Biological properties of mesenchymal Stem Cells from different sources

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Summary

Mesenchymal stem cells (MSCs) are adult, non-hematopoietic, stem cells that were initially isolated from bone marrow. Now they can be isolated from almost every tissue of the body. They have the ability to self-renew and differentiate into multiple cell lineage, including bone, chondrocytes, adipocytes, tenocytes and cardiomyocytes, and it makes them an attractive cell source for a new generation of cell-based regenerative therapies. In this review we try to summarize data on sources and the biological properties of MSCs.

Key words: bone marrow, mesenchymal stem cells, stromal cells, tissue engineering.

Introduction

Mesenchymal stem cells (MSCs) are adult stem cells that can differentiate into several mesenchymal cell lineages and regenerate themself. The International Society for Cellular Therapy define MSCs as cells with a specific immunophenotype, *ex vivo* plastic-adherent growth, and multilineage differentiation[1]. MSCs have been originally isolated from the bone marrow (BM) as precursors of stromal elements, but during the recent years, MSC-like populations have been obtained from a wide range of adult tissues, showing similar properties and minor differences. They have been shown to differentiate into bone[2], skeletal muscle[3], adipose tissue[4], cartilage[5] and tendon[6]. There is evidence that MSCs differentiate into neural cells, such as neurons and glial cells. However, these cells possess many, but not all, of the properties of mature neurons[7].
The history of research on adult stem cells began about 50 years ago. In the 1950s, researchers discovered that the bone marrow contains at least two kinds of stem cells, hematopoietic stem cells and a second population, called bone marrow stromal stem cells or mesenchymal stem cells were discovered a few years later. These non-hematopoietic stem cells make up a small proportion of the stromal cell population in the bone marrow, and can generate bone, cartilage, fat, cells that support the formation of blood, and fibrous connective tissue. The clonal nature of marrow cells was pointed out by McCulloch et al. in the 1960s[8], and MSCs were originally described by Friedenstein and colleagues ten years later[9]. The original criteria used to identify MSCs involved their ability to adhere to plastic substrates, the capacity for substantial clonal expansion. Adherence to tissue culture plastic is a nonspecific cellular property, but it excludes cell subpopulations with hematopoietic functions.
As that they can be isolated from almost every tissue of the body[10], currently some authors thought that these cells form a complex system diffused throughout the body, deriving by a unique cell population originating from the mesoderm[11]. So, in a unifying attempt to explain their origin, it has been hypothesized by some authors that MSCs are a subgroup of vessel-lining pericytes that may contribute to vessel homeostasis by reacting to tissue damage with regenerative processes, by locally modulating the inflammatory reaction, and by entering systemic circulation to relocate according to different cytokine gradients[12]. However no definitive theory on the real origin of MSCs has been postulated.

The stem cells niche

Adult stem cells reside in a special microenvironment called the "niche," which varies in nature and location depending on the tissue type. Stem cell niches (SCN) provide the homeostasis of MSCs, control stem cell proliferative activity and the maintaining of stem cell populations. The "niche" hypothesis was proposed by Schofield in 1978 to describe the physiologically microenvironment that supports stem cells[13]. Historically, "niche" is generally used to describe the stem cell location but it is much more than a simple location, because it is composed of the cellular components of the microenvironment surrounding stem cells as well as the signals emanating from the support cells. A complete definition of niche was given by Scadden[14]. He defined niches as: "specific anatomic locations that regulate how stem-cell participate in tissue generation, maintenance and repair. The niche saves stem cells from depletion, while protecting the host from

over-exuberant stem-cell proliferation. It constitutes a basic unit of tissue physiology, integrating signals that mediate the balanced response of stem cells to the needs of organisms. The interplay between stem cells and their niche creates the dynamic system necessary for sustaining tissues, and for the ultimate design of stem-cell therapeutics. The simple location of stem cells is not sufficient to define a niche. The niche must have both anatomic and functional dimensions".

