

# Stem Cells: Insights into Niche, Classification, Identification, Characterization, Mechanisms of Regeneration by Using Stem Cells, and Applications in Joint Disease Remedy

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## Abstract

**Background:** Stem cell therapy has attracted much interest in the 21<sup>st</sup> century, not only because of the controversy surrounding the ethics involving pluripotent stem cells, but their potential for clinical use.

**Objectives:** The present review highlights the stem cells niche, types, identification, and characterization, mechanisms of regeneration by using stem cells, and applications in joint disease remedy. Stem cells could be well differentiated cells with the potential to display different cell types depending on the host niche. Niche is defined as the cellular microenvironment providing support and stimuli to control the properties of stem cells. It consists of signaling molecules, inter-cell contacts and interaction between stem cells and their extracellular matrix neighbors. Stem cells are classified according to their sources into two main types, the embryonic and non-embryonic. Embryonic stem cells are pluripotent and can differentiate into all germ layers. Non-embryonic stem cells can be sub-classified into fetal stem cells and adult stem cells. Cultured cells can be made to differentiate into exclusive lineages by providing selective media components that can be identified by histochemical staining and quantified by quantitative Real-time polymerase chain reaction. Mesenchymal stem cells (MSCs) can be identified based on the expression of specific proteins called surface antigen phenotype of mesenchymal stem cell markers. MSCs secrete a variety of interleukins, several neurotrophic factors, many cytokines, and growth factors. These secreted bioactive factors have both paracrine and autocrine effects, which are anti-fibrotic and anti-apoptotic, as well as enhance angiogenesis. Furthermore, they stimulate mitosis and differentiation of tissue-intrinsic reparative stem cells. Systemic MSC transplantation can engraft to an injured tissue and promote wound healing through differentiation, and proliferation in synergy with hematopoietic stem cells. MSCs have been shown to express a variety of chemokines and chemokine receptors and can home to sites of inflammation by migrating towards injury or inflammatory chemokines and cytokines. MSCs are proven to have immunomodulatory properties that are among the most intriguing aspects of their biology. The immunosuppressive properties of MSCs inhibit the immune response of naive and memory T cells in a mixed lymphocyte culture and induce mitogen. The systemic infusion of MSCs can be used in immunosuppressive therapy of various disorders. MSCs have become an alternative source of cells that can be drawn from several these cells have been used as treatment to repair cartilage defects at early stages sources. Using the MSCs and directing them into chondrogenic differentiation might lead to the formation of higher quality cartilage, which has a great composition of hyaline, adequate structural reorganization and therefore improved biomechanical properties.

**Conclusion:** It can be concluded that stem cells are classified according to their sources into two main types, the embryonic and non-embryonic. Embryonic stem cells are pluripotent and can differentiate into all germ layers. Non-embryonic stem cells can be sub-classified into fetal stem cells and adult stem cells. MSCs secrete bioactive factors that are anti-fibrotic and anti-apoptotic, as well as enhance angiogenesis. The systemic infusion of MSCs can be used in immunosuppressive therapy of various disorders. These cells have been used as treatment to repair cartilage defects at early stages.

**Keywords:** stem cells, niche, classification, identification, characterization, applications in joint disease remedy

## 1. Introduction

Stem cells are defined as cells that can self-renew indefinitely (dividing continuously). These divisions are asymmetric. One of the two daughter

cells retains the stem cell characteristics, while the other is destined for a limited number of future divisions and will produce a more organ-specific cells) and able to differentiate into various cells when induced appropriately [1]. These cells have many other properties bringing to

attention its application in regenerative medicine. Stem cell therapy has attracted much interest in the 21<sup>st</sup> century, not only because of the controversy surrounding the ethics involving pluripotent stem cells, but their potential for clinical use [2].

## 2. Objectives

The present review highlights the stem cells niche, types, identification, and characterization, mechanisms of regeneration by using stem cells, and applications in joint disease remedy.

