Stem Cell Rev and Rep (2017) 13:575–586
DOI 10.1007/s12015-017-9753-1

Mesenchymal Stem/Progenitor Cells Derived from Articular Cartilage, Synovial Membrane and Synovial Fluid for Cartilage Regeneration: Current Status and Future Perspectives

Yi-Zhou Huang[1] · Hui-Qi Xie[1] · Antonietta Silini[2] · Ornella Parolini[2,3] · Yi Zhang[1] · Li Deng[1] · Yong-Can Huang[4,5,6,7]

Published online: 18 July 2017

Abstract Large articular cartilage defects remain an immense challenge in the field of regenerative medicine because of their poor intrinsic repair capacity. Currently, the available medical interventions can relieve clinical symptoms to some extent, but fail to repair the cartilaginous injuries with authentic hyaline cartilage. There has been a surge of interest in developing cell-based therapies, focused particularly on the use of mesenchymal stem/progenitor cells with or without scaffolds. Mesenchymal stem/progenitor cells are promising graft cells for tissue regeneration, but the most suitable source of cells for cartilage repair remains controversial. The tissue origin of mesenchymal stem/progenitor cells notably influences the biological properties and therapeutic potential. It is well known that mesenchymal stem/progenitor cells derived from synovial joint tissues exhibit superior chondrogenic ability compared with those derived from non-joint tissues; thus, these cell populations are considered ideal sources for cartilage regeneration. In addition to the progress in research and promising preclinical results, many important research questions must be answered before widespread success in cartilage regeneration is achieved. This review outlines the biology of stem/progenitor cells derived from the articular cartilage, the synovial membrane, and the synovial fluid, including their tissue distribution, function and biological characteristics. Furthermore, preclinical and clinical trials focusing on their applications for cartilage regeneration are summarized, and future research perspectives are discussed.

Keywords Mesenchymal stem cells · progenitors · Synovial joint · Synovial membrane · Synovial fluid · Articular cartilage · Regenerative medicine

Introduction

Articular cartilage is a thin connective tissue covering the end of bones to transmit loads to the subchondral bone and to provide a smooth surface for low-friction motion. Many joint disorders, such as intra-articular fracture, can lead to cartilage defects. The repair ability of articular cartilage is very limited because of its avascular nature and the paucity of resident stem cells. Partial-thickness

✉ Li Deng
dengli2000@gmail.com

✉ Yong-Can Huang
y.c.huang@connect.hku.hk

[1] Laboratory of Stem Cell and Tissue Engineering, West China Hospital, Sichuan University, Chengdu, China

[2] Centro di Ricerca E. Menni, Fondazione Poliambulanza-Istituto Ospedaliero, Brescia, Italy

[3] Istituto di Anatomia Umana e Biologia Cellulare, Università Cattolica del Sacro Cuore Facoltà di Medicina e Chirurgia, Rome, Italy

[4] Shenzhen Engineering Laboratory of Orthopaedic Regenerative Technologies, Orthopaedic Research Center, Peking University Shenzhen Hospital, Shenzhen, China

[5] Shenzhen Key Laboratory of Spine Surgery, Department of Spine Surgery, Peking University Shenzhen Hospital, Shenzhen, China

[6] Department of Orthopaedics and Traumatology, The University of Hong Kong, Hong Kong, SAR, China

[7] Beijing Key Laboratory for Genetic Research of Bone and Joint Disease, Central Laboratory, Peking Union Medical College Hospital, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing, China

defects of mature articular cartilage do not heal spontaneously [1, 2], while full-thickness lesions are always repaired with fibrocartilage that has inferior mechanical properties [3, 4]. Much effort has been devoted to cartilage regeneration; nevertheless, current medical interventions, such as microfracture and mosaicplasty, can relieve clinical symptoms to some extent but fail to restore functional and phenotypically stable hyaline cartilage [5–7].

Autologous chondrocyte implantation (ACI) has been considered as a useful repair technique for cartilage defects [8]. In this cell-based technique, chondrocytes are harvested from a non-weight bearing articular cartilage after biopsy, expanded *in vitro* and then implanted into the cartilage defect [9]. Nevertheless, chondrocytes gradually lose their phenotype during *in vitro* expansion [10, 11], and dedifferentiated chondrocytes have been reported to be unable to generate stable hyaline cartilage [12–14]. Therefore, the phenotype changes of expanded chondrocytes may compromise the long-term clinical outcomes of ACI.

Because stem cells can generate hyaline-like cartilage tissue under specific conditions, stem cell-based therapy has been regarded as a promising approach to address the complexity of cartilage injury. Recently, mesenchymal stem cells (MSCs) have attracted increasing attention for cartilage regeneration owing to their ease of isolation, relatively high expansion rates, low immunogenicity, and multipotency. MSC-specific markers are not yet clearly defined, and the phenotype and comparison of MSCs from different tissues isolated using different protocols are still based on the combination of different parameters according to the minimal criteria proposed by the International Society for Cellular Therapy [15].

MSCs are comprised of heterogeneous cell populations, and their regenerative capacity varies among tissue sources [16–18]. MSCs from different tissues have been shown to differ dramatically in chondrogenic potency [19–21]. Hence, the innate tissue-specific characteristics should be taken into consideration when developing MSC-based cartilage regeneration approaches. For instance, bone marrow-derived MSCs (BM-MSCs) are apt to undergo endochondral bone development rather than form stable hyaline cartilage after chondrogenic induction. Without any osteogenic induction, rat BM-MSCs can spontaneously calcify during *in vitro* culture [22]. The subcutaneous implantation of chondrogenically-primed BM-MSCs leads to bone formation, but not stable cartilage formation [23–26]. Furthermore, the transplantation of chondrogenically-primed BM-MSC pellets into an atrophic, non-union environment resulted in pronounced bone regeneration through endochondral ossification [27]. These findings raise considerable concern about the improper differentiation of BM-MSCs during cartilage regeneration.

Recent studies found that stem/progenitor cells derived from synovial joint tissues showed superior chondrogenic ability when compared with those derived from bone marrow and adipose tissue [20, 28, 29], thus suggesting that the resident stem/progenitor cells in synovial joints could be ideal cell sources for cartilage repair. A further understanding of the biology and regenerative potential of these progenitors is likely to yield new therapeutic approaches that could successfully repair cartilage defects. Therefore, in this review, we discussed the distribution and function, preclinical and clinical applications, and future perspectives of stem/progenitor cells isolated from three types of synovial joint tissues: articular cartilage, synovial membrane and synovial fluid.

In vivo Distribution of Progenitors in the Synovial Joint

The synovial joint comprises different tissues enclosed within the joint capsule, including articular cartilage, bone, synovium, ligaments, and the fibrous capsule. The following techniques have been employed to determine the distribution of progenitors in the synovial joint: 1) DNA labeling methods to detect the slow-cycling cells [30–32]; 2) transgenic animal models to track the fate of progenitors [33]; 3) immunohistological analysis to detect the distribution of putative stem cell markers [34–38].

Tissue Distribution of Progenitors in the Synovial Joint

The distribution of stem/progenitor cells in synovial joint tissues has been studied in some rodent species, such as rat, mouse and rabbit. Progenitors are slowly proliferating cells *in vivo*; therefore, DNA labeling agents, such as [^{3}H]-thymidine, bromodeoxyuridine (BrdU) and 5-ethynyl-2′-deoxyuridine (EdU), are useful to identify their localization due to the long-term labeling capacity of these agents in stem/progenitor cells. Usually, the distribution of cells that retain a long-term label is determined by a pulse-chase method.

In embryonic and young rats, cells with long-term [^{3}H]-thymidine-labeling were observed in the proximal portion of the growth plate, the perichondrial ring and the surface of articular cartilage [30]. Similarly, in rabbit joints, long-term BrdU-positive cells were observed in the germinal zone of the growth plate, the perichondrial groove of Ranvier and all the zones of articular cartilage [31]. In the developing synovial joints of mice, long-term EdU-labeled cells were predominantly present at the surface zone of the articular cartilage, but were also detectable in the other areas of articular cartilage, the perichondrium/periosteum and the synovium [32]. Collectively, these studies showed that stem/progenitor cells reside in the articular cartilage and its adjoining tissues.

