Chapter 10

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

Hechmi Toumi, Eric Lespessailles and Marija Mazor

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.68176

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

http://dx.doi.org/10.5772/intechopen.68176

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].

http://dx.doi.org/10.5772/intechopen.68176

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

http://dx.doi.org/10.5772/intechopen.68176

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

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

Author details

Hechmi Toumi*, Eric Lespessailles and Marija Mazor

*Address all correspondence to: hechmi.toumi@chro-orleans.fr

Service de Rhumatologie, Centre Hospitalier Régional d'Orléans, Collegium ST, University of Orleans, Orleans, France

References

[1] Lorenz H, Richter W. Osteoarthritis: Cellular and molecular changes in degenerating cartilage. Progress in Histochemistry and Cytochemistry. 2006;**40**(3):135-163

[2] Jayabalan P, Hagerty S, Cortazzo MH. The use of platelet-rich plasma for the treatment of osteoarthritis. The Physician and Sportsmedicine. 2014;**42**(3):53-62

[3] Eyre DR. Collagens and cartilage matrix homeostasis. Clinical Orthopaedics and Related Research. 2004;(427 Suppl): S118-S122

[4] Pearle AD, Warren RF, Rodeo SA. Basic science of articular cartilage and osteoarthritis. Clinics in Sports Medicine. 2005;**24**(1):1-12

[5] Versier G, Dubrana F, French Arthroscopy S. Treatment of knee cartilage defect in 2010. Orthopaedics and Traumatology Surgery and Research. 2011;**97**(8 Suppl):S140-S153

[6] Longo UG, et al. Stem cells and gene therapy for cartilage repair. Stem Cells International. 2012;**2012**:168385

[7] Alison MR, et al. An introduction to stem cells. The Journal of Pathology. 2002;**197**(4): 419-423

[8] Khan WS, Johnson DS, Hardingham TE. The potential of stem cells in the treatment of knee cartilage defects. Knee. 2010;**17**(6):369-374

[9] Wang L, et al. Progress in stem cells and regenerative medicine. Sheng Wu Gong Cheng Xue Bao. 2015;**31**(6):871-879

[10] Goodell MA, Rando TA. Stem cells and healthy aging. Science. 2015;**350**(6265):1199-1204

[11] Tuan RS, Boland G, Tuli R. Adult mesenchymal stem cells and cell-based tissue engineering. Arthritis Research & Therapy. 2003;**5**(1):32-45

[12] Raveh-Amit H, et al. Tissue resident stem cells: Till death do us part. Biogerontology. 2013;**14**(6):573-590

[13] Pittenger MF, et al. Multilineage potential of adult human mesenchymal stem cells. Science. 1999;**284**(5411):143-147

[14] Liu ZJ, Zhuge Y, Velazquez OC. Trafficking and differentiation of mesenchymal stem cells. Journal of Cellular Biochemistry. 2009;**106**(6):984-991

[15] Friedenstein AJ. Stromal mechanisms of bone marrow: Cloning in vitro and retransplantation in vivo. Haematology and Blood Transfusion. 1980;**25**:19-29

[16] Friedenstein AJ, Chailakhjan RK, Lalykina KS. The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. Cell and Tissue Kinetics. 1970;**3**(4):393-403

[17] Delorme B, et al. Specific plasma membrane protein phenotype of culture-amplified and native human bone marrow mesenchymal stem cells. Blood. 2008;**111**(5):2631-2635

[18] Yang M, Zhang H, Gangolli R. Advances of mesenchymal stem cells derived from bone marrow and dental tissue in craniofacial tissue engineering. Current Stem Cell Research & Therapy. 2014;**9**(3):150-161

[19] Gottipamula S, et al. Isolation, expansion and characterization of bone marrow-derived mesenchymal stromal cells in serum-free conditions. Cell and Tissue Research. 2014;**356**(1):123-135

[20] Laschke MW, et al. In vitro osteogenic differentiation of adipose-derived mesenchymal stem cell spheroids impairs their in vivo vascularization capacity inside implanted porous polyurethane scaffolds. Acta Biomaterialia. 2014;**10**(10):4226-4235

[21] Wu AY. Autologous fat transfer with in-situ mediation (AIM): A novel and compliant method of adult mesenchymal stem cell therapy. Journal of Translational Medicine. 2013;**11**:136

[22] Ozpur MA, et al. Generation of skin tissue using adipose tissue-derived stem cells. Plastic and Reconstructive Surgery. 2016;**137**(1):134-143

