even if perivascular cells share the same *in vitro* mesodermal abilities with MSCs, different cell subpopulations coexist in the perivascular environment. Therefore, based on the isolation procedure used, we shall refer to the isolated population of stem/progenitor cells as cultured pericytes or cultured adventitial cells to distinguish these cell populations from the more heterogeneous population of MSCs obtained exploiting the protocol initially applied to isolate bm-MSCs (Pittenger et al., 1999; Zuk et al., 2001; Sabatini et al., 2005; Dominici et al., 2006).

in vivo role of perivascular cells

The activation of cells of the perivascular compartment has been observed under inflammatory conditions and following induction of tissue damage (Díaz-Flores et al., 2009; Armulik et al., 2011). Studies on a rat model showed that 40% of pericytes migrate from the vessels into the parenchyma after a traumatic brain injury (Dore-Duffy et al., 2000; Chapel et al., 2003). This migratory ability of pericytes is regulated by specific transmembrane proteins that allow pericytes/endothelial cell interactions. One transmembrane protein expressed by pericytes and involved in this interaction is the PDGFR-β (Dellavalle et al., 2007; Crisan et al., 2008; Winkler et al., 2010). Endothelial cells, by releasing platelet-derived growth factor subunit B (PDGF-B), the PDGFR-β ligand, activate a signal transduction that allows the recruitment of PDGFR-β-expressing pericytes during angiogenesis and tissue remodeling (Armulik et al., 2011). The disruption of the PDGFR-β-mediated interaction between endothelium and pericytes leads to aberrant vasculature remodeling (Benjamin et al., 1998) and, during embryonic development, lack of PDGF-B and/or PDGFR-β signaling leads to the development of hematological, renal and placental abnormalities that cause hemorrhages (Levéen et al., 1994; Lindahl, 1997; Hellström et al., 1999). Although PDGF-B deficiency results in pathological detachment of pericytes from the blood vessels of PDGF-B knockout mouse (Lindahl, 1997), it has been argued that the detachment of pericytes from the vessels is required to activate and address pericytes towards the regenerative mechanisms (Caplan and Correa, 2011; Caplan and Hariri, 2015). These latter aspects outline the importance of cell-cell contacts in the perivascular niche and indicate how the regulation of cell-cell interactions, via PDGF-B/PDGFR-β for instance, may affect pericyte recruitment and activation.

Although several studies suggest that cultured

perivascular cells can self-renew and differentiate into functional cell types of a given tissue (see next section), a strict *in vivo* proof of their multipotent abilities is still missing, and the extent of perivascular cells involvement in tissue regeneration is still debated. Lineage tracing studies reported that a specific spinal cord pericyte subtype contributes to scar-forming stromal cells during post-injury responses, and that NG2+ pericytes of the dental pulp can differentiate into odontoblasts during tooth growth and in response to damage (Feng et al., 2011; Goritz et al., 2011; Cano et al., 2017). Notably, Cossu and collaborators reported that direct recruitment of pericytes in tissue regeneration also occurs in skeletal muscle. Using a transgenic mouse model in which AP+ endogenous pericytes are traced by beta-galactosidase expression, these authors demonstrated that skeletal muscle pericytes contribute to both blood vessels and skeletal muscle fiber development during physiological mouse growth (Dellavalle et al., 2011). In contrast with these findings, pericyte involvement in tissue remodeling and/or regeneration has been recently challenged by Evans and colleagues (Guimarães-Camboa et al., 2017). In the study, the authors focused their attention on a specific subpopulation of pericytes expressing Tbx18, a protein expressed in all mural cells (including pericytes) of several, but not all, mouse adult organs. Lineage-tracing experiments, performed on the inducible Tbx18-CreERT2 mouse model, revealed that Tbx18+ pericytes do not differentiate into other cell lineages in aging or during regenerative processes. Accordingly, the authors concluded that adult endogenous pericytes do not behave as multipotent progenitors *in vivo* (Guimarães-Camboa et al., 2017). However, it is worth noting that in the inducible Tbx18-CreERT2 mouse model, Tbx18 positive pericytes could not be detected in kidney, liver, pancreas and gastro-intestinal tract, although the presence of pericytes has been widely reported in these organs (Crisan et al., 2008; Richards et al., 2010; Powell et al., 2011; Hellerbrand, 2013; Stefanska et al., 2016). Taking these data in consideration, it would appear that the Tbx18-CreERT2 mouse model system identifies only a subset of tissue/organ specific pericytes, suggesting that the absence of *in vivo* pericytes with multipotent properties observed by Evans and colleagues (Guimarães-Camboa et al., 2017) may only reflect the properties of a specific subpopulation of Tbx18-positive pericytes. Accordingly, it is important to consider that the results from lineage-tracing experiments have been obtained from formally distinct pericytes (i.e. PDGFR-β, NG2, AP and Tbx18 expressing pericytes), whose differentiation potential towards a definite cell type have been tracked within a specific tissue.