Tissue Distribution of Progenitors in the Articular Cartilage

The articular cartilage consists of histologically distinct zones with different cell populations: the superficial zone, the transition zone, the deep zone, and the calcified zone [39]. During development, articular cartilage grows appositionally from the articular surface, indicating that the superficial zone contains progenitor cells that provide transit-amplifying progeny for growth [40]. Using a transgenic mouse model, Kozhemyakina *et al*. tracked the fate of articular cartilage progenitor cells and found that the $Prg4^+$ cells, a progenitor population for the deeper layers of mature articular cartilage, were observed at the joint surface in the embryo [33].

Several putative stem cell markers have been used to determine the location of progenitors in the cartilage. CD166, a putative marker of articular cartilage stem/progenitor cells, was primarily located in the superficial and middle zones of cartilage [34]. Interestingly, the expression of Notch-1, a cell surface marker of *in vitro* cultured cartilage progenitors, was observed in all zones of human articular cartilage [35]. Likewise, other putative MSCs markers, such as CD90, Stro-1, Oct-3/4, and CD105, were found in various zones of human cartilage [36]. From the superficial to the deep zone of articular cartilage, a high percentage of cells were positive for Notch-1, Stro-1 and VCAM-1 [37]. In view of the low frequency of stem/progenitor cells in the cartilage, these abundantly expressed cell markers may not be sufficient to determine the precise location of stem/progenitor cells; thus, further investigation is needed.

Tissue Distribution of MSCs in the Synovial Membrane

The synovial membrane has two layers: the synovial lining and the sub-intimal layer. The synovial lining is comprised of fibroblast-like synoviocytes and macrophage-like synoviocytes, while the sub-intimal space contains fibrous tissue, blood vessels and immune cells [41]. *In vitro*, the ultrastructural and immunocytochemical features of synovial membrane-derived MSCs (SM-MSCs) are similar to the fibroblast-like synoviocytes, indicating that they may originate from the synovial lining [42].

In a mouse model of joint-surface injury, SM-MSCs have been identified in the lining layer and the subsynovial tissue of synovium [43]. In healthy humans, SM-MSCs were found to be localized in the subintimal zone of the synovial membrane [44]. In patients with osteoarthritis, however, the distribution of SM-MSCs was more diffuse; they have been found around veins in the perivascular matrix [44], as well as in synovial surface projections [38].

Response of Stem/Progenitor Cells to Cartilage Injury

To maintain tissue homeostasis, adult stem cells undergo asymmetric cell division to self-renew and generate functional cells to replenish the dead ones [45]; this process can be intensified by injury signals to restore tissue function. After cartilage injury, stem/progenitor cells in the joint tissues, such as synovium, undergo proliferation and serve as a pool of reparative cells [43, 46–48].

In vivo Studies

Partial-thickness articular cartilage injuries (PTCIs) provide a useful model to study the intrinsic reparative response of resident stem/progenitor cells in the synovial joint [46, 47]. Notably, in the PTCIs model, cartilage defects do not penetrate the subchondral bone; and consequently, there is no infiltration of bone marrow cells from the subchondral bone, which usually serves as the major source of reparative cells in full-thickness cartilage defects [52, 53].

In immature rats, the repair response occurs immediately after PTCIs, and progenitor cells, identified as $CD105^+$ and $CD166^+$ cells, have been found in the superficial and transitional zones of the reparative cartilage tissue [46]. In mature rats, putative progenitor cells, defined as $CD105^+$ or BrdU-label-retaining cells, have been found around the injury sites of PTCIs and increased during the repair process, indicating that cartilage injury activated the proliferation of these progenitors [47]. Although there is no recruitment of BM-MSCs from the subchondral bone in PTCIs, the precise tissue origin of these reparative stem/progenitor cells remains uncertain.

The synovium is highly responsive to cartilage injury. MSCs residing in the synovium have been shown to respond to full-thickness articular cartilage injury through cell proliferation and chondrogenesis [43]. At PTCIs, the recruitment of repair cells from the synovial membrane was evident, as shown by a continuous layer of mesenchymal cells extending from the synovial membrane across the surface of normal articular cartilage into the defect [48]. Additionally, the recruited synovial cells were able to differentiate into chondrocytes when stimulated by TGF-β_1 [49]. Collectively, these data clearly showed that the synovium contributes to articular cartilage repair in both full- and partial-thickness defects.

Synovial fluid (SF) is a clear, viscous, hyaluronic acid-rich liquid in contact with the synovial membrane and articular cartilage [54]. It provides a route for exogenous stem cells to access cartilage defects after intra-articular injection [55]. In normal joints, the number of synovial fluid-derived MSCs (SF-MSCs) is very low, but it increases notably under injury or in osteoarthritic conditions [50, 51].

Ex vivo Studies

Cartilage explants are very helpful for investigating the reparative mechanism of cartilage injury. Following a mechanical injury, two repair mechanisms have been described in porcine cartilage explants: 1) proliferation of cells close to the injured cartilage, and 2) chemotactic migration of cells toward the injured surface followed by binding to the repair tissue [56]. Likewise, in blunt-impact-injured cartilage explants, nonviable areas were repopulated by MSC-like cells migrating from the surrounding matrix [57], and this repair response can be enhanced by stromal cell-derived factor 1 alpha through stimulation of the recruitment of local cartilage progenitors [58]. At the wound edge of explants, cells responsible for neocartilage formation originated from the deep zone of cartilage [59]. Taken together, these studies revealed that tissue-resident progenitors were actively engaged in the repair of injured cartilage.

Biological Characteristics

Articular Cartilage-Derived Mesenchymal Progenitor Cells (AC-MPCs)

Chondrocytes represent a highly differentiated cell type that producing cartilage-specific extracellular matrix, and they have long been regarded as the only cell type in articular cartilage. In 2002, Dowthwaite *et al.* first isolated AC-MPCs, a cell type with multi-differentiation potential, from the surface of bovine articular cartilage by a method of differential cell adhesion to fibronectin-coated dishes (termed as the fibronectin adhesion assay). They observed that AC-MPCs formed large numbers of colonies from an initially small seeding density and expressed $\alpha5\beta1$ integrin and Notch-1 [60]. Subsequently, they found that AC-MPCs exhibited phenotypic plasticity in an embryonic chick tracking system [61]. Similarly, AC-MPCs isolated from human articular cartilage have been shown to be multipotent under specific induction conditions, and to express a panel of cell surface markers typical of MSCs (Table 1). Based on the similarities with MSCs, in literature AC-MPCs are also referred to as articular cartilage-derived mesenchymal stem cells [62, 103, 105].

In addition to the fibronectin adhesion assay, other techniques, as shown in Fig. 1, have been applied to isolate AC-MPCs, including the Hoechst 33,342 exclusion assay and the cell sorting techniques. The Hoechst 33,342 exclusion assay is a valuable technique for identifying and sorting AC-MPCs because of the property of adult stem/progenitor cells to exclude this DNA binding dye. The cell sorting techniques are used to isolate AC-MPCs based on the positive and/or negative expression of specific cell surface markers. Interestingly, due to differences in the isolation methods, variations in some of the biological properties of AC-MPCs, such as differentiation potency, were presented. For instance, AC-MPCs isolated by the Hoechst 33,342 exclusion assay have been shown to be osteogenic and chondrogenic, but not adipogenic, after *in vitro* induction [37]. Additionally, the proliferation and differentiation abilities among AC-MPCs subpopulations have been shown to vary enormously [62].

AC-MPCs have greater chondrogenic potency than BM-MSCs [63] and adipose-derived MSCs [28, 64, 65]. Unlike the full-depth chondrocyte populations, AC-MPCs preserved chondrogenicity after extensive expansion [66]. Notably, AC-MPCs did not obtain a hypertrophic cartilage phenotype after chondrogenic induction, and could thus form stable hyaline cartilage without calcification [67].