[23] Gao X, et al. A comparison of bone regeneration with human mesenchymal stem cells and muscle-derived stem cells and the critical role of BMP. Biomaterials. 2014;**35**(25):6859-6870

[24] Radtke CL, et al. Characterization and osteogenic potential of equine muscle tissue- and periosteal tissue-derived mesenchymal stem cells in comparison with bone marrow- and adipose tissue-derived mesenchymal stem cells. American Journal of Veterinary Research. 2013;**74**(5):790-800

[25] Liu H, et al. Comparison of cellular responses of mesenchymal stem cells derived from bone marrow and synovium on combined silk scaffolds. Journal of Biomedical Materials Research A. 2015;**103**(1):115-125

[26] Dominici M, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006;**8**(4):315-317

[27] Szala S, Wisniewska E, Czapla J. Mesenchymal stromal cells. Postepy Higieny I Medycyny Doswiadczalnej (Online). 2014;**68**:1287-1298

[28] Simmons PJ, Torok-Storb B. Identification of stromal cell precursors in human bone marrow by a novel monoclonal antibody. *STRO-1*. Blood. 1991;**78**(1):55-62

[29] Halfon S, et al. Markers distinguishing mesenchymal stem cells from fibroblasts are downregulated with passaging. Stem Cells and Development. 2011;**20**(1):53-66

[30] Rozemuller H, et al. Prospective isolation of mesenchymal stem cells from multiple mammalian species using cross-reacting anti-human monoclonal antibodies. Stem Cells and Development. 2010;**19**(12):1911-1921

[31] De Schauwer C, et al. Markers of stemness in equine mesenchymal stem cells: A plea for uniformity. Theriogenology. 2011;**75**(8):1431-1443

[32] Kim TH, et al. In vitro and in vivo evaluation of bone formation using solid freeform fabrication-based bone morphogenic protein-2 releasing PCL/PLGA scaffolds. Biomedical Materials. 2014;**9**(2):025008

[33] Moeinzadeh S, Jabbari E. Morphogenic peptides in regeneration of load bearing tissues. Advances in Experimental Medicine and Biology. 2015;**881**:95-110

[34] McMahon MS. Bone morphogenic protein 3 signaling in the regulation of osteogenesis. Orthopedics. 2012;**35**(11):920

[35] Thorpe SD, et al. The response of bone marrow-derived mesenchymal stem cells to dynamic compression following TGF-beta3 induced chondrogenic differentiation. Annals of Biomedical Engineering. 2010;**38**(9):2896-2909

[36] An C, et al. IGF-1 and BMP-2 induces differentiation of adipose-derived mesenchymal stem cells into chondrocytes-like cells. Annals of Biomedical Engineering. 2010;**38**(4):1647-1654

[37] Davidson D, et al. Fibroblast growth factor (FGF) 18 signals through FGF receptor 3 to promote chondrogenesis. The Journal of Biological Chemistry. 2005;**280**(21):20509-20515

[38] Moore EE, et al. Fibroblast growth factor-18 stimulates chondrogenesis and cartilage repair in a rat model of injury-induced osteoarthritis. Osteoarthritis & Cartilage. 2005;**13**(7):623-631

[39] Hocking AM, Gibran NS. Mesenchymal stem cells: Paracrine signaling and differentiation during cutaneous wound repair. Experimental Cell Research. 2010;**316**(14):2213-2219

[40] Doorn J, et al. Therapeutic applications of mesenchymal stromal cells: Paracrine effects and potential improvements. Tissue Engineering Part B Reviews. 2012;**18**(2):101-115

[41] Ronziere MC, et al. Chondrogenic potential of bone marrow—and adipose tissue-derived adult human mesenchymal stem cells. BioMedical Materials and Engineering. 2010;**20**(3):145-158

[42] De Miguel MP, et al. Immunosuppressive properties of mesenchymal stem cells: Advances and applications. Current Molecular Medicine. 2012;**12**(5):574-591

[43] Castro-Manrreza ME, Montesinos JJ. Immunoregulation by mesenchymal stem cells: Biological aspects and clinical applications. Journal of Immunology Research. 2015;**2015**:394917

[44] Freyria AM, Mallein-Gerin F. Chondrocytes or adult stem cells for cartilage repair: the indisputable role of growth factors. Injury. 2012;**43**(3):259-265