## 3. Niche of Stem Cells

Current research is focused on the microenvironment (niche) of stem cells, thus bringing several ideas that provide explanations for the stem cell line and determination of fate. A niche consists of signaling molecules, inter-cell contacts and interaction between stem cells and their extracellular matrix neighbors. The three-dimensional microenvironment is thought to control gene and properties that define the stemness, i.e., its self-renewal and development to committed cells [3]. Further studies of the niche may provide us with more information and a better understanding of the control of stem cell differentiation. Stem cells could be well differentiated cells with the potential to display different cell types depending on the host niche. In addition, stem cells located in completely different niches have the potential to differentiate into different cell types of the new environment [4]. These findings showed possible niche influence and the ability to dedifferentiate into cells from other lineages [5]. This may have clinical implications, such as neural stem cells produced when muscle cells were implanted in skeletal muscle [6] and bone marrow cells into neuronal cells when they were transplanted into an environment of neurons [7, 8]. As well as the liver and pancreas which develop from the same embryological line, specific growth factors and culture techniques can ensure the trans-differentiation of liver cells to islet cells [5]. Overall, Niche is defined as the cellular microenvironment providing support and stimuli to control the properties of stem cells. Further studies of the niche may provide us with more information and a better understanding of the control of stem cell differentiation and other cells trans-differentiation [4].

## 4. Classification of Stem cells

Nowadays, there are many bases to classified stem cells [9] for example, but not limited to

**4.1 Classification of stem cells on the basis of potency:** Stem cells can be classified by the extent to which they can differentiate into different cell types [10, 11]:

- 1) **Totipotential stem cells:** The ability to differentiate into all possible cell types (from the embryo cells & extra embryo cells). Examples are the zygote formed at egg fertilization and the first few cells that result from the division of the zygote [12, 13].
- 2) **Pluripotential stem cells:** The ability to differentiate into almost all cell types. Examples include embryonic stem cells and cells that are derived from the mesoderm, endoderm, and ectoderm germ layers that are formed in the beginning stages of embryonic stem cell differentiation [12, 14].
- 3) **Multipotential stem cells:** The ability to differentiate into a closely related family of cells. Examples include hematopoietic, (adult) stem cells that can become red and white blood cell and platelets [12, 15, 16].

- 4) **Oligopotential stem cells:** The ability to differentiate into a few cells. Examples include (adult) lymphoid or myeloid stem cells [12, 17].
- 5) **Unipotential stem cells:** The ability to only produce cells of their own type, but have the property of self-renewal required to be labeled a stem cell. Examples include (adult) muscle stem cells [12, 15, 18].

## 4.2 Classification of stem cells on the basis of their sources [19]:

- 1) **Embryonic stem cells:** Embryonic stem cells are self-replicating pluripotent cells that are potentially immortal. They are derived from embryos at a developmental stage before the time of implantation would normally occur in the uterus. The embryos from which human embryonic stem cells are derived are typically four or five days old and are a hollow microscopic ball of cells called the blastocyst [20, 21].
- 2) **Non-Embryonic (Adult) stem cells:** Adult stem cells are undifferentiated multipotent cells, found throughout the body after embryonic development that multiply by cell division to replenish dying cells and regenerate damaged tissues. The primary roles of adult stem cells in a living organism are to maintain and repair the tissue in which they are found. The origin of adult stem cells in some mature tissues is still under investigation [22, 23].
- 3) **Cloning (nuclear transfer) stem cells:** Somatic cell nuclear transfer (SCNT) is a technique for cloning. The nucleus is removed from a healthy egg (ova). This egg becomes the host for a nucleus that is transplanted from another cell, such as a skin cell. Then, using electrical shock called Roslin technique [24] or chemical shock called Honolulu technique [25] to start cell division & development. The resulting embryo (zygote/fertilized ova) can be used to generate embryonic stem cells with a genetic match to the nucleus donor [26].
- 4) **Induce Pluripotent (reprogramming) stem cells:** Scientists have engineered stem cell, with properties similar to embryonic stem cells, these induced pluripotent stem cells (iPS cells) by reprogramming technology which manipulating the expression of certain genes with reprogramming factors (oct4, soxy2, klf4 and myc), 'reprogramming' somatic cells back to a pluripotent state [27, 28].

## 4.3 Classification of stem cells on the basis of transplanted stem cells sources for purpose of therapy [29]:

- 1) **Autologous stem cells:** referring to collected and transfer of stem cells from one location to another for transplanted into the same patient [30].
- 2) **Syngeneic stem cells:** referring to collected and transfer of stem cells or tissue or organ from one location of the person's identical twin to transplanted into patient [31].
- 3) **Allogeneic stem cells:** referring to collected and transfer of stem cells from one location of a donor person to transplanted into another person in same species (patient belong to same species) [32].

- 4) **Xenogeneic stem cells:** referring to collected and transfer of stem cells from one location of a donor person to transplanted into another species (patient belong to other species) [33].

