Chapter 10

# Stem Cell-Based Therapies for Osteoarthritis: From Pre-Clinical to Clinical Applications

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**Abstract**

Although many surgical and pharmaceutical interventions are currently available for treating osteoarthritis (OA), restoration of normal cartilage function remains inefficient. In fact, because of the absence of vasculature within the articular cartilage (AC), the self-potential for regeneration is very poor. Recently, researchers and clinicians have been focusing on alternative methods for cartilage preservation and repair. It has been shown that AC contains a population of stem cells or progenitor cells, similar to those found in many other adult tissues that are thought to be involved in the maintenance of tissue homeostasis. In the present chapter, we review the current status of stem cells potential in the treatment of early OA and discuss the possible origin of these cells and the role they might have in cartilage repair. We also review the recent progress in the field of chondroprogenitors in cartilage.

**Keywords:** osteoarthritis, stem cells, chondrocytes, bone marrow, cartilage, progenitors

## 1. Introduction

Articular hyaline cartilage is a tissue whose mechanical properties allow joint movements with a low coefficient of friction and a high absorption of constraints. Degradation of hyaline cartilage, posttraumatic or degenerative, causes functional impairment of the joint, pain, and decreased quality of life. These conditions generally lead to the formation of the most common degenerative orthopedic disease such as osteoarthritis (OA). The OA involves gradual deterioration of cartilage and subchondral bone accompanied by chronic low-grade inflammation of the synovium. These pathological changes lead to destruction of the whole joint organ. Even it is agreed that OA affects entire joint articular cartilage, breakdown remains

the principal characteristic of OA. Unfortunately, since cartilage is a neural tissue, the OA is generally diagnosed in more advanced stages when the majority of cartilage is already degraded. Thus, restoration of normal cartilage function in OA remains challenged despite many surgical and pharmaceutical interventions being currently available [1]. Several treatment options are available to support the knee articular cartilage injury. Painkillers and anti-inflammatory drugs are first prescribed in association with loss of weight or physiotherapy. When these options are not sufficient, intra-articular injections of corticosteroids, hyaluronic acid, or platelet-rich plasma (PRP) [2] represent non-surgical alternatives. Despite drugs used clinically to reduce pain and maintain joint movement, in many cases, surgical substitution with artificial implants is inevitable. A number of surgical treatment strategies are currently available for articular cartilage defect repair. The cartilage repair aims to restore the histological structure of the whole osteochondral structure so that it can restore the original mechanical and functional properties [3, 4]. Restorative procedures include abrasion chondroplasty, subchondral drilling, microfracture, and mosaicplasty arthroscopy. The procedure chosen will depend on the size of the lesion, its depth, the age of the patient, the nature of the symptoms, and the regulations in force in each country. Surgical possibilities routinely used to repair articular cartilage can be separated into three major groups; those conducting subchondral stimulation, reconstruction techniques which transplant mature cartilage, and finally cellular transplants which aim to create a favorable environment for cartilage healing [5]. Recently, both cartilage and bone marrow stromal cells (BMSCs), also known as bone marrow-derived "mesenchymal stem cells" and "mesenchymal stromal cells," with inherent chondrogenic differentiation potential appeared to present a potential for therapeutic use in cartilage regeneration. BMSCs are easy to isolate and expand in culture in an undifferentiated state for therapeutic use. Owing to their potential to modulate local microenvironment via anti-inflammatory and immunosuppressive functions, BMSCs have an additional advantage for allogeneic application.

## 2. Mesenchymal stem cells (MSC) in cartilage repair

### 2.1. Stem cells

Stem cells are the foundation cells for every organ, tissue, and cell in the body [6, 7]. They may be thought of as a blank microchip that can ultimately be programmed to perform any number of specialized tasks. This role is justified based on two key properties: (1) the ability to self-renew, dividing in a way to make copies of themselves and (2) the ability to differentiate, giving rise to the mature types of the cells that make up our organs and tissues [6, 7].