[45] Boyette LB, et al. Human bone marrow-derived mesenchymal stem cells display enhanced clonogenicity but impaired differentiation with hypoxic preconditioning. Stem Cells Translational Medicine. 2014;**3**(2):241-254

[46] Bernardo ME, et al. Human mesenchymal stem cells derived from bone marrow display a better chondrogenic differentiation compared with other sources. Connective Tissue Research. 2007;**48**(3):132-140

[47] Nakagawa Y, et al. Cartilage derived from bone marrow mesenchymal stem Cells expresses lubricin in vitro and in vivo. PLoS One. 2016;**11**(2):e0148777

[48] Murdoch AD, et al. The development of a mature collagen network in cartilage from human bone marrow stem cells in Transwell culture. Matrix Biology. 2016;**50**:16-26

[49] Sakaguchi Y, et al. Comparison of human stem cells derived from various mesenchymal tissues: Superiority of synovium as a cell source. Arthritis and Rheumatism. 2005;**52**(8):2521-2529

[50] Norambuena GA, Khoury M, Jorgensen C. Mesenchymal stem cells in osteoarticular pediatric diseases: An update. Pediatric Research. 2012;**71**(4 Pt 2):452-458

[51] Lubis AM, Lubis VK. Adult bone marrow stem cells in cartilage therapy. Acta Medica Indonesiana. 2012;**44**(1):62-68

[52] Heng BC, Cao T, Lee EH. Directing stem cell differentiation into the chondrogenic lineage in vitro. Stem Cells. 2004;**22**(7):1152-1167

[53] Fafian-Labora J, et al. Influence of age on rat bone-marrow mesenchymal stem cells potential. Scientific Reports. 2015;**5**:16765

[54] Danisovic L, et al. Comparative analysis of mesenchymal stromal cells from different tissue sources in respect to articular cartilage tissue engineering. General Physiology and Biophysics. 2016;**35**(2):207-14.

[55] Chang F, et al. Repair of large full-thickness articular cartilage defects by transplantation of autologous uncultured bone-marrow-derived mononuclear cells. Journal of Orthopaedic Research. 2008;**26**(1):18-26

[56] Yan H, Yu C. Repair of full-thickness cartilage defects with cells of different origin in a rabbit model. Arthroscopy. 2007;**23**(2):178-187

[57] Bai T, et al. Experimental research on repair of rabbit articular cartilage deffects with composite of autologous cell-carriers. Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi. 2008;**22**(4):487-491

[58] Wakitani S, et al. Mesenchymal cell-based repair of large, full-thickness defects of articular cartilage. The Journal of Bone and Joint Surgery American. 1994;**76**(4):579-592

[59] Ding Z, Huang H. Mesenchymal stem cells in rabbit meniscus and bone marrow exhibit a similar feature but a heterogeneous multi-differentiation potential: Superiority of meniscus as a cell source for meniscus repair. BMC Musculoskeletal Disorders. 2015;**16**:65

[60] Tay LX, et al. Treatment outcomes of alginate-embedded allogenic mesenchymal stem cells versus autologous chondrocytes for the repair of focal articular cartilage defects in a rabbit model. The American Journal of Sports Medicine. 2012;**40**(1):83-90

[61] Li WJ, et al. Evaluation of articular cartilage repair using biodegradable nanofibrous scaffolds in a swine model: A pilot study. Journal of Tissue Engineering and Regenerative Medicine. 2009;**3**(1):1-10

[62] Dutton AQ, et al. Enhancement of meniscal repair in the avascular zone using mesenchymal stem cells in a porcine model. The Journal of Bone and Joint Surgery British. 2010;**92**(1):169-175

[63] Mrugala D, et al. Phenotypic and functional characterisation of ovine mesenchymal stem cells: Application to a cartilage defect model. Annals of the Rheumatic Diseases. 2008;**67**(3):288-295

[64] Dorotka R, et al. Repair of articular cartilage defects treated by microfracture and a three-dimensional collagen matrix. Biomaterials. 2005;**26**(17):3617-3629

[65] Araki S, et al. Improved quality of cartilage repair by bone marrow mesenchymal stem cells for treatment of an osteochondral defect in a cynomolgus macaque model. Acta Orthopaedica. 2015;**86**(1):119-126

[66] Vega A, et al. Treatment of knee osteoarthritis with allogeneic bone marrow mesenchymal stem cells: A randomized controlled trial. Transplantation. 2015;**99**(8):1681-1690