## 5. Identification and Characterization of Stem Cells

### 5.1 Stemness

Stemness refers to combines the ability of stem cells to perpetuate their lineage (Self renew), to give rise to differentiated cells, and to interact with their environment to maintain a balance between quiescence, proliferation, and regeneration [34].

### 5.2 Morphology

Mesenchymal stem cells (MSCs) have been shown to adhere to cell culture flask and exhibit fibroblastic-like shape. Many studies have been demonstrated the effects of different culture protocols on the cell phenotype. The reports show little and no significant differences among the cells isolated by any protocol [35-37].

### 5.3 Cell differentiation

Differentiation is the process by which matured cells change to a specialized type. During differentiation, certain genes are turned on and become activated while others are switched off and become inactivated; a complex process tightly regulated, resulting in cell development of specific structures, which perform certain functions. Cultured cells can be made to differentiate into exclusive lineages by providing selective media components that can be identified by histochemical staining and quantified by quantitative Real-time polymerase chain reaction (RT-PCR) [38]. The standard test to confirm the mesenchymal stem cells is differentiation of the cells into other specific cells such as osteoblasts, adipocytes, chondrocytes, myocytes and neurons. MSCs have been seen to even differentiate into neuron-like cells. The process of differentiation normally will occur with the aid of influencing factors and it is considered the test for multipotency of MSCs [39].

### 5.4 Immunophenotyping

Immunophenotyping is using a flow cytometry technique to enable identification of specific cell types from complex biological samples according to the cell surface antigen expression. Mesenchymal stem cells can be identified based on the expression of specific proteins called surface antigen phenotype of mesenchymal stem cell markers. Some of these markers are present on undifferentiated MSCs and disappear during differentiation [40]. Some of these surface antigens may be expressed on the other cells but by using a profile of positive phenotypes which include Stro-1 (Stromal marker) and cluster of differentiation (CD). The specific positive phenotypes include CD105 (Endoglin) CD106 (VCAM-1), CD10 (Antibody of common acute lymphoblastic leukemia antigen: anti-CALLA), CD117 (Stem cell factor receptor), CD120a (TNF receptor Type I), CD120b (TNF receptor Type I), CD13 (Integral membrane glycoprotein), CD15 (SSEA-1, Lewis X), CD166 (ALCAM), CD271 (p57, NGFR/NTR), CD29 (Integrin  $\beta$ 1), CD44 (Pgp-1, HCAM), CD49d (Integrin  $\alpha$ 4), CD49e (Integrin  $\alpha$ 5), CD51 (Integrin  $\alpha$  V), CD71 (Transferrin Receptor), CD73 (Ecto5' nucleotidase), CD90 (Thy-1), Flk-1 (KDR, VEGF-R2, LY-73), Ly-6A/E (Sca-1), (Intermediate filament in Vimentin CO-activator), TAZ (Transcriptional cytoskeleton) and CD14 (Monocyte or macrophage antigen). On the other hand, the negative phenotypes include CD11a (Integrin  $\alpha$  chain), CD11b (Integrin  $\alpha$  M chain), CD31 (PECAMI), CD34 (Mucosalin, gp 105-120) and CD45 (Leukocyte Common Antigen, Ly-5). As can be recalled, it is possible to segregate specific type of cells based on the above-mentioned phenotypes. Many studies have used this experimental technique to identify and separate the stem cells from bone marrow cells as well as tissue derived cells [41-43].

## 6. Mechanisms of Regeneration by Using Stem Cells

### 6.1 Stemness

Stemness encompasses the capability of stem cells for self-renewal and differentiation to exhibit the potential to differentiate into various lineages of cells [44]. In other words, it refers to the capacity of stem cells to self-renew and give rise to progeny capable of differentiating into diverse cell types [45].