The stem cells can be generally divided into three groups: totipotent, pluripotent, and multipotent stem cells. Totipotent stem cells originate from the fertilized egg and give rise to the whole organism. These cells, through the process of proliferation and differentiation, become *pluripotent embryonic stem cells* that form three germ layers: ectoderm, mesoderm, and endoderm [8]. These three germ layers are the embryonic source of all cells of the body (adult organism consists of 200 different cells types). During embryonic development, stem cells

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become specialized, which makes them terminally differentiated with specific function and they are unable to be renewed [9, 10].

Yet, even in the specialized tissue, we can find a pool of cells referred to as "*adult*" or "*somatic*" *stem cells*, which replace injured and dead cells of certain tissue (blood, skin, liver, brain, etc.) [9, 10]. These cells are termed as multipotent as their potential is limited to produce some or all of the mature cell types within a particular tissue where they reside (tissue-specific stem cells) [9–11]. Yet, some of the adult stem cells are less differentiated than the others and can give rise to the several tissue types belonging to the same germ layer. These include hematopoietic stem cells as a source of both red and white blood cells and mesenchymal stem cells (MSC), which may be a potential source of the several mesodermal tissues [10–12].

Based on this, the focus of scientific research became the potential use of adult stem cells for tissue repair but also to generate new tissue under *in vitro* conditions for biological transplantation. The ability to obtain cells with proliferation and differentiation potential without sacrificing potential human life is a highly popular and hopeful tool for modern day researchers.

### 2.2. Phenotype and differentiation potential of MSC

The MSC cells are multipotent—self-renewing cells found in adult tissues, which can be *in vitro* differentiated and form adipocytes, fibroblast, osteocyte, and chondrocytes lineage [13, 14]. These cells had been primarily isolated in the early 1970s when Friedenstein et al. discovered that a specific number of fibroblastic cells isolated from bone marrow have the capacity to form colonies *in vitro* and under appropriate stimulating environmental conditions, small aggregates of bone, and cartilage [15, 16]. Over the years, it has become clear that MSC are not an exclusive feature of the bone marrow [17–19], but can also be isolated from other organs and tissues such as fat [20–22], skeletal muscles [23, 24], and synovium [25].

The isolation and characterization of MSC among the other cell types are based on their properties to adhere and grow on plastic, phenotype characteristics, and differentiation potential [26]. Over the last decades of research, significant effort has been made to establish phenotypic characterization of MSC. Despite all the effort, to date, there is no specific marker or combination of markers which will allow isolation of the homogeneous MSCs pool [27].

Nevertheless, it has been generally agreed that MSCs express specific surface antigens which involve: CD105 (endoglin—type I glycoprotein), CD73 (ecto-5′-nucleotidase), CD44 (HCAM—homing cell adhesion molecule), CD90 (cluster of differentiation 90 [Thy 1]), CD71 (cluster of differentiation 71) and Stro-1 as well as the adhesion molecules CD106 (vascular cell adhesion molecule [VCAM]-1), CD166 (activated leucocyte cell adhesion molecule [ALCAM]), intercellular adhesion molecule (ICAM)-1, neurogenic locus notch homolog protein 3 (NOTCH3), integrin alpha-11 (ITGA11), and CD29 [17, 26, 28–31]. However, they do not express the hematopoietic-specific markers CD79a, CD45, CD11, CD34, CD19, or CD14 and co-stimulatory molecules CD80, CD40, CD86, or the adhesion molecules CD31 (platelet/endothelial cell adhesion molecule [PECAM]-1), CD18 (leucocyte function-associated antigen-1 [LFA-1]), or CD56 (neuronal cell adhesion molecule-1) [26].

Hence, to confirm the presence of MSC and extract them among the other cell types, researchers use the different combinations of these markers.

Another way to identify supposed MSC population is by their differentiation capacity to bone, cartilage, and adipocyte tissue. Herein, MSC has to be cultured in the specific medium composed of the substituent known to stimulate and control these differentiations *in vivo*. These are mostly specific growth factors such as BMPs for osteocytes [32–34] and TGFs, BMPs, and FGFs for chondrocytes [35–38]. To optimize MSC differentiation, cells need to be put under the *in vivo*-like environment. Then MSC aimed to become osteocytes or chondrocytes will be cultured in 3D pellets [32–38] while differentiation to adipocytes will be performed in monolayer.