[67] Orozco L, et al. Treatment of knee osteoarthritis with autologous mesenchymal stem cells: A pilot study. Transplantation. 2013;**95**(12):1535-1541

[68] Wakitani S, et al. Human autologous culture expanded bone marrow mesenchymal cell transplantation for repair of cartilage defects in osteoarthritic knees. Osteoarthritis & Cartilage. 2002;**10**(3):199-206

[69] Wakitani S, et al. Autologous bone marrow stromal cell transplantation for repair of full-thickness articular cartilage defects in human patellae: Two case reports. Cell Transplantation. 2004;**13**(5):595-600

[70] Wakitani S, et al. Repair of articular cartilage defects in the patello-femoral joint with autologous bone marrow mesenchymal cell transplantation: Three case reports involving nine defects in five knees. Journal of Tissue Engineering and Regenerative Medicine. 2007;**1**(1):74-79

[71] Kuroda R, et al. Treatment of a full-thickness articular cartilage defect in the femoral condyle of an athlete with autologous bone-marrow stromal cells. Osteoarthritis & Cartilage. 2007;**15**(2):226-231

[72] Kim YS, et al. Assessment of clinical and MRI outcomes after mesenchymal stem cell implantation in patients with knee osteoarthritis: A prospective study. Osteoarthritis & Cartilage. 2016;**24**(2):237-245

[73] Davatchi F, et al. Mesenchymal stem cell therapy for knee osteoarthritis: 5 years follow-up of three patients. International Journal of Rheumatic Diseases. 2016;**19**(3):219-225

[74] Nejadnik H, et al. Autologous bone marrow-derived mesenchymal stem cells versus autologous chondrocyte implantation: An observational cohort study. The American Journal of Sports Medicine. 2010;**38**(6):1110-1116

[75] Burrage PS, Mix KS, Brinckerhoff CE. Matrix metalloproteinases: Role in arthritis. Frontiers in Bioscience. 2006;**11**:529-543

[76] Giannini S, et al. Cartilage repair evolution in post-traumatic osteochondral lesions of the talus: from open field autologous chondrocyte to bone-marrow-derived cells transplantation. Injury. 2010;**41**(11):1196-1203

[77] Kim YS, et al. Mesenchymal stem cell implantation in osteoarthritic knees: Is fibrin glue effective as a scaffold? The American Journal of Sports Medicine. 2015;**43**(1):176-185

[78] Kim YS, Choi YJ, Koh YG. Mesenchymal stem cell implantation in knee osteoarthritis: an assessment of the factors influencing clinical outcomes. The American Journal of Sports Medicine. 2015;**43**(9):2293-2301

[79] Estes BT, et al. Isolation of adipose-derived stem cells and their induction to a chondrogenic phenotype. Nature Protocols. 2010;**5**(7):1294-1311

[80] Guilak F, et al. Nicolas Andry Award: Multipotent adult stem cells from adipose tissue for musculoskeletal tissue engineering. Clinical Orthopaedics and Related Research. 2010;**468**(9):2530-2540

[81] Hamid AA, et al. Characterization of human adipose-derived stem cells and expression of chondrogenic genes during induction of cartilage differentiation. Clinics (Sao Paulo). 2012;**67**(2):99-106

[82] Diekman BO, Estes BT, Guilak F. The effects of BMP6 overexpression on adipose stem cell chondrogenesis: Interactions with dexamethasone and exogenous growth factors. Journal of Biomedical Materials Research A. 2010;**93**(3):994-1003

[83] Diekman BO, et al. Chondrogenesis of adult stem cells from adipose tissue and bone marrow: Induction by growth factors and cartilage-derived matrix. Tissue Engineering Part A. 2010;**16**(2):523-533

[84] Mehlhorn AT, et al. Differential effects of BMP-2 and TGF-beta1 on chondrogenic differentiation of adipose derived stem cells. Cell Proliferation. 2007;**40**(6):809-823

[85] Wang W, et al. Human adipose-derived mesenchymal progenitor cells engraft into rabbit articular cartilage. International Journal of Molecular Sciences. 2015;**16**(6):12076-12091

[86] Desando G, et al. Intra-articular delivery of adipose derived stromal cells attenuates osteoarthritis progression in an experimental rabbit model. Arthritis Research & Therapy. 2013;**15**(1):R22

[87] Yoshimura H, et al. Comparison of rat mesenchymal stem cells derived from bone marrow, synovium, periosteum, adipose tissue, and muscle. Cell and Tissue Research. 2007;**327**(3):449-462