### 6.2 Trophic Support

MSCs secrete a variety of interleukins such as: (L-3, IL-6, IL-7, IL-8, IL-11, IL-12, IL-14, and IL-15). Also, they secrete several neurotrophic factors such as: Nerve growth factor (NGF), Brain-derived neurotrophic factor (BDNF), and Glial cell- derived neurotrophic factor (GDNF). Besides that, they secrete many cytokines and growth factors such as: Leukemia inhibitory factor (LIF), Vascular endothelial growth factor (VEGF), Hepatocyte growth factor (HGF), Stromal derived factor (SDF), Stem Cell Factor (SCF), Macrophage-colony stimulating factor (M-CSF), Basic fibroblast growth factor (bFGF), insulin-like growth factor binding protein (IGFBP), Oncostatin M (OSM), Macrophage inflammatory protein-1 $\beta$  (MIP-1 $\beta$ ), tissue inhibitor of metalloproteinase: (TIMP-1 & TIMP-2), Transforming growth factor beta: (TGF- $\beta$ 1 & TGF- $\beta$  2), Platelet-derived growth factor (PDGF), Epidermal growth factor (EGF), and Keratinocyte Growth Factor (KGF) [46, 47]. These secreted bioactive factors have both paracrine and autocrine effects, which are anti-fibrotic and anti-apoptotic, as well as enhance angiogenesis [46, 48, 49]. Furthermore, they stimulate mitosis and differentiation of tissue-intrinsic reparative stem cells. These effects known as trophic effects are distinct from direct differentiation of MSCs in tissue repair. Several studies that tested the use of MSCs in different models such as myocardial infarction (heart injury), stroke (brain), or models of regeneration of the meniscus are reviewed in the context of MSC- mediated trophic effects in tissue repair [48].

### 6.3 Anti-inflammatory

MSCs have been proposed to have anti-inflammatory properties, which in recent years have generated much interest. The anti-inflammatory properties of MSCs are achieved through decrease or inhibition of pro-inflammatory mediators like IL-1, IL-6, TNF- $\alpha$ , and IFN- $\gamma$ , as well as increase in anti-inflammatory mediators like IL-4, IL-10, IL-11 and IL-13. These effectively reduce inflammation. There are many studies that demonstrate the beneficial effects of MSC application for restoration of the stem cell niche in damaged tissues with its anti-inflammatory activity [50-53].

### 6.4 Differentiation into Tissue

MSCs are thought to be multipotent cells that mainly present in bone marrow (BM) [41, 54]. Many reports have indicated that MSCs that have the potential to differentiate into a wide range of tissues, including bone, cartilage, adipose tissue, muscle tissue and both in vivo and ex vivo [35, 55]. These adult stem cells could be induced to differentiate exclusively into the adipocytic, chondrocytic, or osteocytic lineages. Individual stem cells were identified that, when expanded to colonies, retained their multi-lineage potential. In response to experimental conditions, MSCs could also differentiate into cells of three germ layers [56, 57]. This type of cross-line age differentiation is known as trans-differentiation, which implies that adult stem cells contain multi-differentiation potentials. It has been shown in vivo that MSCs can generate epithelial cell types in skin, lung, and other tissues [58, 59]. Many studies suggest that systemic MSC transplantation can engraft to an injured tissue and promote wound healing through differentiation, proliferation, and in synergy with hematopoietic stem cells [60]. A recent report suggested that the transplantation of MSCs could rebuild damaged tissues [61].

## 6.5 Homing to Injury Site

The homing to injury or inflammation site is a unique property for MSCs that has sparked particular interest for clinical applications aiming at using noninvasive systemic cell administration to treat injury or inflammation. MSCs have been shown to express a variety of chemokines and chemokine receptors and can home to sites of inflammation by migrating towards injury or inflammatory chemokines and cytokines [62, 63]. According to studies, the heterogeneity in the expression of surface receptors was observed, which is probably due to differences in growing conditions and limits of detection techniques, Homing of cultured MSCs, however, is inefficient compared with leukocytes. This inefficiency has been attributed to a lack of cell adhesion and chemokine receptors, but also to the size of MSCs that promote cellular passive trapping and reduce traffic [64]. Moreover, the evidence that host MSCs can mobilize in response to inflammation or injuries can be seen in the ability of systemically infused MSCs to be observed within the bone marrow or damaged tissues [65].