The fact that MSCs can be differentiated into several different cells types *in vitro* clearly makes MSC and MSC-like cells (progenitors) a promising cell source for tissue repair and regeneration. Moreover, MSCs are known to secrete a large number of growth factors (GFs), cytokines, and chemokines for mediating various functions including anti-inflammatory, anti-apoptotic, anti-fibrotic, angiogenic, mitogenic, and wound-healing through paracrine activity [27, 39, 40]. All these features are highly desired and support their candidature for therapeutic purpose.

### 2.3. MSC potential for cartilage repair

Current research into cartilage tissue engineering focuses on the use of adult MSCs as an alternative to autologous chondrocytes [41]. The advantage of MSC over chondrocytes is their ability to self-renew without loss of differentiation capacity. Likewise, MSCs may retain immunomodulatory activity in recipient tissue due to lack of human leucocyte antigen (HLA) class II expression [42, 43]. These properties make MSC promising for a diversity of clinical applications including *in vitro* development of the cartilage tissue and its transplantation into the joint defect.

To date, research has demonstrated that bone marrow, adipose, and synovial-derived MSCs are mostly relevant as MSC sources for cartilage repair [8].

#### *2.3.1. Bone marrow-derived MSC in cartilage repair*

##### *2.3.1.1. In vitro studies*

Since the Friedenstein study in the early 1970s to date, numerous reports confirmed the multipotency of MSC isolated from bone marrow (BMSC) [16, 44–48]. Although, they represent a minor fraction of the total nucleated cell population (1 MSC/5 $\times 10^3$ mononuclear cells), they could significantly increase their number through *in vitro* expansion [44, 49–51]. Sakaguchi et al. confirmed that BMSC potential to divide persists even after 10 *in vitro* passages [49]. This is a significant achievement as the high cells number is required to fill the cartilage defects. Note that, as opposed to chondrocytes, MSC retain chondrogenetic potential even after long monolayer expansion [46, 52]. When a sufficient cell number is reached, cells are placed in the differentiation-specific medium. The quality of BMSC-derived chondrocytes and the formed cartilage tissue is then estimated [46, 52].

The obtained tissue exhibited high positive staining for cartilage ECM components: glycosaminoglycans, collagen II, and lubricin [45–48]. Note that, however, positive staining was also obtained for the collagen X, which is well-known as a marker of hypertrophic chondrocytes and produces calcified cartilage [45].

In a comparative study of MSC isolated from versus tissues, BMSC showed greater chondrogenetic potential over the fetal lung MSC or placenta MSC [45, 46]. Nevertheless, BMSC-derived cartilage pellets exhibited significantly higher expression of collagen X than those derived from the two other sources [46]. Moreover, the capacity of BMSC to differentiate into chondrocytes was reduced by passaging of the cells [46]. This has been recently confirmed on the animal model [53]. The results showed that proliferative, differentiation, and metabolism profile of BMSC significantly decreases by age increase [53]. In the other comparative study from 2016, authors did not observe any preference in *in vitro* chondrogenesis among MSC derived from bone marrow, adipose tissue, and umbilical cord [54].

*2.3.1.2. Pre-clinical studies in animal model*

To investigate cartilage repair by MSC *in vivo*, most of these pre-clinical studies have been performed in rabbit models treated with MSCs combined with appropriated scaffold materials and environmental factors [55–57]. The histological outcomes confirmed formation of the hyaline cartilage-like tissue expressing collagen type II [55, 56, 58, 59] as well as collagen type I [55, 56, 58]. Note that, the latter is a marker of fibrocartilaginous tissue. However, compared to the traditional ACI, the MSC therapy of cartilage defect resulted in regenerated hyaline cartilage-like tissue and restored a smooth cartilage surface, while the chondrocyte-seeded constructs produced mostly fibrocartilage-like tissue with a discontinuous superficial cartilage contour [60].

This finding has been further tested in large animal models. The study on swine model confirmed the beneficial effect of MSC over the ACI [61, 62]. Moreover, ovine MSCs have been isolated from bone marrow, expanded, characterized, and injected with transforming growth factor (TGF) b3 in a fibrin clot [63]. Two months after implantation, histological analysis revealed chondrocyte-like cells surrounded by a hyaline-like cartilaginous matrix that was integrated to host cartilage [63, 64]. Similar findings had been observed in the *Cynomolgus macaque* OA-model. The 2 months postoperative evaluation confirmed regular surface integration with neighboring native cartilage, and reconstruction of trabecular subchondral bone in the BMSC filled defects [65].