Additional data suggest that perivascular cells can further participate in tissue regeneration by acting as recruiters, modulators and activators of other cell types (Caplan and Correa, 2011; Somoza et al., 2016). By exerting a trophic effect on cells at site of injuries, perivascular cells can therefore contribute to form a regenerative microenvironment that supports the healing process (Chen et al., 2009; García-Gómez et al., 2010; Morigi et al., 2010; Caplan and Correa, 2011). Indeed, given their localization around vessels, perivascular cells have the possibility to interact and recruit many different cell types ranging from cells of the immune system to resident stem cells. Moreover, the blood flow might allow factors secreted by perivascular cells to spread through the entire organism, activating a systemic response to tissue damage. The cross talk between perivascular cells and other cell types within a given tissue may thus represent a key factor for tissue regeneration and maintenance (Geevarghese and Herman, 2014; Rohban et al., 2017).

Distinctive properties of MSCs and pericytes isolated from different tissues and with different procedures

The possibility to isolate MSCs from almost all organs, the evidence that the perivascular compartment of different tissues contains cells with mesodermal differentiation potential and the evidence that pericytes and adventitial cells, although distinguishable *in situ* by different markers, both express MSCs markers, suggests the association between MSCs and perivascular cells, but also indicates a certain level of heterogeneity within these cells (Crisan et al., 2008, 2012; Corselli et al., 2012; Zimmerlin et al., 2013).

The use of different techniques for MSCs isolation from the perivascular compartment may further contribute to increase the level of heterogeneity among cultured MSCs populations. Initial protocols were only based on the enzymatic digestion of a given tissue using collagenase, which is able *per se* to dissociate perivascular cells from the basal membrane of the vessels. Afterward, it has been shown that perivascular cells, irrespective of the tissue of origin, can also be obtained without enzymatic digestion (Tonlorenzi et al., 2007; Cossu et al., 2015; Pierantozzi et al., 2015, 2016). This isolation protocol yields cells that present a MSCs phenotype once expanded i*n vitro*, but also express pericyte markers. Alternative protocols have been applied to isolate subpopulations of perivascular cells on the base of the expression of specific surface markers such as CD146, CD34 or AP from a bulk population (Dellavalle et al., 2007; Corselli et al., 2012; Vezzani et al., 2016).

It is therefore not surprising that differences can be observed between cultured perivascular cells and MSCs obtained by exploiting different isolation procedures (Bieback et al., 2008; Chen et al., 2015; Günther et al., 2015; Pierantozzi et al., 2015; Herrmann et al., 2016; Sacchetti et al., 2016; Vezzani et al., 2016). In a recent study, the mesodermal differentiation efficiency of MSCs isolated from adipose tissue through enzymatic digestion and of isogenic pericytes obtained following spontaneous outgrowth, was compared (Pierantozzi et al., 2015). The authors reported that the latter cell population was more prone to differentiate towards

osteogenic, adipogenic and myogenic lineage than the former one. In addition, the properties of pericytes also appear to depend, at least in part, on the tissue of origin (Pierantozzi et al., 2016; Sacchetti et al., 2016). Pericytes prepared from adipose tissue, skeletal and smooth muscle, showed distinct differentiation abilities (Pierantozzi et al., 2016). In fact, although pericytes isolated from all tissues presented a similar phenotype, only cells from skeletal muscle and adipose tissue were able to differentiate into adipocytes, chondrocytes and osteocytes. Even more surprising was the evidence that only pericytes from skeletal muscle were able to spontaneously fuse and form skeletal muscle myotubes, while putative pericytes prepared from smooth muscle were only able to differentiate into smooth muscle cells. Similarly, pericytes isolated from cardiac muscle are committed to cardiomyocyte differentiation, while they display a null skeletal myogenic potential (Chen et al., 2015). These findings suggest that cultured pericytes from different tissues are not equal in terms of differentiation potential, as they appear to retain a preferential commitment to differentiate into cells of the tissue from which they have been isolated (Vezzani et al., 2016). In agreement with these findings, perivascular cells from different tissues have been shown to display a different transcriptomic signature, which parallels the distinct differentiation abilities observed following both *in vitro* differentiation assays and cell transplantation in mouse models (Sacchetti et al., 2016). It is therefore evident that, although similar in terms of morphology and marker expression, when evaluated in light of multipotent properties, cultured pericytes display clear differences that reflect the tissue of origin and/or the isolation procedure.