The first studies regarding stem cells niche have been performed on more simple organisms such as Drosophila[15] and Caenorhabditis elegans[16]. Only recently, significant progress regarding stem cells and their surrounding microenvironments in a variety of mammalian models has been made[17,18]. The stem cell niche is composed of a group of cells in a special tissue location for the maintenance of stem cells. The structure of the niche is variable, and different cell types can provide the niche environment. A primary function of the niche is to anchor stem cells. Many types of adhesion molecules including integrin may play an important role in the interaction of MSCs with the microenvironment. A second function of the niche is to generate extrinsic factors that control stem cell fate and number. Many signal molecules have been shown to be involved in regulation of stem cell behavior. Bone Morphogenetic Proteins (BMPs) haves been recognized to be an important signal pathway for controlling stem cell self-renewal. Than the stem cell niche exhibits an asymmetric structure. Upon division, one daughter cell is maintained in the niche as a stem cell while the other daughter cell leaves the niche to proliferate and differentiate, eventually becoming a functionally mature cell[19].

The exact locations of SCN are poorly understood. They are located in different site in different tissues, but there is evidence suggesting that MSCs are located within the vicinity of vessel walls[20]. It has also been postulated by some authors that the cells with properties of MSCs derive from pericytes a close relationship with perycites[21]. The hematopoietic SCN is located on the endosteal surfaces of trabecular bone, skeletal muscles SCN reside adjacent to the myofiber plasma membrane, Tendon derived-MSC niches is located within the interfibrillar spaces. Adipose Derived -MSC niche is like to be perivascular and animal studies showed that they are correlated with vascular density[22].

Scientists are studying the various components of the niche and trying to replicate the *in vivo* niche conditions *in vitro*. Adult stem cells remain in an undifferentiated state throughout adult life. However, when they are cultured *in vitro*, they often undergo an 'aging' process in which their morphology is changed and their proliferative capacity is decreased. It is believed that correct culturing conditions of adult stem cells needs to be improved so that adult stem cells can maintain their stemness over time.

Sources of MSCs

There are increasing reports that MSCs can be isolated from various adult mesenchymal tissues both in adult tissues and fetal tissues. MSCs have been isolated from different tissues including trabecular and cortical bone, synovial membranes, adipose tissue, tendons, skeletal muscle, peripheral blood, periosteum, umbilical cord blood and Wharton's jelly, skin and the nervous system, in addition to bone marrow[23]. The clinical use of Bone Marrow-MSCs has presented some problems, including pain, morbidity, and low cell number upon harvest, and this has led many authors to investigate alternate sources for MSCs.

Bone marrow MSCs

Bone marrow MSCs (BM-MSCs) are a subpopulation of the stromal cells that line the endosteal surface of the marrow space. Cells in many respects identical to BM-MSCs can be isolated from trabecular and compact bone[24,25] and from non-hematopoietic bone marrow sites, such as the femoral head[26]. MSCs have been first isolated from the bone marrow. They have been defined as non-hematopoietic, multipotential cells that support hematopoietic stem cells expansion in vitro and can differentiate into cells of various connective tissues. They are easy to harvest and they are hold in bone marrow in relatively high concentration, so for these reasons, bone marrow is still a commonly used source of MSCs[27]. The iliac crest has long been the preferred source of autograft material, but graft harvest is associated with frequent donor site morbidity. Chronic pain at the donor site has being reported in up to 39% of cases[28]. So others sources of BM-MSCs have been investigated by many authors. The vertebral body (VB) has been proved to contain a heterogenous population of osteogenic precursor cells and it should provide progenitor cell concentrations similar to those of the iliac crest[29]. The osteogenic cellular concentrations obtained via vertebral body aspiration has been found to be comparable to those obtained via aspiration of the iliac crest[30]. So progenitor cells from the vertebral bone marrow have been proposed for osseous graft supplementation in spinal fusion procedures. In fact they can be easily collected during the surgical procedure and may further reduce the time of surgery and morbidity associated with iliac crest harvest. Another possible source of BM-MSCs described in literature is humeral head. Mazzocca reported the isolation of BM-MSCs from the humeral head during arthroscopic rotator cuff repair[31]. He stated that it was a safe and reproducible procedure, without intraoperative complications nor increase patient morbidity and postoperative complication rate. Than MSCs harvested form humeral head have been shown to comparable to MSCs from the vertebral body and iliac crest[30]. Cells that exhibited stem cell-like characteristics such as a stable undifferentiated phenotype, and the ability to proliferate extensively and differentiate into osteoblastic, adipogenic and chondrogenic lineages have been isolated also from trabecular bone[32] (TB-MSCs) and periosteum[33] (P-MSCs). Sottile et al. demonstrated that cultures of TB-MSCs are equivalent to cultures of bone marrow-derived stem cells in terms of proliferation and multipotent differentiation capabilities[34]. P-MSCs are essential for bone repair. BM-MSCs have been also harvested from femoral head in animal studies.