[88] Shirasawa S, et al. In vitro chondrogenesis of human synovium-derived mesenchymal stem cells: Optimal condition and comparison with bone marrow-derived cells. Journal of Cellular Biochemistry. 2006;**97**(1):84-97

[89] Isobe Y, et al. Comparison of human mesenchymal stem cells derived from bone marrow, synovial fluid, adult dental pulp, and exfoliated deciduous tooth pulp. International Journal of Oral and Maxillofacial Surgery. 2016;**45**(1):124-131

[90] Koga H, et al. Comparison of mesenchymal tissues-derived stem cells for in vivo chondrogenesis: Suitable conditions for cell therapy of cartilage defects in rabbit. Cell and Tissue Research. 2008;**333**(2):207-215

[91] Suzuki S, et al. Properties and usefulness of aggregates of synovial mesenchymal stem cells as a source for cartilage regeneration. Arthritis Research & Therapy. 2012;**14**(3):R136

[92] Ando W, et al. Detection of abnormalities in the superficial zone of cartilage repaired using a tissue engineered construct derived from synovial stem cells. European Cells & Materials. 2012;**24**:292-307

[93] Mamidi MK, et al. Mesenchymal stromal cells for cartilage repair in osteoarthritis. Osteoarthritis & Cartilage. 2016;**24**(8):1307-16

[94] Li D, et al. Role of mechanical factors in fate decisions of stem cells. Regenerative Medicine. 2011;**6**(2):229-240

[95] Wang YK, Chen CS. Cell adhesion and mechanical stimulation in the regulation of mesenchymal stem cell differentiation. Journal of Cellular and Molecular Medicine. 2013;**17**(7):823-832

[96] Ishii I, et al. Healing of full-thickness defects of the articular cartilage in rabbits using fibroblast growth factor-2 and a fibrin sealant. The Journal of Bone and Joint Surgery British. 2007;**89**(5):693-700

[97] Chubinskaya S, et al. Effects induced by BMPS in cultures of human articular chondrocytes: Comparative studies. Growth Factors. 2008;**26**(5):275-283

[98] Danisovic L, Varga I, Polak S. Growth factors and chondrogenic differentiation of mesenchymal stem cells. Tissue and Cell. 2012;**44**(2):69-73

[99] Sekiya I, et al. Comparison of effect of BMP-2, -4, and -6 on in vitro cartilage formation of human adult stem cells from bone marrow stroma. Cell and Tissue Research. 2005;**320**(2):269-276

[100] Markway BD, et al. Enhanced chondrogenic differentiation of human bone marrow-derived mesenchymal stem cells in low oxygen environment micropellet cultures. Cell Transplantation. 2010;**19**(1):29-42

[101] Khan WS, Adesida AB, Hardingham TE. Hypoxic conditions increase hypoxia-inducible transcription factor 2alpha and enhance chondrogenesis in stem cells from the infrapatellar fat pad of osteoarthritis patients. Arthritis Research & Therapy. 2007;**9**(3):R55

[102] Khan WS, et al. Bone marrow-derived mesenchymal stem cells express the pericyte marker 3G5 in culture and show enhanced chondrogenesis in hypoxic conditions. Journal of Orthopaedic Research. 2010;**28**(6):834-840

[103] Merceron C, et al. Differential effects of hypoxia on osteochondrogenic potential of human adipose-derived stem cells. American Journal of Physiology Cell Physiology. 2010;**298**(2):C355-364

[104] Murphy CL, et al. Hypoxia. HIF-mediated articular chondrocyte function: Prospects for cartilage repair. Arthritis Research & Therapy. 2009;**11**(1):213

[105] van Osch GJ, et al. Cartilage repair: Past and future—lessons for regenerative medicine. Journal of Cellular and Molecular Medicine. 2009;**13**(5):792-810

[106] Pacifici M, et al. Hypertrophic chondrocytes. The terminal stage of differentiation in the chondrogenic cell lineage? Annals of the New York Academy of Sciences. 1990;**599**:45-57

[107] Kolf CM, Cho E, Tuan RS. Mesenchymal stromal cells. Biology of adult mesenchymal stem cells: Regulation of niche, self-renewal and differentiation. Arthritis Research & Therapy. 2007;**9**(1):204

[108] Kuroda R, et al. Cartilage repair using bone morphogenetic protein 4 and muscle-derived stem cells. Arthritis and Rheumatism. 2006;**54**(2):433-442