SM-MSCs

The surface markers of SM-MSCs are similar to those of BM-MSCs (Table 1), but the expression is influenced by various factors such as cell passage number [68, 69]. SM-MSCs are multipotent, and their differentiation ability is not influenced by donor age, cell passage or cryopreservation [70]. SM-MSCs are highly clonogenic, with a clone forming efficiency more than 100-fold higher than that of BM-MSCs [71, 72]. Furthermore, SM-MSCs isolated by different methods (Fig. 1) have robust *in vitro* expandability; when subcultured at low density, SM-MSCs retained proliferation ability after extensive expansion [71].

SM-MSCs possess greater chondrogenic ability than MSCs derived from extra-joint tissues, such as the adipose tissue [73], bone marrow [71] and umbilical cord [20]. It is reported that gene expression profiles of chondrocytes and SM-MSCs are closer to each other than those of extra-articular tissue-derived MSCs [74]. At the single-cell level, SM-MSCs are heterogeneous in chondrogenic potency [75]. Therefore, as shown in several studies, an enriched subpopulation of SM-MSCs could be more efficient for chondrogenic differentiation than the mixed SM-MSCs populations [69, 76, 77].

SF-MSCs

SF-MSCs are fibroblast-like cells with a phenotype similar to that of BM-MSCs (Table 1). SF-MSCs are usually isolated by the method of direct cell seeding in the culture flask (Fig. 1), and they are highly proliferative *in vitro*. For instance, most bovine SF-MSCs could expand for at least 1 million-fold [78]. The proliferation ability and the expression of pluripotent transcription factors of SF-MSCs were higher than BM-MSCs [79].

In contrast to the proliferation ability, SF-MSCs were inferior in adipogenic, osteogenic and neurogenic differentiation

Table 1 Multipotency and cell surface markers of AC-MPCs, SM-MSCs and SF-MSCs

Cells	Isolation method	Species	Sample	Differentiation	Cell markers	Ref.
AC-MPCs	Fibronectin adhesion assay	Calf	Normal AC	Phenotypic plasticity in an embryonic chick tracking system	Positive: Notch-1, α5β1 integrin subunits	[60] [61]
	Fibronectin adhesion assay	Human	Normal and OA AC	Osteo, Chondro and Adipo	Positive: CD90, STRO-1, Notch-1, Delta 1, Jagged 1, collagen type II (Col II), 2B6, aggrecan, Sox9, collagen type I (Col I)	[62] [66]
	Immunomagnetic selection for $CD105^+/CD166^+$ cells	Human	Normal and OA AC	Osteo, Chondro and Adipo	Positive: CD105, CD166, aggrecan, Col I; Negative: Col II, cartilage-derived morphogenetic protein 1	[101]
	FACS selection for $CD9^+/CD90^+/CD166^+$ cells	Human	OA AC	Osteo, Chondro and Adipo	Not analyzed	[102]
	FACS selection for $CD146^+$ cells	Human	OA AC	Osteo, Chondro and Adipo	Positive: CD44, CD146, CD73, CD90, CD105, HLA-ABC; Negative: CD34, CD45, HLA-DR	[28]
	Hoechst 33,342 exclusion assay	Calf	Normal AC	Chondro	Not analyzed	[103]
	Hoechst 33,342 exclusion assay	Human	Normal and OA AC	Osteo and Chondro	Not analyzed	[37]
	Single cell sorting combined with clonogenicity screening	Cattle	Normal AC	Osteo, Chondro and Adipo	Positive: ATP-binding cassette sub-family G member 2 (ABCG2), Notch-1	[104]
	Direct cell seeding after enzymatic cell release	Human	Embryonic and fetal AC	Osteo, Chondro, Adipo, neurons and islet-like cells	Positive: Oct4, Nanog, Sox2, SSEA-3, SSEA-4, CD29, CD44, CD90, CD105, CD73, Stor-1; Negative: CD34, CD117, CD271	[63] [105]
SM-MSCs	Direct cell seeding after enzymatic cell release	Human	SM of healthy, injured and OA joints	Osteo, Chondro and Adipo	Positive: CD44, CD73, CD90, CD105, CD147, CD166; Negative: CD14, CD34, CD45, CD117, CD31	[68] [70] [71] [106]
	Tissue culture method	Horse	SM of healthy, osteochondritis dissecans and OA joints	Osteo, Chondro and Adipo	Positive: CD90, CD44; Negative: Oct4, Nanog, CD105, CD34	[107]
	Direct cell seeding after enzymatic cell release	Sheep	SM of normal joints	Osteo, Chondro and Adipo	Positive: CD44, MHC-I; Negative: CD11b, CD45	[108]
	Hoechst 33,342 exclusion assay	Calf	SM of normal joints	Osteo, Chondro and myogenic differentiation	Positive: CD34, Flk-1, c-Kit, Abcg-2, Mdr-1	[109]
	Separation of CD14-negative cells	Pig	SM of knee joints	Chondro	Positive: CD44, CD90, cadherin-11; Negative: CD14	[76]
SF-MSCs	Direct cell seeding in the culture flask	Human	SF from normal, OA, RA and osteochondral lesion patients	Osteo, Chondro and Adipo	Positive: CD13, CD105, CD55, CD10, CD90; Negative: CD45, CD14, CD34	[80] [110]
	Direct cell seeding in the culture flask	Cow	SF of normal joints	Osteo, Chondro and Adipo	Positive: CD44, CD166; Negative: CD45	[78]
	Direct cell seeding in the culture flask	Pig	SF of normal joints	Osteo, Chondro, Adipo, and neuronal differentiation	Positive: CD29, CD44, CD90; Negative: CD34, CD45, MHC-II	[79]

AC: articular cartilage; Adipo: adipogenic differentiation; Chondro: chondrogenic differentiation; FACS: fluorescence-activated cell sorting; OA: osteoarthritis; Osteo: osteogenic differentiation; RA: rheumatoid arthritis; Ref.: reference(s); SM: synovial membrane; SF: synovial fluid

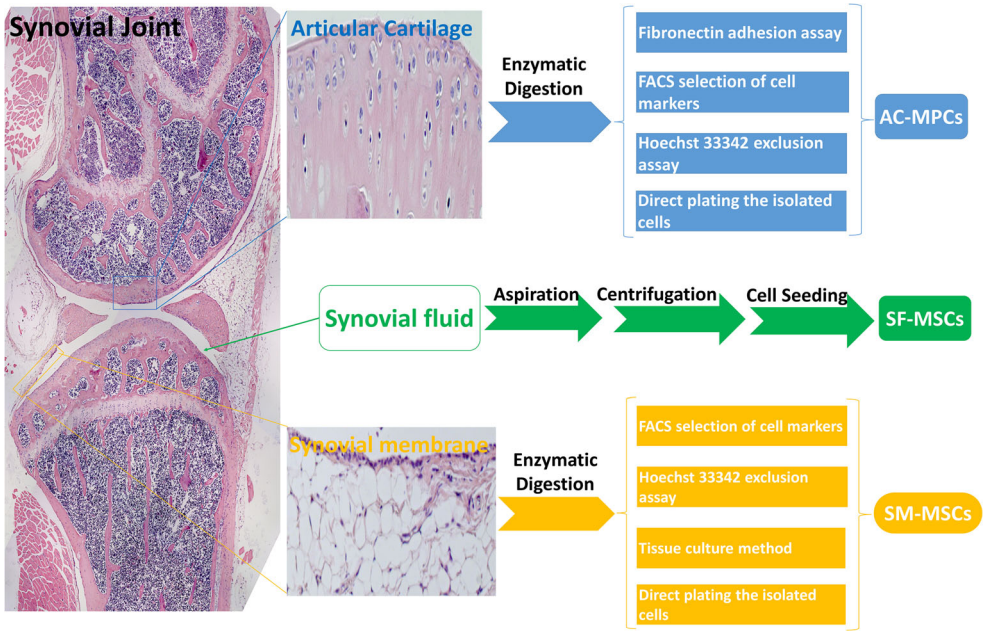

Fig. 1 Schematic representation of the isolation of stem/progenitor cells from articular cartilage, synovial membrane and synovial fluid. AC-MPCs: articular cartilage-derived mesenchymal progenitor cells; SF-MSCs: synovial fluid-derived mesenchymal stem cells; SM-MSCs: synovial membrane-derived mesenchymal stem cells; FACS: fluorescence-activated cell sorting

when compared to BM-MSCs [78, 79]. However, the chondrogenic potential of SF-MSCs was comparable to SM-MSCs [29]. The detailed mechanism of cell-source-dependent variant in the biological properties of MSCs has not been fully understood. Gene profiles indicated that SF-MSCs were more similar to SM-MSCs than to BM-MSCs and adipose tissue-derived MSCs [80].