In addition, it must be taken into account that pericyte differentiation abilities, as for the vast majority of stem cells, are influenced also by aging (Goodell and Rando, 2015). Indeed, a recent study on AP+ pericytes isolated from human skeletal muscle of young and elderly donors showed that ”aged” pericytes exhibit a reduced myogenic differentiation ability, both *in vivo* and *in vitro*, in favor of a higher adipogenic potential (Rotini et al., 2018). Moreover, the same authors also reported that AP+ pericyte abundancy was reduced in elderly skeletal muscles. Interestingly, the correlation between aging and decrease of pericyte content has also been described in mouse kidney (Stefanska et al., 2015). These findings suggest that the age of the donor represents an additional variable that can affect the multipotent properties of pericytes.

MSCs and perivascular cells in clinical applications

The increased interest in stem cells observed in recent years, based on their potential applications in regenerative medicine, has resulted in a large number of clinical trials, where MSCs have been used in cell-based therapies aimed to treat a variety of diverse diseases. Currently, only a fraction of these clinical trials have been fully completed, and the advancements achieved in using MSCs in this therapeutic field are not as striking as expected (Bianco, 2014; Cagliani et al., 2017). On these bases, as described in previous sections, additional studies aimed at obtaining a better understanding of MSCs properties, especially in terms of their *in vivo* origin and physiological role, have been started. As an outcome of the clinical studies completed in the past years using MSCs obtained from either bone marrow, adipose tissue or umbilical cord, encouraging results have been obtained for the treatment of liver diseases (Lee et al., 2017; Tsuchiya et al., 2017), immune associated diseases (Cagliani et al., 2017), bowel diseases (Mao et al., 2017), cartilage defects (De Windt et al., 2017; Paschos and Sennett, 2017), bone defects (Paduano et al., 2017) and in the enhancement of hematopoietic stem cell transplantation (Kallekleiv et al., 2016; Zhao and Liu, 2016; Najar et al., 2018). Currently, according to ClinicalTrials.gov, 260 clinical studies, mainly phase I and phase II, are ongoing or recruiting patients to test the use of MSCs for the treatment of additional pathological conditions. These studies aim to evaluate the effectiveness of either autologous or allogenic MSCs transplantation in the treatment of several conditions affecting heart, brain, bone/cartilage, lungs, kidneys, liver, and immune system. MSCs used in these clinical trials are mainly derived from bone marrow (40%), umbilical cord (17%) and adipose tissue (11%) (clinicaltrials.gov).

The first evidence of *in vivo* regenerative potential of cultured pericytes was observed after intra-arterial delivery of healthy fetal cultured pericytes in α-sarcoglycan null dystrophic mice (Sampaolesi et al., 2003). Further studies showed that cultured pericytes from a variety of fetal and adult tissue are able to regenerate human myofibers once transplanted into injured muscles (Crisan et al., 2008). Cultured pericyte transplantation not only leads to muscle fiber recovery in terms of structure, but also recovers muscle functionality (Dellavalle et al., 2007). The effectiveness of transplantation of skeletal muscle derived pericytes has been shown also in a myocardial infarction mouse model. Human CD146+ cells after transplant improved the contractility of the damaged heart and released specific trophic factors involved in the overall regenerative process (Chen et al., 2013, 2015). These promising results, further confirmed also in a canine pre-clinical model of muscular dystrophy (Sampaolesi et al., 2006), led to cultured pericytes transplantation on pediatric patients affected by Duchenne muscular dystrophy. Unfortunately, results obtained in the preclinical models were not reproduced in human patients (Cossu et al., 2015). The observed lack of skeletal muscle regeneration in pediatric patients as compared with preclinical trials on animal models could be explained by at least three different events: 1) patients were undergoing anti-inflammatory and immuno-suppressive therapies that might affect pericyte extravasation and engraftment; 2) humans, at variance

with mice or dogs, use also limb girdle and dorsal muscles to sustain posture and motility, while cell delivery targeted only upper and lower limbs; 3) the total cell number injected may not have been sufficient. Nevertheless, this clinical trial showed promising results regarding the safety of intra-arterial transplantation of HLA-matched cultured pericytes, indicating that a cell therapy based on the transplantation of cultured pericytes can be considered safe, even though improvements of the procedure are required (Cossu et al., 2015; Thomas et al., 2017).