Synovial MSCs

MSCs can be isolated from synovial membrane or from synovial fluid itself and they are an alternative attractive cell source.

Synovial MSCs (S-MSCs) are capable of considerable proliferative expansion and have multi-lineage differentiation potential. Yoshimura et al. found that rat S-MSCs were superior to bone-marrow-, adipose tissue-, periosteum-, and muscle-derived stem cells in terms of colony number per nucleated cell, colony number per adherent cell, and cell number per colony[35]. Multipotent MSCs have been isolated also from the synovial membrane of human knee joints[36]. These cells showed the ability to proliferate extensively in culture, and they maintain their multi lineage differentiation potential *in vitro*, establishing their progenitor cell nature. S-MSCs can be induced to differentiate *in vitro* toward chondrogenesis, osteogenesis, myogenesis, and adipogenesis. An advantage for clinical use is that synovium can be obtained arthroscopically with a low degree of invasiveness and without causing complications at the donor site due. Many authors consider the synovium a very attractive source of MSCs during joint surgical procedures.

Adipose Tissue MSCs

Adipose tissue is one of the richest sources of MSCs and they have been first isolated by Zuk et al. in 2001[37]. Adipose-derived mesenchymal stem cells (AD-MSCs) have become an attractive alternative to BM-MSCs in recent years, due to the ease of tissue collection, high initial cell yields and robust in vitro proliferative capacity[38,39]. Adipose tissue is easier to get in larger volumes, the harvest is less painful and at lower risks, and yield more stem cells compared to bone marrow[40]. They can be obtained from either liposuction aspirates or excised fat, or under local anesthesia if we need small amounts of adipose tissue. There is more than 50 times more stem cells in 1 gr. of fat when compared to 1 gr. of aspirated bone marrow. One gr. of adipose tissue yields approximately 5,000 stem cells, whereas the yield from BM-derived MSCs is 100 to 1,000 cells/mL of marrow[41]. AD-MSCs show similar properties to BM-MSCs. They seem to be able to differentiate *in vitro* toward the osteogenic, adipogenic, myogenic, and chondrogenic lineages[37]. These similarities led some authors to think that these MSCs are simply an MSC population located within the adipose compartment and the result of infiltration of MSCs from the peripheral blood supply. In 2002 Zuk et al. found several distinctions between AD-MSC and BM-MSC populations[42]. Immunofluorescence analysis identified differences in CD marker profile because both AD-MSCs and BM-MSCs expressed marker profile which is considered peculiar for MSCs, such as CD29, CD44, CD71, CD90, CD105/SH2 and SH3, but they did not find the expression of the hematopoietic lineage markers (CD31, CD34, and CD45)[43]. Than MSCs derived from adipose tissue did not show chondrogenic or myogenic differentiation under the conditions used in the study, suggesting distinctions in differentiation capacities. So the authors concluded this two MSCs populations are similar but not identical, and that adipose tissue contains stem cells which are distinct from those present in the bone marrow.

Tendon-Derived MSCs

Traditionally tendons are considered only to contain tenocytes that are responsible for the homeostasis of the extracellular matrix, the remodeling and healing of the tendons, but recently stem cells, which are called tendon-derived mesenchymal stem cells (TD-MSCs), have been identified both in human and animal tendons. Bi and colleagues demonstrated the existence of MSCs in both murine and human tendon in 2007[44]. More recently TD-MSCs have been identified in the horse superficial digital flexor tendon[45]. TD-MSCs niche is located within the interfibrillar spaces. These cells have high clonogenic properties and proliferating potential, they show particular affinity for extracellular tendon matrix, and *in vivo* spontaneously regenerate tendon-like tissue structures[46]. They have multi-lineage potential and show great tenogenic, osteogenic, chondrogenic, and adipogenic differentiation potential[47]. For these reasons, some authors consider TD-MSCs a good model for stem cell biology and very useful for future tendon regenerative medicine investigations.

