

# Stem Cell Therapy for Articular Cartilage Repair

## Review of the Entity of Cell Populations Used and the Result of the Clinical Application of Each Entity

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**Background:** Following successful preclinical studies, stem cell therapy is emerging as a candidate for the treatment of articular cartilage lesions. Because stem cell therapy for cartilage repair in humans is at an early phase, confusion and errors are found in the literature regarding use of the term *stem cell therapy* in this field.

**Purpose:** To provide an overview of the outcomes of cartilage repair, elucidating the various cell populations used, and thus reduce confusion with regard to using the term *stem cell therapy*.

**Study Design:** Systematic review.

**Methods:** The authors systematically reviewed any studies on clinical application of mesenchymal stem cells (MSCs) in human subjects. A comprehensive search was performed in MEDLINE, EMBASE, the Cochrane Library, CINAHL, Web of Science, and Scopus for human studies that evaluated articular cartilage repair with cell populations containing MSCs. These studies were classified as using bone marrow–derived MSCs, adipose tissue–derived MSCs, peripheral blood–derived MSCs, synovium–derived MSCs, and umbilical cord blood–derived MSCs according to the entity of cell population used.

**Results:** Forty-six clinical studies were identified to focus on cartilage repair with MSCs: 20 studies with bone marrow–derived MSCs, 21 studies with adipose tissue–derived MSCs, 3 studies with peripheral blood–derived MSCs, 1 study with synovium–derived MSCs, and 1 study with umbilical cord blood–derived MSCs. All clinical studies reported that cartilage treated with MSCs showed favorable clinical outcomes in terms of clinical scores or cartilage repair evaluated by MRI. However, most studies were limited to case reports and case series. Among these 46 clinical studies, 18 studies erroneously referred to adipose tissue–derived stromal vascular fractions as “adipose-derived MSCs,” 2 studies referred to peripheral blood–derived progenitor cells as “peripheral blood–derived MSCs,” and 1 study referred to bone marrow aspirate concentrate as “bone marrow–derived MSCs.”

**Conclusion:** Limited evidence is available regarding clinical benefit of stem cell therapy for articular cartilage repair. Because the literature contains substantial errors in describing the therapeutic cells used, researchers need to be alert and observant of proper terms, especially regarding whether the cells used were stem cells or cell populations containing a small portion of stem cells, to prevent confusion in understanding the results of a given stem cell–based therapy.

**Keywords:** cartilage; repair; entity; mesenchymal stem cells; cell concentrate; stromal vascular fraction

Clinical application of stem cells for cartilage lesions in humans is increasing. Articular cartilage is known to have poor potential for spontaneous healing, and any damage from trauma or degeneration can lead to focal cartilage lesions and osteoarthritis (OA).<sup>5</sup> Current cartilage repair techniques include bone marrow (BM) stimulation (microfracture), cell-based techniques, and cell plus scaffold–

based transplant techniques.<sup>8,27,100</sup> Research has shown that BM stimulation technique resulted in the formation of fibrous cartilage within the defect rather than the normal hyaline cartilage at the knee joint.<sup>78</sup> Autologous chondrocyte implantation has been shown to improve structural and functional outcomes at long-term follow-up,<sup>10,74,80</sup> but this technique has the disadvantages of requiring an additional surgery, lack of availability of sufficient chondrocytes, senescence or dedifferentiation of the proliferated chondrocytes, and donor site morbidity.<sup>76,91</sup> In this regard, the use of mesenchymal stem cells (MSCs) is emerging as a potential strategy for cartilage repair due to properties of self-

renewal, multilineage differentiation potential, and immunomodulatory capacity.<sup>90</sup> Moreover, recent studies have reported that MSCs supported a healing process of the host through paracrine action.<sup>72,77</sup> Chondrocytes often lose their phenotype after passaging in culture,<sup>31</sup> while MSCs can retain their properties even after culture expansion.<sup>42</sup> Although BM has traditionally been used as a source for MSCs, advances in cell isolation techniques have allowed MSCs to be isolated from human tissues like adipose tissue, umbilical cord blood, synovial membrane, synovial fluid, periosteum, dermis, trabecular bone, and muscle; such cells have similar phenotypic characteristics but different tendencies in proliferation and differentiation when induced.<sup>70</sup>

With a rapidly increasing interest in MSCs for cartilage repair, confusion has arisen in use of the term *stem cell therapy* in clinical research with regard to the entity of cell population used for the studies. Some investigators have used the term *stem cells*, which are relatively homogeneous by culture expansion, interchangeably with the term *cell concentrates*, which are heterogeneous, containing only a small fraction of stem cells. Such mislabeling practices may result in misunderstanding and confusion to other readers and researchers. To use the term *stem cell therapy*, the cells should have been isolated from a pellet of cell concentrate, followed by culture expansion,<sup>122</sup> and then characterized for the following parameters: self-renewal, expression of specific cell surface markers, and multilineage differentiation capacity.<sup>23</sup> Thus, the obtained cell population would be relatively homogeneous<sup>30</sup> and can be referred to as “MSCs used for stem cell therapy.” This stands in contrast to stem cells derived from cell concentrates, which should be described as “cell source aspirate concentrate,” “cell source-derived cells,” or “cell source-derived stromal vascular fraction cells.”