## 6.6 Immune System Modulation

MSCs are proven to have immunomodulatory properties that are among the most intriguing aspects of their biology [66]. As is widely known, the expression of major histocompatibility complex (MHC) molecules on all cells of the body allows the immune system to distinguish self from non-self [35, 41, 67-69]. Therefore, non-self-cells or tissues are rejected by the immune system through direct or indirect pathways. Cells expressing MHC molecules stimulate T cells directly only if they have the co-stimulatory molecules, or by activating T cells through an indirect pathway where their MHC antigens are presented by professional antigen presenting cells (APC). At the end, they will be rejected by immune defenses of the body through stimulating an immune response. However, in contrast, undifferentiated MSCs fail to induce immune responses, even in the presence of APCs or after provision of co-stimulatory signals. This is because the immunosuppressive properties of MSCs inhibit the immune response of naive and memory T cells in a mixed lymphocyte culture (MLC) and induce mitogen [66, 70-78]. Previous studies showed that MSCs inhibit the division of stimulated T cells via preventing their entry into the S phase of the cell cycle and through mediating an irreversible G0/G1 phase arrest [79]. There are several contrasting studies that showed that MSCs not only appear to down-regulate the immune-reactivity of a variety of effector cells, but also escape immune rejection through escape lysis by CD8+ cytotoxic lymphocytes [66, 71, 80]. Indeed, there are reports suggesting that the systemic infusion of MSCs can be used in immunosuppressive therapy of various disorders [65].

## 7. Applications of Mesenchymal Stem Cells in Joint Disease Remedy

Over the last decade, MSCs have become an alternative source of cells that can be drawn from several sources. These cells have been used as treatment to repair cartilage defects at early stages. The MSC-based cartilage repair has been attempted in animal models using various carrier matrices and synthetic polymers such as poly alpha-hydroxy esters (P alpha-hydroxy E), poly lactic acid (PLA), poly glycolic acid (PGA) and their copolymer poly lactic-co-glycolic acid (PLGA) [81-83]. Delivering stem cells by using techniques like micro-fracture are performed by penetration of the subchondral bone. When the tourniquet is released, the possible recruitment of stem cells from the underlying bone marrow leads to the formation of a super clot [84, 85]. Report showed 11% of biopsies being predominantly hyaline cartilage and 17% of a mixture of fibrocartilage and hyaline [86]. However, this technique is not sufficient for large lesions and the results have not always been consistent [87]. Using the MSCs and directing them into chondrogenic differentiation might lead to the formation of higher quality cartilage, which has a great composition of hyaline, adequate structural reorganization and therefore improved

biomechanical properties. Wakitani *et al.* [88] successfully used MSCs gel type I collagen to repair chondral o caprine model and subsequently translated it into clinical practice. Autologous bone marrow derived MSC transplantation has been used to repair full-thickness defects of articular cartilage in the knee caps of two patients [89]. Wakitani *et al.* [90] also reported on twelve patients suffering from knee OA (Osteoarthritis) who received MSCs injected into cartilage defects of the medial femoral condyle at the time of high tibial osteotomy. These were then covered by periosteum. Although the clinical improvement was not significantly different, MSCs treated patients had better arthroscopic and histological grading scores. In rabbit model, MSCs have been cultured then transplanted into defective cartilages, which have been created in the left medial femoral condyle. The repair tissue approximated intact cartilage and was Superior to osteo-chondral auto-grafts and repair by innate mechanisms [91]. These findings suggest that repair of cartilage defects in the rabbit can be enhanced by the implantation of cultured MSCs [92]. In the near future, a new approach for OA could be the use of MSCs to inhibit disease progression. In OA, it has been demonstrated that stem cells are depleted and/or have reduced proliferation and differentiation capacity [93]. Thus, systemic or local administration of stem cells could increase the population of regenerative cells and possibly induce repair or inhibit the progression of the disease. Local delivery of adult MSCs suspended in sodium hyaluronan into injured joints of a caprine OA model showed that the MSCs stimulated regeneration off meniscal tissue. Degenerated cartilage, osteophytic remodeling, and subchondral sclerosis were reduced in the cell-treated joints compared to controls [94]. These experiments implicate that MSCs hold exciting promise for regenerating meniscus and preventing OA. A group of researchers from Singapore have used this procedure clinically, with promising results [8]. In a sheep model of OA treated with autologous BMSCs cultured in chondrogenic medium, there was Clear evidence of articular cartilage regeneration [95].

## 8. Conclusion

It can be concluded that stem cells could be well differentiated cells with the potential to display different cell types depending on the host niche. Stem cells are classified according to their sources into two main types, the embryonic and non-embryonic. Embryonic stem cells are pluripotent and can differentiate into all germ layers. Non-embryonic stem cells can be sub-classified into fetal stem cells and adult stem cells. MSCs secrete bioactive factors that are anti-fibrotic and anti-apoptotic, as well as enhance angiogenesis. The systemic infusion of MSCs can be used in immunosuppressive therapy of various disorders. These cells have been used as treatment to repair cartilage defects at early stages.

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