Muscle-Derived MSCs

Postnatal skeletal muscle tissue contains two different types of stem cells, which are called Muscle-Derived Stem Cells (MD-MSCs) and Satellite Cells. They both function as muscle precursors, but satellite cells are committed and unipotent cells. They form a small population of adult stem cells positioned under the basal lamina of muscle fibers, but they solve an important function for postnatal muscle regeneration[48]. MD-MSCs have been isolated from canine muscle and they presented all the characteristics of stem cells[49].

Circulating MSCs

Several studies have identified low concentrations of multipotent MSCs in the blood samples of laboratory mammals and from humans[50]. It is not known whether these circulating mesenchymal stem cells (C-MSCs) are derived from cells mobilized from bone marrow or other sites, or represent a small population of dedicated intravascular MSCs. The clinical applications C-MSCs, respect to the other sources, presents some problems. First the number of MSCs present in the peripheral blood is very low. In fact the number of MSCs within the bone marrow stroma is low and is likely to be even lower in the peripheral blood[51]. Than none efficient technique to separate and concentrate circulating MSCs from whole blood has developed until now. Ahern et al. assessed the utility of apheresis to concentrate MSCs within the mononuclear cell fraction of peripheral blood, but they found that the apheresis process removed or inactivated the MSCs[52].

Umbilical Cord Blood

MSCs can be obtained also from the umbilical cord tissue. The umbilical cord MSCs (UC-MSCs) have more primitive properties than adult MSCs, which might make them a useful source of MSCs for clinical applications. They are easily obtained after the birth of the newborn and they resolve the ethical and political issues related to the use of embryonic stem cell. MSCs have been isolated from four different compartments of the umbilical cord, from Wharton's jelly, from tissue surrounding the umbilical vessels, from umbilical cord blood, and from the subendothelium of umbilical vein. They can be induced to form adipose tis-

sue, bone, cartilage, skeletal muscle cells, cardiomyocyte-like cells, and neural cells. Although few articles are published in the literature, several differences between fetal MSCs and adult MSCs can be detected. UC-MSCs appear to have greater expansion ability in vitro and faster doubling time than adult MSCs and they have a different physiology that is likely due to their naïve status[53]. There are also difference between cells obtained from different parts of the umbilical cord. In fact MSCs are found in much higher concentration in the Wharton's jelly compared to the umbilical cord blood, while umbilical cord blood is a rich of hematopoietic stem cells[54].

Proliferative capacity

Although MSCs are capable of considerable cell division, this capacity is not unlimited. After a certain number of cell divisions MSCs enter senescence, which is defined as irreversible growth arrest. This phenomenon was first described in the 1960s by Hayflick[55]. Colter et al. found that the single-cell-derived colonies of MSC can be expanded up to as many as 50 population doublings (PDs) in about 10 weeks[56]. Other authors reported that MSCs can be expanded up to 30 PDs in about18 weeks[57,58]. The absence of senescence phenomena after prolonged expansion suggests the neoplastic transformation of MSCs. The molecular mechanisms that underlie senescence are still poorly understood. Two fundamental ways have been hypothesized how this process may be governed: replicative senescence might either be the result of a purposeful program driven by genes or rather be evoked by stochastic or random, accidental events[57]. Senescence is characterized by both morphological and functional changes of MSCs. From the morphological point of view, cells present an enlarged and irregular shape. Progressive shortening of the telomeres or modified telomeric structure has been associated with replicative senescence[59]. The effect of aging has been investigated on the differentiation potential of MSC. A gradual reduced differentiation potential and a dropped in the capacity of differentiation in the late-passages has been found[60]. Than changes of surface marker expression suggests some authors that consistent changes in the global gene expression should also take part[61].