A major limit of cell based therapies is therefore represented also by the need to transplant a large number of cells. The use of MSCs from umbilical cord or cultured pericytes from skeletal muscle, implies their *in vitro* expansion in order to increase the number of cells obtained from the initial biopsies. Alternatively, the possibility to isolate an appropriate amount of cells, avoiding cell culture, has been proved using adipose tissue as supplier. During the past years, adipose tissue has been recognized as a conspicuous source of MSCs due to its easy accessibility through minimally invasive surgical procedures, such as liposuction (Yoshimura et al., 2006). The cellular fraction obtained after enzymatic dissociation of adipose tissue, referred to as stromal vascular fraction (SVF), contains different cell types including pre-adipocytes, endothelial cells, leucocytes and perivascular cells (Zimmerlin et al., 2010). Due to the presence of perivascular cells, SVF thus represents a source of MSCs (West et al., 2016). The use of SVF, as coadjuvant therapy, has been exploited in orthopedic conditions, wound healing, diabetes, radiotherapy derived disorders, bowel disease and ulcer (Amos et al., 2010; James et al., 2012; Shukla et al., 2015; König et al., 2016; Tawonsawatruk et al., 2016; Zollino et al., 2016; Esteves and Donadeu, 2017; Klar et al., 2017).

A potential different approach for the use of MSCs in clinical applications derives from studies on the ability of these cells to secrete soluble factors that may stimulate resident cells. Interestingly, it has been shown that MSCs secrete cytokines, exosomes and other trophic factors that are known to be involved in the activation of endogenous mechanisms, from immunomodulation to post-injury tissue remodeling (including fibrosis) (Caplan and Correa, 2011; Caplan, 2016). The immunomodulatory function of MSCs has come under particular attention in the recent years (Abdi et al., 2008; Gao et al., 2016). MSCs can regulate both T- and B-cell-mediated immune response, either through the secretion of soluble factors or through cell-contact dependent mechanisms (Jiang et al., 2005; Jarvinen et al., 2008; Ren et al., 2010; Yagi et al., 2010). Additional studies reported that MSCs are endowed with macrophage-like non-professional antigen-presenting cell characteristics (Pardridge et al., 1989; Shepro and Morel, 1993; Balabanov et al., 1996; Navarro et al., 2016), a feature that can prevent the development of autoimmune activities (Caplan, 2013). Notably, MSCs release exosomes, and secrete chemokines and other bioactive molecules, which not only can modulate inflammation, but also promote and regulate cell proliferation, apoptosis, matrix remodeling and angiogenesis (Kögler et al., 2005; Liu and Hwang, 2005; Tögel et al., 2005; Lai et al., 2010; Morigi et al., 2010; Li et al., 2013; Lin et al., 2013; Murphy et al., 2013; Shabbir et al., 2015). This trophic and immunomodulatory role, fundamental to support homeostasis and tissue regeneration, has led to the proposal of converting the acronym MSCs from Mesenchymal Stem Cells into Medicinal Signaling Cells. This new meaning aims at underlining the prevalence of the stimulatory effect provided by MSCs on resident cells, rather than their ability to provide differentiated cells in cell-based therapies (Caplan, 2010, 2013; Caplan and Correa, 2011; Somoza et al., 2016; Boregowda et al., 2018).

The ability of MSCs to synthesize and release a broad spectrum of growth factors, cytokines, antiapoptotic and angiogenic factors, which can either affect cells in their proximity or can be released in the blood stream, suggested that the identification of specific factors that could be used for clinical purposes may be obtained from the characterization of MSCs secretome (Haynesworth et al., 1996; Caplan and Dennis, 2006; Salgado et al., 2010; Montemurro et al., 2011; Kapur and Katz, 2013; Yu et al., 2014; Rani et al., 2015; Vizoso et al., 2017). Interestingly, the secretome profile of MSCs appears to be tissue-specific. In fact, a study conducted by Pires and colleagues, proved that the secretome of human MSCs isolated from bone marrow, adipose tissue, and umbilical cord have different expression profiles. This finding suggests a possible way to identify the best MSCs subtype for the treatment of a specific conditions, according to the factors secreted (Pires et al., 2016).