Taken together, animal studies indicated that MSC may be a promising approach for cartilage repair. However, animal models could not completely mimic OA pathogenesis in humans. In human primary OA, disease generally develops as a result of disturbed cell homeostasis, which leads to misbalance in synthesis and degradation of cartilage and subchondral bone matrix. These pathological changes are widely spread in OA cartilage at advanced stages when OA is generally diagnosed. Unfortunately, at this stage of the disease, there is only a slight amount of normal cartilage left. In contrast, experimental OA induced by mechanical trauma represents cartilage lesion surrounded by healthy tissue. The implanted cartilage

construct may interact differently with healthy tissue than with a damaged surrounding tissue. Thus, repair techniques performed on the OA experimental model may not be sufficient to predict outcomes of this technique in humans.

*2.3.1.3. Clinical studies*

The clinical reports of cartilage defects treated by bone marrow MSC did show promising results. The symptoms improvement was mostly expressed through the pain relief and progress in physical mobility [66, 67]. However, quality of regenerated tissue evaluated by MRI and histology vary with respect to the time elapsed since surgery [68–72].

Autologous BMSC embedded in a collagen gel were transplanted into articular cartilage defects and covered with autologous periosteum [68–71]. Six weeks follow-up revealed better arthroscopic and histological scores in the cell-transplanted compared to the cell-free control group [68]. The repaired defects were filled with hyaline-like cartilage tissue confirmed by positive Safranin O staining [71]. Moreover, pain and walking abilities have been improved significantly [69]. Nevertheless, 1-year follow-up analysis detected formation of fibrocartilaginous tissue instead of hyaline cartilage tissue in the repaired lesions [57, 70]. This has been further confirmed by a 5-year follow-up study, where in the first 6 months after surgery pain, walking, stairs climbing, patella crepitus, and flection contractures were all improved. However, after the 6 months, they started gradually to deteriorate [73].

In the comparative study of autologous BMSC and autologous chondrocyte implantation (ACI), it has been shown that older patients showed significantly lower improvement compared to the younger in the ACI group. Nevertheless, age did not make any difference for the patients treated by autologous BMSC [74]. This finding may indicate that cellular senescence downgraded chondrocytes molecular pathways that are involved in regulation of cell activity, which affected their ability to form functional cartilage tissue [75].

Yet, these results did not confirm significant improvement between ACI and MSC therapies [74, 76]. Moreover, the same as for ACI, being overweight and large lesion size are significant predictors of poor clinical and arthroscopic outcomes after MSC therapy [77, 78].

*2.3.2. Adipose tissue-derived MSC in cartilage repair*

*2.3.2.1. In vitro studies*

Even the BMSC were commonly investigated and used in treatment of cartilage defects, the harvesting of bone marrow is painful and followed by risk of wound infection. Moreover, the BMSC number in bone marrow is very low which requires extended *in vitro* expansion and may cause loss of cells regenerative potential [8]. Given that, the adipose tissue became a novel source of adult stem cells due to easier harvesting procedure from the wasted tissue after the liposuction treatment.

Moreover, the proportion of the AMSCs in adipose tissue is several times higher than of MSCs in bone marrow. Results have confirmed their potential for chondrogenesis, osteogenesis, adipogenesis, myogenesis, and some aspects of neurogenesis [79, 80].

Chondrogenesis of human AMSCs has shown significantly higher expression of chondrogenic markers after 1 week under appropriate conditions [81]. However, a significantly elevated expression of collagen type X was observed after 3 weeks of chondrogenic induction [41, 81]. The tendency of the AMSCs to differentiate in hypertrophic chondrocytes had been further confirmed by the other studies. These studies showed positive staining of the collagen I and X in newly formed tissue even after the stimulation with chondrogenic growth factors [82–84]. This indicates that the regulation of cellular activity by growth factors, scaffolds, and even gene therapy merits further investigation.