Interestingly, the health state of the donor greatly influences the chondrogenic potential of SF-MSCs. For example, SF-MSCs derived from normal joints did not require a micro-mass step for efficient chondrogenesis, while those obtained from osteoarthritic joints needed the micro-mass procedure [81].

Animal Studies

AC-MPCs

Very few animal studies have been conducted to determine the cartilage repair potential of AC-MPCs (Table 2). To determine the *in vivo* plasticity of AC-MPCs, fluorescent PKH26 labeled cells were injected into the thigh muscle of severe-combined immunodeficient (SCID) mice; at two weeks post implantation, no robust cartilage pellet, but only diffuse cartilage nodules, were found, suggesting that AC-MPCs required further signals for chondrogenic differentiation after ectopic implantation [82].

Nevertheless, in a full-thickness cartilage defect model, the transplantation of autologous AC-MPCs resulted in improved reparative tissue and significantly reduced central osteophyte formation [83]. Moreover, the transplantation of AC-MPCs showed histological repair scores similar to those of full-depth chondrocytes, and both groups showed evidence of collagen type II-positive repair tissue [66].

SM-MSCs

Compared with AC-MPCs, more animal studies have been performed to explore the cartilage repair potential of SM-MSCs (Table 2). After induction in a traditional chondrogenic medium, primed SM-MSCs were unable to form stable hyaline cartilage after ectopic implantation [84, 85]. Interestingly, when transplanted into cartilage defects, SM-MSCs showed high cartilage repair ability in both small and large animal experiments [86–92], indicating that the microenvironment of graft sites influenced greatly on the fate and behaviors of grafted cells.

In a rat osteochondral defect model, it was observed that articular cartilage defects could be repaired by grafted SM-MSCs [86]. Likewise, in rabbit full-thickness osteochondral defects, transplantation of SM-MSCs improved cartilage repair [87–90]. Interestingly, placing a suspension of SM-MSCs on the surface of cartilage defects resulted in rapid adherence of grafted cells and an improved cartilage repair outcome in full-thickness osteochondral defects [91, 92]. Due to the promising outcomes and the simple treatment procedure, this cell delivery strategy is attractive for clinical application.

In partial-thickness chondral defects (PTCDs), however, transplanted cells have difficulty in attaching to the surface of lesions, which may be due to the anti-adhesive properties of proteoglycan, a rich component of the cartilage matrix

Table 2 Animal studies of AC-MPCs, SM-MSCs and SF-MSCs for cartilage repair

Cells	Animal model	Transplantation method	Results	Ref.
AC-MPCs	SCID mice	Intramuscular injection of cell suspension.	Cells survived post-implantation, but failed to create a robust cartilage pellet; diffuse cartilage nodules were found.	[82]
	Equine FTODs	Cells plus fibrin were grafted at the defect sites.	Autologous AC-MPCs group showed better results than the empty-defect group, the fibrin only group and the allogenic AC-MPCs group.	[83]
	Goat FTODs	Cells were seeded on collagen membrane and then grafted at the defect sites.	The AC-MPCs group and the full-depth chondrocyte group showed comparable histological repair scores.	[66]
SM-MSCs	Porcine PTCDs	Scaffold-free cell/matrix complex was implanted at the defect sites.	The repair tissue stained positive for Safranin O and collagen II, exhibited mechanical property similar to that of normal cartilage.	[94]
	Rabbit FTODs	Defects were filled with cells and covered with periosteum.	SM-MSCs improved histological scores, produced abundant cartilage matrix, and changed over a time course according to the tissue microenvironment.	[87]
	Rabbit FTODs	Cell suspension was placed on the cartilage defects for 10 min.	Placing an SM-MSC suspension on the cartilage defect for 10 min resulted in adherence of >60% of cells to the defect and promoted cartilage regeneration.	[91]
	Rabbit FTODs	Defects were filled with premature cartilage engineered by SM-MSCs.	Defects were repaired with hyaline-like cartilage that integrates with the native tissue.	[88]
	Porcine PTCDs	Defects were implanted with tissue-engineered constructs (TECs) of allogenic SM-MSCs.	TECs promoted the repair of chondral lesions in both immature and mature pigs without immune reaction; the repaired tissue exhibited similar viscoelastic properties to normal cartilage regardless of skeletal maturity.	[95]
	Rat FTODs	Intra-articular injection of cell suspension.	SM-MSCs accumulated at the osteochondral defect; the regeneration of the articular cartilage was confirmed.	[86]
	Porcine PTCDs	TECs derived from allogenic SM-MSCs were implanted at the defects.	The superficial zone of TEC-mediated tissue was fibrocartilage-like and exhibited compromised mechanical properties; the middle or deep zones of TEC-mediated tissue were more hyaline cartilage-like.	[97]
	Porcine FTODs	Cell suspensions were placed on the defects for 10 min.	Transplantation of SM-MSCs resulted in better outcomes than the control knees (without cell transplantation).	[92]
	Rabbit FTODs	Defects were filled with SM-MSC aggregates.	Transplantation of SM-MSC aggregates at relatively low density achieved successful cartilage regeneration, while high density transplantation failed to regenerate cartilage.	[89]
	Rabbit FTODs	Defects were filled with SM-MSCs embedded in PRP.	SM-MSCs embedded in PRP gel successfully resurfaced the defect with cartilage and restored the subchondral bone.	[90]
	Rabbit PTCDs	Intra-articular injection of cell suspension.	Exposure to fibronectin enhanced the attachment of SM-MSCs to partial-thickness chondral defects; the tissue regenerated by SM-MSCs was not hyaline cartilage.	[93]
	Porcine PTCDs	TECs derived from allogenic SM-MSCs were implanted at the defects.	The repair tissue was well integrated with the adjacent host cartilage; no significant differences in histological scores between the integration boundary and the center of the repair tissue.	[96]
SF-MSCs	Porcine FTODs	SF-MSC-laden PRP was used to fill in the defects.	SF-MSC-laden PRP increased cell growth and maturation of chondrocytes compared with the PRP only group.	[98]

PTCDs: partial-thickness chondral defects; FTODs: full-thickness osteochondral defects; Ref.: reference(s)

existing at the surface of PTCDs [93]. Nakamura and his colleagues evaluated the reparative ability of allogenic SM-MSCs in a porcine PTCDs model [94–97]. They first generated 3-dimensional scaffold-free tissue engineered constructs (TECs) from allogenic SM-MSCs *in vitro* and then implanted TECs into the PTCDs; the results showed that TECs promoted the repair of chondral lesions, and the reparative tissue exhibited mechanical properties similar to normal cartilage in static compression, friction and unconfined compression tests [94, 95]. However, further analysis revealed some compromised mechanical properties of the reparative tissue: 1) the surface stiffness of the reparative tissue, measured by a micro-indentation analysis, was significantly lower than that of normal cartilage [97], and 2) the tensile strength of the integration boundary between native cartilage and reparative tissue was significantly lower than that of uninjured cartilage [96]. Hence, further investigations are needed to improve the integrity of reparative tissue.

SF-MSCs

Only one report has determined the cartilage repair ability of SF-MSCs (Table 2). In this study, the SF-MSC-laden platelet-rich plasma (PRP) hydrogel possessed better therapeutic

potential than PRP hydrogel alone in repairing porcine osteochondral defects, which was evidenced by an increase in cell growth and the maturation of chondrocytes [98].