The rate and persistence of MSC proliferation appears to vary between tissue sources, age donor and culture conditions[62]. The great expansion ability of BM-MSCs has been proved by many authors[63]. In a Human study, bone marrow, synovium, periosteum, adipose tissue, and skeletal muscle were obtained during anterior cruciate ligament reconstruction surgery for ligament injury, and their expandability have been compared[64]. The authors conclude that MD-MSCs and AD-MSCs had a lower proliferation potential than the other MSCs, while SD-MSCs had a great expansion ability which is compared favorably with that of BM-MSCs. S-MSCs possessed high self-renewal capacity with limited senescence over at least 10 PDs[36]. A significant inverse correlation between the donor age, the number of progenitor cells and expansion ability has been demonstrated reported both in animal[65] and human studies[57,66]. Huibregtse et al. reported reduction in cell concentration harvesting from the iliac crest of rabbits, and a decrease in colony-forming efficiency with increasing age of animals[65]. Stenderup et al. showed that donor age affects rate of *in vitro* senescence in MSC[58]. They fund that MSCs harvested from people older than 66 showed lower proliferative ability than those harvested from donors younger than 30 years. More recently a study performed by Mareschi et al. compared the *in vitro* replicative capacity of MSCs isolated from pediatric and adult donors[67]. They reported a negative correlation between donor age and the number and the proliferative capacity of MSCs, and showed that MSCs isolated from pediatric donors have a faster growth rate than MSCs isolated from adult donors. Furthermore the culture condition may also influence the pace of senescence of MSCs. Extended expansion of MSCs in an up-scalable three dimensional culture system produce more cells than expansion of MSCs in two-dimensional plate culture, and increase potential for stem cell homing ability and osteogenic and adipogenic differentiation[68]. Colter et al. reported that single cell derived MSC clones could be expanded up to 50 population doublings in about 10 weeks if cultured by repeated passage at low density whereas cells stopped growing after 15 passages if passed at high cell density[56]. Other authors suggested that lower oxygen concentrations could enhance the maximal number of population doublings[69].

Multi-Lineage Differentiation

The ability for differentiation along several mesenchymal cell lineages is an important feature of MSCs. Although it is a fundamental property of MSCs, the differentiation of MSCs highly depends on the tissue source, and culture leads to a substantial loss of multi-potentiality *in vitro*, probably due to cellular senescence. Many study showed significant differences in the properties of MSCs depending on cell source, donor and experimental variation. In Table 1 the sources and the differentiation ability of MSCs are summarized.

BM-MSCs are capable of multi-lineage differentiation. A recent review showed that BM-MSC clones differentiate into the 3 mesenchymal lineages (osteoblastic, chondrocytic and adipocytic)[70]. Multipotent cells isolated from bone marrow are capable of supporting hematopoiesis *in vivo* upon *de novo* bone formation[71]. The osteogenic differentiation of human BD-MSCs has been improved by adding glucose in culture[68]. *In vitro* cartilage formation by BM-MSCs was first described by Johnstone et al.[72]. Recent studies indicate that BM-MSCs have a greater chondrogenesis and adipogenesis potential and compared to other MSCs[64]. Violini et al. demonstrated that horse BM-MSCs have capability to differentiate into tenocytes by in vitro exposure to BMP-12[73]. Mazzocca et al. showed that BM-MSCs treated with insulin differentiated into cells with characteristics consistent with tenocytes[74]. They showed an increased synthesis of tendon-specific type I and type III collagen, decorin, and tenascin C compared with control cells. Others authors showed that this cells also have the ability to differentiate into the vascular smooth muscle cell lineage[75]. Periosteum has been proved to contain

Table 1. Sources and differentiation ability of MSCs.