MSCs conditioned medium is also enriched in exosomes that represent a new intercellular communication system. These nanovescicles are involved in many different biological processes (Shabbir et al., 2015), and can be easily isolated from serum, plasma and cell culture media. Exosomes isolated from MSCs conditioned medium possess MSCs features, such as tissue damage repair, inflammatory response suppression, and immune system modulation (Ludwig and Giebel, 2012; Lin et al., 2013; Blazquez et al., 2014; Kordelas et al., 2014; Yu et al., 2014; Kang et al., 2015; Nakamura et al., 2015; Shabbir et al., 2015; Zhang et al., 2016). Accordingly, the efficacy of *in vivo* administration of MSCs derived exosomes in restoring pathological conditions has been reported in different mouse models (Lai et al., 2010; Li et al., 2013). When injected, exosomes are more stable compared to cells, and have a lower possibility of rejection after allogenic administration. Taken together, these findings suggest a possible novel approach in regenerative medicine based on the use of MSCs derived exosomes instead of MSCs (Yu et al., 2014; Gimona et al., 2017). However, the major limit of the use of MSCs or MSCs derived exosomes in clinical practice is the manipulative process that is needed for cell/exosomes isolation. We already

outlined how enzymatic digestion, or culture expansion could affect MSCs behavior and marker expression (Pierantozzi et al., 2015; Guimarães-Camboa et al., 2017).

In the past decade, subcutaneous adipose tissue transplant became widely used in aesthetic surgery as a filler for soft tissue reconstruction (Simonacci et al., 2017; Spiekman et al., 2017). Beneficial results obtained with autologous lipofilling in the treatment of scars suggested the possible application of this technique for the treatment of other conditions in regenerative medicine (Coleman, 2006; Oberbauer et al., 2015). Accordingly, different studies have reported that adipose tissue injections can improve not only scar regression, but can also reduce the consequences, like fibrosis, woody induration, and hypovascularity, which are frequently observed in skin and subcutaneous tissue after radiation treatments (Pinski and Roenigk, 1992; Cooper and Lee, 2009; Phulpin et al., 2009). Currently, it is accepted that these valuable effects might be ascribed to the residing perivascular cells.

Recently, different companies started developing non-enzymatic systems to obtain mechanically dissociated adipose tissue ready for transplant (Bianchi et al., 2013; Oberbauer et al., 2015). In fragmented adipose tissue, the perivascular niche is left in its physiological state, and pericytes are still wrapped around the microvasculature in direct contact with endothelial cells. To date, micro fragmented adipose tissue autologous transplant has been used with promising results for the treatment of different conditions, such as cartilage defect repair, incontinence, glottiC insufficiency and soft tissue regeneration (Cestaro et al., 2015; Benzi et al., 2015; Raffaini and Pisani, 2015; Saibene, 2015; Bosetti et al., 2016). *in vitro* studies revealed that microfragmented adipose tissue secretes a higher amount of exosomes, compared to the enzymatically digested counterpart (Garcia-Contreras et al., 2014). This difference outlines once more how enzymatic dissociation affects the physiological state of cells, affecting also their regenerative potential. Since cell-cell contact, interactions with the extracellular matrix and secretion of cytokines control the behavior of cells (Watt, 2000), it can be envisioned that treatments that leave the perivascular niche unperturbed may result in increased regenerative abilities of resident pericytes.

Conclusions

Results obtained over the last decades have provided evidence that MSCs and cells of the perivascular compartment significantly contribute to tissue regeneration either by differentiating into specialized cells, or by recruiting, activating and modulating resident cells in the complex process of tissue regeneration. Many studies have addressed the mechanisms of MSCs regenerative potential, and more recently have also focused on their trophic activity and their effects on immune cells. Due to their potential, MSCs from bone marrow, umbilical cord and adipose tissue have been widely used for the treatment and restoration of different pathological conditions.

Based on the knowledge that enzymatic digestion and *in vitro* cell culture conditions dramatically affect cell fate, regenerative medicine perspectives are driving towards a less manipulative approach, in order to preserve the original properties of the cell. Accordingly, paying attention to maintain the perivascular niche of mesenchymal progenitor/stem cells unperturbed seems to represent a promising approach to enhance perivascular cells contribution to regeneration.

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