Clinical Trials

Clinical trials focusing on cartilage regeneration by articular cartilage-, synovial membrane- and synovial fluid-derived stem/progenitor cells are very limited. According to the clinical trials database (www.clinicaltrials.gov), to date (Jun 1, 2017), there is only one completed trial (Identifier: NCT01879046) that explored the cartilage repair potential of SF-MSCs, while there are no trials investigating AC-MPC- or SM-MSC-based cartilage repairs.

In literature, two studies have reported the clinical application of AC-MPCs and SM-MSCs for cartilage repair [99, 100], but there were no clinical results regarding SF-MSC-based cartilage regeneration. For AC-MPCs, Jiang and colleagues obtained a population of AC-MPCs from fully differentiated human articular chondrocytes and evaluated their repair ability for large knee cartilage defects in 15 patients; the clinical outcomes of AC-MPCs implantation were highly encouraging [99]. Concerning SM-MSCs, it was reported that transplantation of SM-MSCs could improve the clinical outcomes of patients with a symptomatic single cartilage lesion of the femoral condyle, in terms of magnetic resonance imaging score, qualitative histology and clinical evaluation scores [100].

Current Challenges and Future Perspectives

The high chondrogenic potential of AC-MPCs, SM- and SF-MSCs makes them promising graft cells for cartilage repair. However, many questions need to be addressed before extensive clinical application. Particular attention should be paid to the following unanswered questions: 1) How can a sufficient amount of the aforementioned stem/progenitor cells with high therapeutic potential be obtained? 2) How can the therapeutic potential of grafted cells be promoted/enhanced? 3) What is the repair mechanism? 4) How safe and efficient are these strategies?

First, obtaining a clinically relevant number of cells is an important premise for cell-based therapy. The small number of progenitors in articular cartilage and the synovial fluid hampers the acquisition of a sufficient number of cells. Furthermore, clinical-grade cell expansion protocols for the aforementioned stem/progenitor cells have yet to be successfully developed. Hence, further studies are needed to establish protocols that comply with good manufacturing practices; and it is necessary to determine the quality and therapeutic potential of cells after extensive expansion. For instance, detailed evaluations of the genetic stability, phenotype, differentiation potential, migration ability, and paracrine effects are suggested.

Second, it is necessary to develop new methods that can induce stable cartilage formation *in vivo*. The ability of grafted cells to maintain a chondrocyte phenotype and thus to produce hyaline cartilage-specific extracellular matrix is critical for articular cartilage repair. Traditional chondrogenic induction protocols often result in transient cartilage formation. To promote the therapeutic potential of graft cells, smart strategies that mimic the development of articular cartilage, such as a combination of mechanical stimulation and growth factors, are required to produce permanent articular cartilage.

Third, many questions need to be answered at the preclinical level, especially the safety and efficiency of these cell-based cartilage repair approaches. Although previous animal studies have shown improved outcomes after cell transplantation, the fate and repair mechanism of grafted progenitors remains largely unclear. A combination of stable cell labeling techniques and non-invasive cell tracking methods, such as magnetic resonance imaging, is proposed to monitor the fate of implanted cells for a long-term period.

Finally, high quality clinical trials are still missing. Although promising results have been shown in some pilot clinical cases, the clinical evidence is still very limited due to a small patient population and a short-term follow-up [99, 100]. Therefore, the efficiency of these cell-based treatments needs to be further confirmed by reliable clinical data from double-blind, controlled, prospective and multicenter studies with long-term follow-up, especially clinical studies comparing the aforementioned stem cell-based strategies with traditional treatments, such as the arthroscopic procedures.

Acknowledgements This work was financially supported by the National Natural Science Foundation of China (Grant Nos. 31600792, U1613224 and 31570970).

Compliance with Ethical Standards

Disclosures The authors declare no conflicts of interest.

References

1. Yamamoto, T., Wakitani, S., Imoto, K., et al. (2004). Fibroblast growth factor-2 promotes the repair of partial thickness defects of articular cartilage in immature rabbits but not in mature rabbits. *Osteoarthritis and Cartilage, 12*(8), 636–641.
2. Hembry, R. M., Dyce, J., Driesang, I., et al. (2001). Immunolocalization of matrix metalloproteinases in partial-thickness defects in pig articular cartilage. A preliminary report. *Journal of Bone & Joint Surgery, American, 83-A*(6), 826–838.
3. Masahiko, T., Damle, S., Penmatsa, M., et al. (2012). Temporal changes in collagen cross-links in spontaneous articular cartilage repair. *Cartilage, 3*(3), 278–287.