Compared to the BMSC, cartilage obtained from the adipose-derived MSC did not express significantly higher levels of hypertrophic markers: collagen X and MMP-13 [41]. The recent study from 2016 has emphasized that MSCs from bone marrow, adipose tissue, and umbilical cord share similar biological properties and that their chondrogenic potential does not vary [54].

Based on the *in vitro* studies, it is not clear if the AMSCs are the best choice for the cartilage repair. Even though their chondrogenic potential had been clearly justified, their predisposition to form hypertrophic and fibrous tissue should not be neglected.

#### *2.3.2.2. Pre-clinical studies*

*In vitro* studies on animal models demonstrated that adipocyte-derived MSCs were able to restore symptoms of OA-induced cartilage. The improvement had been observed macroscopically where cartilage lesion had been covered by repaired tissue and the surface was relatively smooth. The histological assessment revealed only a few fissures, few cracks, and an almost continuous superficial zone [85]. Another study showed that injected AMSC migrated to the synovial membrane and meniscus, however not in cartilage. Nevertheless, reduced OA progression had been observed [86]. The benefits obtained by AMSCs treatment could be due to a trophic mechanism of action by the release of growth factors and cytokines [86]. Taken together, these few pre-clinical studies are in favor of AMSCs-based cartilage repair.

### *2.3.3. Synovium-derived MSC in cartilage repair*

#### *2.3.3.1. In vitro studies*

Another source of adult stem cells is synovium (synovium-derived stem cells (SDSC)). The comparative study of stem cells from five different sources (bone marrow, synovium, skeletal muscle, periosteum, and adipose tissue) confirmed that SDSC have proliferation and differentiation capacity similar to BMSC [49]. Moreover, the pellets derived from synovium were heavier than those from other tissues, because of their higher secretion of cartilage matrix [87–89]. This makes synovium-derived MSC potentially superior to bone marrow-derived MSC.

#### *2.3.3.2. In vivo studies*

The transplantation of the implant composed of MSC from different sources into the full-thickness cartilage defects of rabbits showed that synovium and bone marrow MSCs had greater *in vivo* chondrogenic potential than adipose and muscle MSCs [89]. Moreover, synovium MSCs

had the advantage of the highest proliferation potential [90]. This study also noted that cartilage repair by synovium-derived MSC requires injection of a high number of these cells into the defect [90]. By contrast, another group reported that the aggregates with a high density of synovium-derived MSCs failed to regenerate cartilage due to cell death and nutrient deprivation into the core of the aggregates. Though, aggregates with relatively low-cartilage density successfully regenerated damaged tissue [91]. When compared to the healthy cartilage, tissue regenerated by constructs composed of the synovium-derived MSCs showed more fibrocartilage-like characteristics mostly in the superficial zone of the repair tissue [92].

This finding needs to be further confirmed by more *in vitro* and *in vivo* studies before introducing these cell types in clinical trials.

### 2.4. Regulation of the MSC chondrogenesis

It has been proposed that *in vitro* chondrogenic differentiation of MSCs mimics *in vivo* embryonic cartilage development. Hence, *in vitr*o MSC expansion phase may correspond to the initial proliferation of mesenchymal cells before condensation. Switching over to the high-density MSC pellet cultures mimics the *in vivo* MSCs condensation steps and early stage chondrogenesis during embryonic development [93]. It has been shown that mechanical forces employed on the cell mass during chondrogenesis may promote the cells differentiation and secretion of the matrix-specific molecule. These biomechanical applications mimic the natural articular cartilage *in vivo* conditions [94, 95].

#### *2.4.1. MSC isolation and in vitro culturing conditions*

The MSC to be subjected to the cartilage formation first need to be isolated from their native tissue. To date, bone marrow, fat, and synovium tissue presents the most suitable sources of adult stem cells [8] with each tissue necessitating a specific isolation procedure [6]. BMSC are aspired by syringe from bone shafts, while ADMS are released and collected due to enzymatic digestion of the tissue [6]. Subsequently, these cells are *in vitro* expanded in order to obtain sufficient cell numbers for the following experimental procedures [6]. After the proliferation step, expanded cells need to be cultured under the 3D conditions in order to stimulate chondrogenesis. Thus, they are cultivated in micromass (pellets) or in scaffold materials, such as polymers, alginate beads, collagen sponges or hydrogels, and microspheres for 2–3 weeks in special chondrogenic medium enriched by growth factors [96]. Growth factors enhance expression of chondrocyte markers and support formation of cartilage tissue [35, 44, 97–99]. Moreover, hypoxic conditions seem to be the logical choice to stimulate chondrogenesis as it is present in *in vivo* articular tissue [100–104]. It has been shown that hypoxia induces expression of crucial genes for cartilage formation like SOX9, SOX6, and SOX5 as well as secretion of ECM molecules typical for hyaline cartilage [44, 100–104].