MSCs Sources	Multilineage differentiation potential	Differentiation ability	Authors
BM-MSCs	Osteoblast	+++	Charbord et al.[70]
	Chondrocyte	+++	Johnstone et al.[72]
	Adipocyte	+++	Charbord et al.[70]
	Tenocyte	++	Violini et al.[73]
	Vascular Smooth Muscle Cells	++	Delorme et al.[75]
P-MSCs	Osteoblast	+++	Yoshimura et al.[35]
	Chondrocyte	+++	Johnstone et al.[72]
S-MSCs	Osteoblast	++++	Yoshimura et al.[35]
	Chondrocyte	++++	Yoshimura et al.[35]
	Adipocyte	++++	Sakaguchi et al.[64]
	Skeletal Muscle cells	++	De Bari et al.[77]
AD-MSCs	Osteoblast	+	Sakaguchi et al.[64]
	Chondrocyte	++	Danisovic et al.[79]
	Adipocyte	++++	Sakaguchi et al.[65]
TD-MSCs	Osteoblast	++++	Rui et al.[84]
	Chondrocyte	++++	Tan et al.[83]
	Tenocyte	++++	Stewart et al.[82]
MD-MSCs	Skeletal Muscle Cells	+++	Relaix et al.[48]
C-MSCs	Osteoblast	++	
	Adipocyte	++	Zvaifler et al.[93]
	Fibroblast	++	
UC-MSCs	Osteoblast	++++	
	Chondrocyte	++++	
	Adipocyte	+++	
	Skeletal Muscle Cells	+++	Conconi et al.[85]
	Endothelian Cells	+++	Wu et al.[86]
	Cardiomyocytes-like Cells	+++	
	Neuron	++++	

cells with chondro-osteogenic potential[33]. Yoshimura et al. found that rat P-MSCs showed high osteogenic differentiation potential[35]. The osteogenic potential of P-MSCs is further supported by Perka et al., who used P-MSCs seeded into polyglycolid-polylactid acid scaffolds to treat ulnar defects in New Zealand white rabbits[76]. Than P-MSC showed also chondrogenic differentiation *in vitro*. Johnstone et al. successfully used P-MSCs to repair an experimental cartilage defect[72]. S-MSCs have a great osteogenetic and adipose potential. In a comparative study between human MSCs derived from different mesenchymal tissue, Sakaguchi et al. demonstrated that cells from synovial fluid aspirates and from synovial membrane showed the greatest chondrogenesis potential[64]. De Bari et al. reported that S-MSCs had myogenic potential both *in vitro* and *in vivo*[77], suggesting the high multipotentiality of synovium-derived MSCs. S-MSCs have been considered by some authors an excellent source of MSCs because they are easy to obtain arthroscopically, and they show an high proliferative capacity and differentiation ability. AD-MSCs are capable of multi-lineage differentiation, both *in vitro* and *in vivo*[78]. Compare to BM-MSCs and other MSC populations, AD-MSCs are biosynthetically less capable of generating osseous tissues, but they are prone to differentiate easily into adipocytes[64]. AD-MSCs are able to differentiate into chondrocytes, even if their chondrogenic potential of AD-MSCs was slightly decreased in comparison with bone marrow-derived MSCs[79]. These MSCs in fact shows an altered responsiveness to transforming growth factor-β (TGF- β) and BMP ligand, and the supplementation of BMP-6 significantly increases chondrogenic differentiation AD-MSCs. An in vitro study showed that AD-MSCs underwent chondrogenic differentiation in a scaffold derived from native cartilage and with the addiction of growth factors[80]. Further, there is also evidence that the regenerative capacities of AD MSCs vary with location. In fact AD MSCs from the infrapatellar fat pad appear to be specifically preprogrammed for chondrogenic differentiation[81].

TD-MSCs with high multi-lineage differentiation potential have been isolated. Many study compared the differentiation ability of TD-MSCs to BM-MSCs. Animal studies indicate that TD MSCs colonize tendon matrix explants with greater efficiency than BM-MSCs and synthesize greater quantities of ECM proteins after colonization[82]. Tan et al. compared clonogenicity, proliferative capacity, and multilineage differentiation potential of rat TD-MSCs and BM-MSCs *in vitro*[83]. They found that TD-MSCs showed higher clonogenicity and proliferative capacity, and had greater tenogenic, osteogenic, chondrogenic, and adi-

pogenic markers and differentiation potential than BM-MSCs. The authors concluded that TD-MSCs might be a better cell source than BM-MSCs for musculoskeletal tissue regeneration. A recent study by Rui et al. showed that TD-MSCs exhibited higher osteogenic differentiation compared with BM-MSCs, and they concluded that TD-MSCs could be an attractive source for tendon-bone junction repair compared with BMSCs[84]. MSCs isolated from Wharton's jelly have been induced to form bone, cartilage, adipose cells and skeletal muscle cells. In an animal study, Wharton's jelly MSCs have been injected into a rat muscle damage, and they showed a differentiation into skeletal muscle lineage[85]. It has been also shown that human Wharton's jelly MSCs can be differentiated successfully into endothelial cells after the addition of vascular endothelial growth factor (VEGF)[86].