4. Sellers, R. S., Zhang, R., Glasson, S. S., et al. (2000). Repair of articular cartilage defects one year after treatment with recombinant human bone morphogenetic protein-2 (rhBMP-2). *Journal of Bone & Joint Surgery, American, 82*(2), 151–160.
5. Mithoefer, K., McAdams, T., Williams, R. J., Kreuz, P. C., & Mandelbaum, B. R. (2009). Clinical efficacy of the microfracture technique for articular cartilage repair in the knee: an evidence-based systematic analysis. *The American Journal of Sports Medicine, 37*(10), 2053–2063.
6. Case, J. M., & Scopp, J. M. (2016). Treatment of articular cartilage defects of the knee with microfracture and enhanced microfracture techniques. *Sports Medicine & Arthroscopy Review, 24*(2), 63–68.
7. Richter, D. L., Schenck Jr., R. C., Wascher, D. C., & Treme, G. (2016). Knee Articular Cartilage Repair and Restoration Techniques: A Review of the Literature. *Sports Health, 8*(2), 153–160.
8. Brittberg, M., Lindahl, A., Nilsson, A., Ohlsson, C., Isaksson, O., & Peterson, L. (1994). Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. *The New England Journal of Medicine, 331*(14), 889–895.
9. Brittberg, M. (2008). Autologous chondrocyte implantation–technique and long-term follow-up. *Injury, 39*(Suppl 1), S40–S49.
10. Schnabel, M., Marlovits, S., Eckhoff, G., et al. (2002). Dedifferentiation-associated changes in morphology and gene expression in primary human articular chondrocytes in cell culture. *Osteoarthritis and Cartilage, 10*(1), 62–70.
11. Darling, E. M., & Athanasiou, K. A. (2005). Rapid phenotypic changes in passaged articular chondrocyte subpopulations. *Journal of Orthopaedic Research, 23*(2), 425–432.
12. Dell'Accio, F., De Bari, C., & Luyten, F. P. (2001). Molecular markers predictive of the capacity of expanded human articular chondrocytes to form stable cartilage in vivo. *Arthritis & Rheumatology, 44*(7), 1608–1619.
13. Dell'Accio, F., De Bari, C., & Luyten, F. P. (2003). Microenvironment and phenotypic stability specify tissue formation by human articular cartilage-derived cells in vivo. *Experimental Cell Research, 287*(1), 16–27.
14. Rackwitz, L., Djouad, F., Janjanin, S., Nöth, U., & Tuan, R. S. (2014). Functional cartilage repair capacity of de-differentiated, chondrocyte- and mesenchymal stem cell-laden hydrogels in vitro. *Osteoarthritis and Cartilage, 22*(8), 1148–1157.
15. Dominici, M., Le Blanc, K., Mueller, I., et al. (2006). Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy, 8*(4), 315–317.
16. Chen, J. Y., Mou, X. Z., Du, X. C., & Xiang, C. (2015). Comparative analysis of biological characteristics of adult mesenchymal stem cells with different tissue origins. *Asian Pacific Journal of Tropical Medicine, 8*(9), 739–746.
17. Somal, A., Bhat, I. A., B, I., et al. (2016). A comparative study of growth kinetics, in vitro differentiation potential and molecular characterization of fetal adnexa derived caprine mesenchymal stem cells. *PloS One, 11*(6), e0156821.
18. Liu, R., Chang, W., Wei, H., & Zhang, K. (2016). Comparison of the Biological Characteristics of Mesenchymal Stem Cells Derived from Bone Marrow and Skin. *Stem Cells International, 2016*, 3658798.
19. Li, C. Y., Wu, X. Y., Tong, J. B., et al. (2015). Comparative analysis of human mesenchymal stem cells from bone marrow and adipose tissue under xeno-free conditions for cell therapy. *Stem Cell Research & Therapy, 6*, 55.
20. Islam, A., Hansen, A. K., Mennan, C., & Martinez-Zubiaurre, I. (2016). Mesenchymal stromal cells from human umbilical cords display poor chondrogenic potential in scaffold-free three dimensional cultures. *European Cells & Materials Journal, 31*, 407–424.
21. Bernardo, M. E., Emons, J. A., Karperien, M., et al. (2007). Human mesenchymal stem cells derived from bone marrow display a better chondrogenic differentiation compared with other sources. *Connective Tissue Research, 48*(3), 132–140.
22. Huang, Y. C., Zhu, H. M., Cai, J. Q., et al. (2012). Hypoxia inhibited the spontaneous calcification of bone marrow derived mesenchymal stem cells. *Journal of Cellular Biochemistry, 113*(4), 1407–1415.
23. Farrell, E., Both, S. K., Odörfer, K. I., et al. (2011). In-vivo generation of bone via endochondral ossification by in-vitro chondrogenic priming of adult human and rat mesenchymal stem cells. *BMC Musculoskeletal Disorders, 12*, 31.
24. Pelttari, K., Winter, A., Steck, E., et al. (2006). Premature induction of hypertrophy during in vitro chondrogenesis of human mesenchymal stem cells correlates with calcification and vascular invasion after ectopic transplantation in SCID mice. *Arthritis & Rheumatology, 54*(10), 3254–3266.
25. Yang, W., Both, S. K., van Osch, G. J., Wang, Y., Jansen, J. A., & Yang, F. (2015). Effects of in vitro chondrogenic priming time of bone-marrow-derived mesenchymal stromal cells on in vivo endochondral bone formation. *Acta Biomaterialia, 13*, 254–265.
26. Serafini, M., Sacchetti, B., Pievani, A., et al. (2014). Establishment of bone marrow and hematopoietic niches in vivo by reversion of chondrocyte differentiation of human bone marrow stromal cells. *Stem Cell Research, 12*(3), 659–672.
27. Van der Stok, J., Koolen, M. K., Jahr, H., et al. (2014). Chondrogenically differentiated mesenchymal stromal cell pellets stimulate endochondral bone regeneration in critical-sized bone defects. *European Cells & Materials Journal, 27*, 137–148.
28. Su, X., Zuo, W., Wu, Z., et al. (2015). CD146 as a new marker for an increased chondroprogenitor cell sub-population in the later stages of osteoarthritis. *Journal of Orthopaedic Research, 33*(1), 84–91.
29. Ando, W., Kutcher, J. J., Krawetz, R., et al. (2014). Clonal analysis of synovial fluid stem cells to characterize and identify stable mesenchymal stromal cell/mesenchymal progenitor cell phenotypes in a porcine model: a cell source with enhanced commitment to the chondrogenic lineage. *Cytotherapy, 16*(6), 776–788.
30. Ohlsson, C., Nilsson, A., Isaksson, O., & Lindahl, A. (1992). Growth hormone induces multiplication of the slowly cycling germinal cells of the rat tibial growth plate. *Proceedings of the National Academy of Sciences of the United States of America, 89*(20), 9826–9830.
31. Karlsson, C., Thornemo, M., Henriksson, H. B., & Lindahl, A. (2009). Identification of a stem cell niche in the zone of Ranvier within the knee joint. *Journal of Anatomy, 215*(3), 355–363.
32. Candela, M. E., Cantley, L., Yasuaha, R., Iwamoto, M., Pacifici, M., & Enomoto-Iwamoto, M. (2014). Distribution of slow-cycling cells in epiphyseal cartilage and requirement of β-catenin signaling for their maintenance in growth plate. *Journal of Orthopaedic Research, 32*(5), 661–668.
33. Kozhemyakina, E., Zhang, M., Ionescu, A., et al. (2015). Identification of a Prg4-expressing articular cartilage progenitor cell population in mice. *Arthritis & Rheumatology, 67*(5), 1261–1273.
34. Pretzel, D., Linss, S., Rochler, S., et al. (2011). Relative percentage and zonal distribution of mesenchymal progenitor cells in human osteoarthritic and normal cartilage. *Arthritis Research & Therapy, 13*(2), R64.
35. Ustunel, I., Ozenci, A. M., Sahin, Z., et al. (2008). The immunohistochemical localization of notch receptors and ligands in human articular cartilage, chondroprogenitor culture and ultrastructural characteristics of these progenitor cells. *Acta Histochemica, 110*(5), 397–407.