Reported *in vitro* conditions provide MSC differentiation to chondrocytes, nevertheless, do not stop chondrogenesis at the pre-hypertrophic stage, while cells undergo terminal differentiation to hypertrophic chondrocytes. These cells produce calcified instead of hyaline cartilage [105]. This remains crucial, a limitation in the formation of functional articular cartilage, as calcified cartilage has different biomechanical characteristics compared to hyaline cartilage [105, 106].

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*2.4.2. Role of growth factors in cartilage repair*

Chondrogenic differentiation of MSCs is induced by various intrinsic and extrinsic factors [107]. Growth factors play the most important role in this process [107]. The importance of growth factors in the maintenance and production of cartilage *in vivo* had been explained previously. Hence, introduction of these factors in *in vitro* controlled chondrogenesis was the logical choice. Below are listed studies that clarified the importance of growth factors in treatment of cartilage defects with MSC. Keep in mind that TGF-β superfamily (TGF-β 1 & 2 and bone morphogenic proteins—BMPs), insulin-like growth factors (IGFs), and fibroblast growth factors (FGFs) are the major factors regulating chondrogenesis and synthesis of cartilage matrix.

Porcine MSCs encapsulated in agarose hydrogels after treatment with TGF-b3 increase the sulfated glycosaminoglycans in surrounding culture media, highlighting their role in cartilage ECM anabolism [35]. Moreover, the expression of BMP4 in transgenic MSC enhances their chondrogenesis in rat model through the positive regulation of main cartilage component, collagen type II [108]. Moreover, after 24 weeks, animals treated with BMP-4 showed significantly better cartilage repair than untreated animals [108]. Nevertheless, better results were obtained in chondrogenesis of MSC when TGF-b1, IGF-1, BMP-2, and BMP-7 were combined [36]. Also, intra-articular application of another growth factor, FGF-18-induced dose-dependent, increases the cartilage thickness of tibial plateau in rat OA model [37]. Similar effect to FGF-18 has FGF-2 which stimulates [38, 109] increase in glycosaminoglycan and collagen type II after its application on MSC culture in chondrogenic medium [38]. Overall, growth factors appear to be one of the main components in improving clinical cartilage regeneration, but they must be precisely combined and loaded on appropriate scaffold materials to simulate the conditions and three-dimensional (3D) structure most similar to the *in vivo* condition.

## 3. Chondroprogenitors in cartilage

### 3.1. Chondrogenesis

Chondrogenesis is a complex process that is initiated by MSC crowding and condensing on the bone-forming site, followed by maturation into terminally differentiated chondrocytes [110, 111]. This pathway is accompanied by stage-specific ECM production, synchronized by cellular interactions with the matrix, growth, and differentiation factors [110]. The latter initiate or suppress cellular signaling pathways and transcription of specific genes in a spatial-temporal manner [110, 111]. The anti-inflammatory and immunosuppressive properties of BMSCs suggest that these cells reduce inflammation in the joint. Moreover, BMSCs may initiate the repair process by differentiating into chondrocytes or by inducing proliferation and differentiation of the remaining healthy chondroprogenitor into mature chondrocytes or both. In addition, other features such as transcription factors, biological modulators, and extracellular matrix proteins expressed or produced by BMSCs may play an important role in enhancing cartilage formation.