[109] Stewart AA, et al. Effect of fibroblast growth factor-2 on equine mesenchymal stem cell monolayer expansion and chondrogenesis. American Journal of Veterinary Research. 2007;**68**(9):941-945

[110] DeLise AM, Fischer L, Tuan RS. Cellular interactions and signaling in cartilage development. Osteoarthritis & Cartilage. 2000;**8**(5):309-334

[111] Shimizu H, Yokoyama S, Asahara H. Growth and differentiation of the developing limb bud from the perspective of chondrogenesis. Development Growth & Differentiation. 2007;**49**(6):449-454

[112] Zuscik MJ, et al. Regulation of chondrogenesis and chondrocyte differentiation by stress. The Journal of Clinical Investigation. 2008;**118**(2):429-438

[113] Lefebvre V, Smits P. Transcriptional control of chondrocyte fate and differentiation. Birth Defects Research Part C Embryo Today. 2005;**75**(3):200-212

[114] Akiyama H, et al. The transcription factor Sox9 has essential roles in successive steps of the chondrocyte differentiation pathway and is required for expression of Sox5 and Sox6. Genes & Development. 2002;**16**(21):2813-2828

[115] Zhou G, et al. Dominance of SOX9 function over RUNX2 during skeletogenesis. Proceedings of the National Academy of Sciences of the United States of America. 2006;**103**(50):19004-19009

[116] Smits P, et al. The transcription factors L-Sox5 and Sox6 are essential for cartilage formation. Developmental Cell. 2001;**1**(2):277-290

[117] Vinatier C, et al. Cartilage engineering: A crucial combination of cells, biomaterials and biofactors. Trends in Biotechnology. 2009;**27**(5):307-314

[118] Topol L, et al. Sox9 inhibits Wnt signaling by promoting beta-catenin phosphorylation in the nucleus. The Journal of Biological Chemistry. 2009;**284**(5):3323-3333

[119] Carlevaro MF, et al. Vascular endothelial growth factor (VEGF) in cartilage neovascularization and chondrocyte differentiation: Auto-paracrine role during endochondral bone formation. Journal of Cell Science. 2000;**113**(Pt 1):59-69

[120] O'Sullivan J, et al. Mesenchymal chondroprogenitor cell origin and therapeutic potential. Stem Cell Research & Therapy. 2011;**2**(1):8

[121] Dowthwaite GP, et al. The surface of articular cartilage contains a progenitor cell population. Journal of Cell Science. 2004;**117**(Pt 6):889-897

[122] Hiraoka K, et al. Mesenchymal progenitor cells in adult human articular cartilage. Biorheology. 2006;**43**(3-4):447-454

[123] Chang HX, et al. Age-related biological characterization of mesenchymal progenitor cells in human articular cartilage. Orthopedics. 2011;**34**(8):e382-388

[124] McCarthy HE, et al. The comparison of equine articular cartilage progenitor cells and bone marrow-derived stromal cells as potential cell sources for cartilage repair in the horse. Veterinary Journal. 2012;**192**(3):345-351

[125] Grogan SP, et al. Mesenchymal progenitor cell markers in human articular cartilage: Normal distribution and changes in osteoarthritis. Arthritis Research & Therapy. 2009;**11**(3):R85

[126] Pretzel D, et al. Relative percentage and zonal distribution of mesenchymal progenitor cells in human osteoarthritic and normal cartilage. Arthritis Research & Therapy. 2011;**13**(2):R64

[127] Nelson L, et al. Evidence of a viable pool of stem cells within human osteoarthritic cartilage. Cartilage. 2014;**5**(4):203-214

[128] Alsalameh S, et al. Identification of mesenchymal progenitor cells in normal and osteoarthritic human articular cartilage. Arthritis and Rheumatism. 2004;**50**(5):1522-1532

[129] Fickert S, Fiedler J, Brenner RE. Identification of subpopulations with characteristics of mesenchymal progenitor cells from human osteoarthritic cartilage using triple staining for cell surface markers. Arthritis Research & Therapy. 2004;**6**(5):R422-R432

[130] Khan IM, et al. Clonal chondroprogenitors maintain telomerase activity and Sox9 expression during extended monolayer culture and retain chondrogenic potential. Osteoarthritis & Cartilage. 2009;**17**(4):518-528

[131] Ozbey O, et al. Characterization f colony-forming cells in adult human articular cartilage. Acta Histochemica. 2014;**116**(5):763-770