36. Ozbey, O., Sahin, Z., Acar, N., et al. (2014). Characterization of colony-forming cells in adult human articular cartilage. *Acta Histochemica, 116*(5), 763–770.
37. Grogan, S. P., Miyaki, S., Asahara, H., D'Lima, D. D., & Lotz, M. K. (2009). Mesenchymal progenitor cell markers in human articular cartilage: normal distribution and changes in osteoarthritis. *Arthritis Research & Therapy, 11*(3), R85.
38. Giurea, A., Rüger, B. M., Hollemann, D., Yanagida, G., Kotz, R., & Fischer, M. B. (2006). STRO-1+ mesenchymal precursor cells located in synovial surface projections of patients with osteoarthritis. *Osteoarthritis and Cartilage, 14*(9), 938–943.
39. Klein, T. J., Malda, J., Sah, R. L., & Hutmacher, D. W. (2009). Tissue engineering of articular cartilage with biomimetic zones. *Tissue Engineering Part B Reviews, 15*(2), 143–157.
40. Hayes, A. J., MacPherson, S., Morrison, H., Dowthwaite, G., & Archer, C. W. (2001). The development of articular cartilage: evidence for an appositional growth mechanism. *Anatomy and Embryology (Berlin), 203*(6), 469–479.
41. Bartok, B., & Firestein, G. S. (2010). Fibroblast-like synoviocytes: key effector cells in rheumatoid arthritis. *Immunological Reviews, 233*(1), 233–255.
42. Vandenabeele, F., De Bari, C., Moreels, M., et al. (2003). Morphological and immunocytochemical characterization of cultured fibroblast-like cells derived from adult human synovial membrane. *Archives of Histology and Cytology, 66*(2), 145–153.
43. Kurth, T. B., Dell'accio, F., Crouch, V., Augello, A., Sharpe, P. T., & De Bari, C. (2011). Functional mesenchymal stem cell niches in adult mouse knee joint synovium in vivo. *Arthritis & Rheumatology, 63*(5), 1289–1300.
44. Hermida-Gómez, T., Fuentes-Boquete, I., Gimeno-Longas, M. J., et al. (2011). Quantification of cells expressing mesenchymal stem cell markers in healthy and osteoarthritic synovial membranes. *The Journal of Rheumatology, 38*(2), 339–349.
45. Chen, C., Fingerhut, J. M., & Yamashita, Y. M. (2016). The in-s(ide) and outs(ide) of asymmetric stem cell division. *Current Opinion in Cell Biology, 43*, 1–6.
46. Mukoyama, S., Sasho, T., Akatsu, Y., et al. (2015). Spontaneous repair of partial thickness linear cartilage injuries in immature rats. *Cell and Tissue Research, 359*(2), 513–520.
47. Zhang, K., Shi, J., Li, Y., et al. (2016). Chondrogenic cells respond to partial-thickness defects of articular cartilage in adult rats: an in vivo study. *Journal of Molecular Histology, 47*(3), 249–258.
48. Hunziker, E. B., & Rosenberg, L. C. (1996). Repair of partial-thickness defects in articular cartilage: cell recruitment from the synovial membrane. *Journal of Bone & Joint Surgery, American, 78*(5), 721–733.
49. Hunziker, E. B. (2001). Growth-factor-induced healing of partial-thickness defects in adult articular cartilage. *Osteoarthritis and Cartilage, 9*(1), 22–32.
50. Morito, T., Muneta, T., Hara, K., et al. (2008). Synovial fluid-derived mesenchymal stem cells increase after intra-articular ligament injury in humans. *Rheumatology (Oxford, England), 47*(8), 1137–1143.
51. Matsukura, Y., Muneta, T., Tsuji, K., Koga, H., & Sekiya, I. (2014). Mesenchymal stem cells in synovial fluid increase after meniscus injury. *Clinical Orthopaedics and Related Research, 472*(5), 1357–1364.
52. Anraku, Y., Mizuta, H., Sei, A., et al. (2009). Analyses of early events during chondrogenic repair in rat full-thickness articular cartilage defects. *Journal of Bone and Mineral Metabolism, 27*(3), 272–286.
53. Chuma, H., Mizuta, H., Kudo, S., Takagi, K., & Hiraki, Y. (2004). One day exposure to FGF-2 was sufficient for the regenerative repair of full-thickness defects of articular cartilage in rabbits. *Osteoarthritis and Cartilage, 12*(10), 834–842.
54. Swan, A., Amer, H., & Dieppe, P. (2002). The value of synovial fluid assays in the diagnosis of joint disease: a literature survey. *Annals of the Rheumatic Diseases, 61*(6), 493–498.
55. Delling, U., Brehm, W., Metzger, M., Ludewig, E., Winter, K., & Jülke, H. (2015). In vivo tracking and fate of intra-articularly injected superparamagnetic iron oxide particle-labeled multipotent stromal cells in an ovine model of osteoarthritis. *Cell Transplantation, 24*(11), 2379–2390.
56. Skagen, P. S., Kruse, H. A., & Horn, T. (2014). Repair Mechanisms in Articular Cartilage—A Porcine in Vitro Study. *Microscopy Research, 2*(2), 67–80.
57. Seol, D., McCabe, D. J., Choe, H., et al. (2012). Chondrogenic progenitor cells respond to cartilage injury. *Arthritis & Rheumatology, 64*(11), 3626–3637.
58. Yu, Y., Brouillette, M. J., Seol, D., Zheng, H., Buckwalter, J. A., & Martin, J. A. (2015). Use of recombinant human stromal cell-derived factor 1α-loaded fibrin/hyaluronic acid hydrogel networks to achieve functional repair of full-thickness bovine articular cartilage via homing of chondrogenic progenitor cells. *Arthritis & Rheumatology, 67*(5), 1274–1285.
59. Bos, P. K., Kops, N., Verhaar, J. A., & van Osch, G. J. (2008). Cellular origin of neocartilage formed at wound edges of articular cartilage in a tissue culture experiment. *Osteoarthritis and Cartilage, 16*(2), 204–211.
60. Dowthwaite, G. P., Bishop, J. C., Redman, S. N., Thomson, B., & Archer, C. W. (2002). Characterisation of articular cartilage progenitor cells. *European Cells & Materials Journal, 4*, 35–36.
61. Dowthwaite, G. P., Bishop, J. C., Redman, S. N., et al. (2004). The surface of articular cartilage contains a progenitor cell population. *Journal of Cell Science, 117*(Pt 6), 889–897.
62. Nelson, L., McCarthy, H. E., Fairclough, J., Williams, R., & Archer, C. W. (2014). Evidence of a Viable Pool of Stem Cells within Human Osteoarthritic Cartilage. *Cartilage, 5*(4), 203–214.
63. Choi, W. H., Kim, H. R., Lee, S. J., et al. (2016). Fetal cartilage-derived cells have stem cell properties and are a highly potent cell source for cartilage regeneration. *Cell Transplantation, 25*(3), 449–461.
64. Salamon, A., Jonitz-Heincke, A., Adam, S., et al. (2013). Articular cartilage-derived cells hold a strong osteogenic differentiation potential in comparison to mesenchymal stem cells in vitro. *Experimental Cell Research, 319*(18), 2856–2865.
65. Li, Y., Zhou, J., Yang, X., Jiang, Y., & Gui, J. (2016). Intermittent hydrostatic pressure maintains and enhances the chondrogenic differentiation of cartilage progenitor cells cultivated in alginate beads. *Development Growth & Differentiation, 58*(2), 180–193.
66. Williams, R., Khan, I. M., Richardson, K., et al. (2010). Identification and clonal characterisation of a progenitor cell sub-population in normal human articular cartilage. *PloS One, 5*(10), e13246.
67. McCarthy, H. E., Bara, J. J., Brakspear, K., Singhrao, S. K., & Archer, C. W. (2012). The comparison of equine articular cartilage progenitor cells and bone marrow-derived stromal cells as potential cell sources for cartilage repair in the horse. *The Veterinary Journal, 192*(3), 345–351.
68. Fickert, S., Fiedler, J., & Brenner, R. E. (2003). Identification, quantification and isolation of mesenchymal progenitor cells from osteoarthritic synovium by fluorescence automated cell sorting. *Osteoarthritis and Cartilage, 11*(11), 790–800.
69. Li, J., Campbell, D. D., Bal, G. K., & Pei, M. (2014). Can arthroscopically harvested synovial stem cells be preferentially sorted using stage-specific embryonic antigen 4 antibody for cartilage, bone, and adipose regeneration? *Arthroscopy: The Journal of Arthroscopic & Related Surgery, 30*(3), 352–361.
70. De Bari, C., Dell'Accio, F., Tylzanowski, P., & Luyten, F. P. (2001). Multipotent mesenchymal stem cells from adult