Initially, MSCs express adhesion molecules including N-cadherin, N-CAM (Ncam1), tenascin-C (Tnc), and versican, which are involved in the compaction and condensation of MSCs regulated by different BMP factors [112]. Through progression of the condensation process, MSCs begin to express early cartilage markers collagen type II, aggrecan, and FGF receptor leading to chondrocytes progenitors stage of chondrogenesis [113]. Process of MSC condensation and chondrogenesis is triggered and positively regulated by major transcriptional factor, Sox 9. It is highly expressed in MSC before condensation and remains highly expressed in all stages of chondrogenesis through prechondrocytes to mature chondrocytes, while it is switched off when cells undergo hypertrophy [113, 114]. The formation of chondrocytes over osteocytes is regulated by combined action of Sox 9 and other transcriptional factors Pax/Nkx/Barx2, Sox 9 through inhibition of Runx2 (Cbfa1) as a domain transcriptional factor required for osteoblast differentiation [113, 115]. Moreover, Sox 9 positively regulates two other Sox family members Sox 5 and Sox 6, which play a significant role in activation of cartilage-specific genes: type II, IX, and XI collagen, aggrecan, and cartilage oligomeric matrix protein [114, 116, 117]. The role and spatio-temporal expression of Sox 5 and Sox 6 in chondrogenesis has been studied through single and double null mutations in mice model. Single gene deletion resulted in moderate skeletal abnormalities; however, double mutation induced animal death caused by systemic chondrodysplasia and skeletal deformity. These results indicate simultaneous action of these two transcription factors in formation of functional skeletal system. Nevertheless, in the double mutant low level of cartilage, specific extracellular matrix component was sustained by normal Sox 9 expression, but it was insufficient to support proper MSC differentiation and formation of cartilage [116]. This implies that synchronized action of Sox 5, 6, and 9 trios is required to maintain sufficient ECM component expression and normal matrix composition. Furthermore, these three genes promote the chondrogenesis by inhibition of hypertrophic and osteogenic differentiation [113]. Chondrocytes maturation to hypertrophic chondrocytes is repressed by Sox 9 modulation of the Wnt/beta-catenin signaling pathway with beta-catenin degradation or inhibition of beta-catenin transcriptional activity without affecting its stability [118]. In addition, Sox 5 and Sox 6 delay chondrocyte hypertrophy by down-regulating Ihh signaling, FGFR3, and Runx2 and up-regulating BMP6 [115].

Further maturation of chondrocytes is essential for the final remodeling of the cartilage into bone. Terminal chondrocytes differentiation into the hypertrophic chondrocytes is promoted by upregulation of Runx 2 and calcified cartilage markers collagen X and MMP13 [113, 117]. Later, hypertrophic and terminal chondrocytes express angiogenic factors, including VEGF, which provide the genesis for vascularization and formation of primary ossification centers within osteoblasts, osteocytes, and hematopoietic cells [119]. Equally, terminal chondrocytes undergo apoptosis by release of collagen types X and I and mineralization of the ECM [117]. Contrary to growth plate chondrogenesis, normal articular chondrocytes never undergo hypertrophic differentiation, except at the tidemark [113].

### 3.2. Chondroprogenitors potential in cartilage repair

Recent research reported the presence of MSC and their progenitors in cartilage itself [104]. These cells possess characteristics similar to stem cells isolated from other adult tissues

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involving proliferation and differentiation potential under appropriate *in vitro* conditions [120–123]. They were subjected to the process of isolation, expansion, and identification in order to confirm their stem cells phenotype previously established on MSC from other adult tissues [121–124]. To date, studies investigated the presence of these cells in normal and OA cartilage. Interestingly, several authors observed that OA cartilage contains higher number of mesenchymal progenitors compared to normal [122, 125–129].

Subpopulation of cells determined as cartilage progenitor cells (ACPCs) possess high-colony forming efficiency and express surface antigens specific to MSC (Notch 1, CD 105 & CD 166) [121–123]. Moreover, after the cultivation in specific chondrogenic medium, they showed capacity to differentiate into cartilage in 3D pellet cultures [130]. The expression of MSC markers and differentiation potential confirmed presence of multipotential mesenchymal progenitor cells in articular cartilage [122]. Comparative study of ACPCs and BMSCs revealed positive expression of adult stem cells markers (Notch 1, Stro 1, CD105, and CD 166) on both cell types. Nevertheless, chondrogenesis of BMSCs resulted in hypertrophic cartilage tissue confirmed by positive staining of collagen X, while this marker was not detected in tissue obtained from ACPCs [124]. Similar was reported by Alsalameh et al. where $CD105^+$ and $CD166^+$ cells showed no signs of hypertrophic chondrocytes and osteogenesis in chondrogenic micromass cultures after 3 weeks [128].