Discussion and conclusion

MSCs have been originally described more than 40 years ago by Friedenstein et al[9]. After their disclosure in bone marrow, they have been recognized in many adult tissues of the human body, where they play and active role in the homeostasis of tissues and organs, and they become the subject of great interest in the biomedical research community.

Adult MSCs are organized in particular structures called niches which provide support to the cell population, the soluble and cell contact-mediated signals required to maintaining stem cell functions, and control the proliferation, differentiation and self-renewal of stem cells. The exact locations of SCN are poorly understood, but there is evidence suggesting a close relationship with perycites[20].

The self-renewal capacity of MSCs is remarkable but MSCs can be passaged in vitro for a limited number of times before they become senescent and stop proliferation. Many studies reported that BM-MSCs and SD-MSCs have a great expansion ability, while proliferation ability was lost at passage 7 in adipose tissue-derived cells and at passage 4 in muscle derived cells[63,64].

The proliferation ability of MSCs depends from many factors such as tissue sources, age donor and culture conditions. Different stromal cell compartment contain a different number of MSCs and differences in growth rate may also reflect culture heterogeneity with variable proportion of self-renewing versus lineage-committed cells[87]. There is evidence that suggest a tendency for higher cumulative population doublings in MSC form younger donors. Cell density of cultures and the spatial conformation culture system seems also to be very important for the proliferation of cells[88].

Although MSCs are multipotent cells buy definition they do not differentiate all into the same cellular lineages. It has been proved that BM-MSC undergo osteogenic differentiation more efficiently when compared to other MSCs. S-MSCs have a great osteogenetic and adipose potential and these cells attracted the interest of many researchers because they showed higher chondrogenic differentiation ability than other common MSCs sources, suggesting that this MSC source is particularly relevant for cartilage regeneration and repair[35,64]. This differentiation variability is probably do to the presence of cells which are predisposed to undergoing preferably a distinct differentiation pathway. Another explanation could be the concern that MSCs from different sources are contaminated to a variable degree with fibroblasts, smooth muscle cells, osteoblasts, or other differentiated mesenchymal cells. It is also now clear that the microenviroment in which MSCs are transplanted (ie, growth factors and local cellular interactions) plays a pivotal role in determining both MSC biology and clinical improvement.

Traditionally it is believed that the therapeutic effects of MSCs derived from their ability to differentiate into the appropriate tissue type and directly stimulate the regeneration of the damage tissue. In other words the expectation was that the implanted MSCs would colonized the injury tissue, differentiate into the appropriate cells and repair the lesion. Although the *in vitro* differentiation into different mesenchymal cells lineage is a fundamental feature of MSCs, the *in vitro* differentiation capacity does not reflect their mechanism of action *in vivo*. Than few studies demonstrated the long-term persistence *in situ* of MSC-derived differentiated cells. Therefore other mechanism should be involved in tissue repair. Paracrine effects by a large number of biologically relevant molecules and cytokines produced by MSCs have been advocated to explain the functional benefits achieved in animal models and treated patients[23], and the critical importance of MSC paracrine activities is now being recognized. MSCs secrete many different cytokines, growth factors and chemokines that can influence the healing of the tissue. Other authors showed that MSCs have immunomodulatory properties and produce mediator of inflammation and cell-adhesion molecules which allow stem cells to survive and migrate to the damaged area[89]. It has been shown in cardiac[90,91] and neurologic models[92] that MSCs secrete factors that promote angiogenesis, protect compromised host cells form apoptosis, inhibit inflammation and reduce scar formation, and finally recruit and stimulate resident stem cells. Although the effect of MSCs might not result in a real regeneration of tissues, there is evidence that they reduce scar formation and improve the quality and the functionality of tissue repair. These complex paracrine mechanisms have attracted a great deal of interest in recent years, but are still not fully understood.

In conclusion significant differences in the properties of MSCs depending on cell source, beyond donor and experimental variation. These results provide important information for selecting the optimal mesenchymal tissue as a source of cells, to enhance clinical utility. The choice of cell source should be based on the aim of clinical application.

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