human synovial membrane. *Arthritis & Rheumatology, 44*(8), 1928–1942.
71. Sakaguchi, Y., Sekiya, I., Yagishita, K., & Muneta, T. (2005). Comparison of human stem cells derived from various mesenchymal tissues: superiority of synovium as a cell source. *Arthritis & Rheumatology, 52*(8), 2521–2529.
72. Yoshimura, H., Muneta, T., Nimura, A., Yokoyama, A., Koga, H., & Sekiya, I. (2007). Comparison of rat mesenchymal stem cells derived from bone marrow, synovium, periosteum, adipose tissue, and muscle. *Cell and Tissue Research, 327*(3), 449–462.
73. Mochizuki, T., Muneta, T., Sakaguchi, Y., et al. (2006). Higher chondrogenic potential of fibrous synovium- and adipose synovium-derived cells compared with subcutaneous fat-derived cells: distinguishing properties of mesenchymal stem cells in humans. *Arthritis & Rheumatology, 54*(3), 843–853.
74. Segawa, Y., Muneta, T., Makino, H., et al. (2009). Mesenchymal stem cells derived from synovium, meniscus, anterior cruciate ligament, and articular chondrocytes share similar gene expression profiles. *Journal of Orthopaedic Research, 27*(4), 435–441.
75. Karystinou, A., Dell'Accio, F., Kurth, T. B., et al. (2009). Distinct mesenchymal progenitor cell subsets in the adult human synovium. *Rheumatology (Oxford, England), 48*(9), 1057–1064.
76. Bilgen, B., Ren, Y., Pei, M., Aaron, R. K., & Ciombor, D. M. (2009). CD14-negative isolation enhances chondrogenesis in synovial fibroblasts. *Tissue Engineering Part A, 15*(11), 3261–3270.
77. Gullo, F., & De Bari, C. (2013). Prospective purification of a subpopulation of human synovial mesenchymal stem cells with enhanced chondro-osteogenic potency. *Rheumatology (Oxford, England), 52*(10), 1758–1768.
78. Jones, E. A., Crawford, A., English, A., et al. (2008). Synovial fluid mesenchymal stem cells in health and early osteoarthritis: detection and functional evaluation at the single-cell level. *Arthritis & Rheumatology, 58*(6), 1731–1740.
79. Lee, W. J., Hah, Y. S., Ock, S. A., et al. (2015). Cell source-dependent in vivo immunosuppressive properties of mesenchymal stem cells derived from the bone marrow and synovial fluid of minipigs. *Experimental Cell Research, 333*(2), 273–288.
80. Kim, Y. S., Lee, H. J., Yeo, J. E., Kim, Y. I., Choi, Y. J., & Koh, Y. G. (2015). Isolation and characterization of human mesenchymal stem cells derived from synovial fluid in patients with osteochondral lesion of the talus. *The American Journal of Sports Medicine, 43*(2), 399–406.
81. Krawetz, R. J., Wu, Y. E., Martin, L., Rattner, J. B., Matyas, J. R., & Hart, D. A. (2012). Synovial fluid progenitors expressing $CD90^{+}$ from normal but not osteoarthritic joints undergo chondrogenic differentiation without micro-mass culture. *PloS One, 7*(8), e43616.
82. Marcus, P., De Bari, C., Dell'Accio, F., & Archer, C. W. (2014). Articular chondroprogenitor cells maintain chondrogenic potential but fail to form a functional matrix when implanted into muscles of SCID mice. *Cartilage, 5*(4), 231–240.
83. Frisbie, D. D., McCarthy, H. E., Archer, C. W., Barrett, M. F., & McIlwraith, C. W. (2015). Evaluation of articular cartilage progenitor cells for the repair of articular defects in an equine model. *Journal of Bone & Joint Surgery, American, 97*(6), 484–493.
84. De Bari, C., Dell'Accio, F., & Luyten, F. P. (2004). Failure of in vitro-differentiated mesenchymal stem cells from the synovial membrane to form ectopic stable cartilage in vivo. *Arthritis & Rheumatology, 50*(1), 142–150.
85. Vinardell, T., Sheehy, E. J., Buckley, C. T., & Kelly, D. J. (2012). A comparison of the functionality and in vivo phenotypic stability of cartilaginous tissues engineered from different stem cell sources. *Tissue Engineering Part A, 18*(11–12), 1161–1170.
86. Hori, J., Deie, M., Kobayashi, T., Yasunaga, Y., Kawamata, S., & Ochi, M. (2011). Articular cartilage repair using an intra-articular magnet and synovium-derived cells. *Journal of Orthopaedic Research, 29*(4), 531–538.
87. Koga, H., Muneta, T., Ju, Y. J., et al. (2007). Synovial stem cells are regionally specified according to local microenvironments after implantation for cartilage regeneration. *Stem Cells, 25*(3), 689–696.
88. Pei, M., He, F., Boyce, B. M., & Kish, V. L. (2009). Repair of full-thickness femoral condyle cartilage defects using allogeneic synovial cell-engineered tissue constructs. *Osteoarthritis and Cartilage, 17*(6), 714–722.
89. Suzuki, S., Muneta, T., Tsuji, K., et al. (2012). Properties and usefulness of aggregates of synovial mesenchymal stem cells as a source for cartilage regeneration. *Arthritis Research & Therapy, 14*(3), R136.
90. Lee, J. C., Min, H. J., Park, H. J., Lee, S., Seong, S. C., & Lee, M. C. (2013). Synovial membrane-derived mesenchymal stem cells supported by platelet-rich plasma can repair osteochondral defects in a rabbit model. *Arthroscopy: The Journal of Arthroscopic & Related Surgery, 29*(6), 1034–1046.
91. Koga, H., Shimaya, M., Muneta, T., et al. (2008). Local adherent technique for transplanting mesenchymal stem cells as a potential treatment of cartilage defect. *Arthritis Research & Therapy, 10*(4), R84.
92. Nakamura, T., Sekiya, I., Muneta, T., et al. (2012). Arthroscopic, histological and MRI analyses of cartilage repair after a minimally invasive method of transplantation of allogeneic synovial mesenchymal stromal cells into cartilage defects in pigs. *Cytotherapy, 14*(3), 327–338.
93. Lee, J. C., Min, H. J., Lee, S., Seong, S. C., & Lee, M. C. (2013). Effect of chondroitinase ABC on adhesion and behavior of synovial membrane-derived mesenchymal stem cells in rabbit partial-thickness chondral defects. *Journal of Orthopaedic Research, 31*(8), 1293–1301.
94. Ando, W., Tateishi, K., Hart, D. A., et al. (2007). Cartilage repair using an in vitro generated scaffold-free tissue-engineered construct derived from porcine synovial mesenchymal stem cells. *Biomaterials, 28*(36), 5462–5470.
95. Shimomura, K., Ando, W., Tateishi, K., et al. (2010). The influence of skeletal maturity on allogenic synovial mesenchymal stem cell-based repair of cartilage in a large animal model. *Biomaterials, 31*(31), 8004–8011.
96. Fujie, H., Nansai, R., Ando, W., et al. (2015). Zone-specific integrated cartilage repair using a scaffold-free tissue engineered construct derived from allogenic synovial mesenchymal stem cells: Biomechanical and histological assessments. *Journal of Biomechanics, 48*(15), 4101–4108.
97. Ando, W., Fujie, H., Moriguchi, Y., et al. (2012). Detection of abnormalities in the superficial zone of cartilage repaired using a tissue engineered construct derived from synovial stem cells. *European Cells & Materials Journal, 24*, 292–307.
98. Chiang, C. W., Chen, W. C., Liu, H. W., & Chen, C. H. (2014). Application of synovial fluid mesenchymal stem cells: platelet-rich plasma hydrogel for focal cartilage defect. *Journal of Experimental & Clinical Medicine, 6*(4), 118–124.
99. Jiang, Y., Cai, Y., Zhang, W., et al. (2016). Human cartilage-derived progenitor cells from committed chondrocytes for efficient cartilage repair and regeneration. *Stem Cells Translational Medicine, 5*(6), 733–744.
100. Sekiya, I., Muneta, T., Horie, M., & Koga, H. (2015). Arthroscopic transplantation of synovial stem cells improves clinical outcomes in knees with cartilage defects. *Clinical Orthopaedics and Related Research, 473*(7), 2316–2326.
101. Alsalameh, S., Amin, R., Gemba, T., & Lotz, M. (2004). Identification of mesenchymal progenitor cells in normal and osteoarthritic human articular cartilage. *Arthritis & Rheumatology, 50*(5), 1522–1532.

102. Fickert, S., Fiedler, J., & Brenner, R. E. (2004). Identification of subpopulations with characteristics of mesenchymal progenitor cells from human osteoarthritic cartilage using triple staining for cell surface markers. *Arthritis Research & Therapy, 6*(5), R422–R432.
103. Hattori, S., Oxford, C., & Reddi, A. H. (2007). Identification of superficial zone articular chondrocyte stem/progenitor cells. *Biochemical and Biophysical Research Communications, 358*(1), 99–103.
104. Yu, Y., Zheng, H., Buckwalter, J. A., & Martin, J. A. (2014). Single cell sorting identifies progenitor cell population from full thickness bovine articular cartilage. *Osteoarthritis and Cartilage, 22*(9), 1318–1326.
105. Fu, C., Yan, Z., Xu, H., et al. (2015). Isolation, identification and differentiation of human embryonic cartilage stem cells. *Cell Biology International, 39*(7), 777–787.
106. Djouad, F., Bony, C., Häupl, T., et al. (2005). Transcriptional profiles discriminate bone marrow-derived and synovium-derived mesenchymal stem cells. *Arthritis Research & Therapy, 7*(6), R1304–R1315.
107. Prado, A. A., Favaron, P. O., da Silva, L. C., Baccarin, R. Y., Miglino, M. A., & Maria, D. A. (2015). Characterization of mesenchymal stem cells derived from the equine synovial fluid and membrane. *BMC Veterinary Research, 11*, 281.
108. Godoy, R. F., Alves, A. L., Gibson, A. J., Lima, E. M., & Goodship, A. E. (2014). Do progenitor cells from different tissue have the same phenotype? *Research in Veterinary Science, 96*(3), 454–459.
109. Teramura, T., Fukuda, K., Kurashimo, S., et al. (2008). Isolation and characterization of side population stem cells in articular synovial tissue. *BMC Musculoskeletal Disorders, 9*, 86.
110. Jones, E. A., English, A., Henshaw, K., et al. (2004). Enumeration and phenotypic characterization of synovial fluid multipotential mesenchymal progenitor cells in inflammatory and degenerative arthritis. *Arthritis & Rheumatology, 50*(3), 817–827.