Likewise, cells positive for other markers that have been identified in MSC $CD9^+/CD90^+/CD166^+$ [131], $CD105^+/CD166^+$ [128], and Notch-$1^+$/Stro-$1^+$ [125] were capable of differentiating in chondrocytes and formed cartilage tissue *in vitro*. MCS differentiation into hypertrophic cartilage is the major limitation in hyaline functional cartilage production [105]. ACPCs may therefore be considered superior to MSCs from other tissues in cartilage repair [124, 125, 128, 129].

These results indicate the opportunity for using OA cartilage as a potential source of cells with cartilage-forming potential. Yet, further investigations are required to explore chondrogenesis regulation *in vitro*.

## 4. Conclusion

Based on self-repair and multilineage potentials, MCS provide hyaline cartilage regeneration opportunities. Studies on cartilage regeneration with adult mesenchymal stem cells have shown that BMSC are the most commonly used cell types to address cartilage regeneration. However, although short-term results appear satisfactory, hypertrophic chondrocyte and fibrocartilage formation emerge thereafter with hypertrophically differentiated MSC. Note that fibrocartilage provides a molecular pattern secreted by hypertrophic chondrocytes, leading to different biomechanical characteristics compared with hyaline cartilage.

Furthermore, harvesting bone marrow is a painful procedure with donor-site morbidity and risk of wound infection and sepsis. Hence, both AMSCs and synovium-derived stem cells have been considered as alternatives. However, results using these two cell lines have been similar to those obtained employing the bone marrow approach. In fact, although a high

expression of chondrogenic markers was initially obtained, they appear to be expressed as collagen type X confirming the presence of hypertrophy.

Therefore, further investigations regarding the regulation of cellular activity by growth factors, scaffolds and even gene therapy remain viable options. Recently, one more potential source of MSC and progenitors for cartilage repair engineering from the cartilage itself has been tested. Cells isolated from the surface zone of articular cartilage have the capacity to differentiate into cartilage in 3D pellet culture. Moreover, no signs of hypertrophic chondrocytes and osteogenesis were observed. Thus, ACPCs could be considered more adequate than MSC in cartilage repair.

## Abbreviations

| | |
|---|---|
| OA | Osteoarthritis |
| AC | Articular cartilage |
| PRP | Platelet-rich plasma |
| ECM | Extra-cellular matrix |
| MSC | Mesenchymal stem cells |
| BMSCs | Bone marrow stromal cells |
| ACI | Autologous chondrocytes implantation |
| COMP | Cartilage oligometric matrix protein |
| TGF-β | Transforming growth factors-beta superfamily |
| IGFs | Insulin-like growth factors |
| FGFs | Fibroblast growth factors |
| BMPs | Bone morphogenetic proteins |
| ALK | Activin receptor like-kinase |
| IHH | Indian hedgehog protein |
| IRS | Insulin receptor-substrate family |
| FGF | Fibroblast growth factors |
| FGFR | Fibroblast growth factor receptor |
| CD105 | Endoglin-type I glycoprotein |
| CD73 | Ecto-5′-nucleotidase |
| CD90 (Thy) | Cluster of differentiation 90 |
| CD106 (VCAM-1) | Vascular cell adhesion molecule-1 |
| CD166 (ALCAM) | Activated leucocyte cell adhesion molecule |
| CD106 (ICAM-1) | Intercellular adhesion molecule-1 |
| NOTCH | Neurogenic locus notch homolog protein |
| ITGA11 | Integrin alpha-11 |

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|---|---|
| CD31 (PECAM-1) | Platelet/endothelial cell adhesion molecule-1 |
| CD18 (LFA-1) | Leucocyte function-associated antigen-1 |
| CD56 | Neuronal cell adhesion molecule-1 |
| GFs | Growth factors |
| HLA | Human leucocyte antigen |
| BMSC | Bone marrow-derived mesenchymal stem cells |
| AMSC | Adipose-derived mesenchymal stem cells |
| ACPC | Articular cartilage progenitor cells |

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