Recently, many review articles have provided an overview of safety and efficacy of stem cell therapy in articular cartilage lesions. But we could find no review article attempting to clarify whether such studies used stem cells (MSCs) or cell concentrates containing a small amount of MSCs. Therefore, in this review, we intended to strictly differentiate between MSCs, being relatively homogeneous by culture expansion, and other cell concentrates that are heterogeneous cell populations containing a small fraction of MSCs and containing other cell types as well; our aim is to help researchers provide more reliable information for proper understanding of the clinical outcomes of current stem cell therapies for articular cartilage repair.

## METHODS

### Data and Literature Sources

A comprehensive search of literature was undertaken in several databases (MEDLINE, EMBASE, the Cochrane Library, CINAHL, Web of Science, and Scopus). The search was conducted on October 1, 2016, and entailed all articles published by September 30, 2016. Only abstracts for articles published in English were reviewed. The search specifics were (“mesenchymal stem cell” OR “mesenchymal stromal cell”) AND (“restoration of cartilage” OR “reproduce cartilage” OR cartilage) AND (human or clinical) NOT animal. A manual search for additional eligible studies that were not found by the above search was performed on reference lists of the included studies and the relevant review articles. Articles identified were then assessed individually for inclusion.

### Study Selection

Studies were eligible if they assessed cartilage regeneration or cartilage repair after administration of a cell population containing MSCs. Only in vivo studies and clinical human studies were included. In vitro and animal studies were excluded for detailed review. Long-term follow-up studies of previously published studies were also included so as to report on all in vivo clinical studies on humans. The title and abstract of each publication were independently screened by 2 authors (Y.B.P., C.W.H.) for eligibility. Subsequently, the same 2 authors individually performed the full-text analysis. Disagreements about inclusion of a given study were solved by consensus or consultation with a third author (H.J.L.), and consensus was assigned to another author (J.H.R.) for the disagreed articles for judgement of inclusion.

### Assessment of Literature Quality

The level of evidence (LOE) of all included studies was assessed by 2 authors using previously published criteria.<sup>75</sup> The quality of each study's method was assessed by 2 authors using the Modified Coleman Methodology Score (MCMS).<sup>61</sup> The MCMS ranges from 0 to 100, where a score greater than 85 represents an excellent study; between 70 and 84, a good study; between 55 and 69, a fair study; and less than 55, a poor study.

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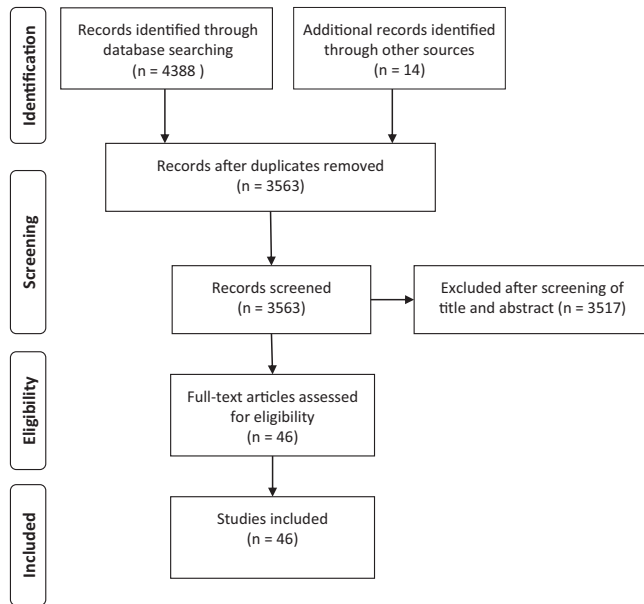
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**Figure 1.** Flow diagram of articles during selection process.

## Data Extraction

Two authors (Y.B.P., C.W.H.) independently recorded data from each study using a predefined data extraction form. Data on cell populations used, number of cases, delivery methods, joint locations, pathological findings, duration of follow-up, outcome variables, and clinical results were extracted. Cell populations were classified as bone marrow–derived mesenchymal stem cells, adipose tissue–derived mesenchymal stem cells, peripheral blood–derived mesenchymal stem cells, synovium–derived mesenchymal stem cells, and umbilical cord blood–derived mesenchymal stem cells according to the entity of cell source.

## RESULTS

After the selection process, 46 studies were included. The selection process for the studies is shown in a flow diagram in Figure 1. All of the included clinical studies were reviewed in detail regarding the entity of cell populations used and the result of the clinical application of each entity. The cell population, source tissue, source site, harvesting technique, culture expansion, and advantages and disadvantages are summarized in Table 1.

### Level of Evidence and Quality of Evidence

There were 6 studies of LOE 1, 12 studies of LOE 2, 7 studies of LOE 3, and 21 studies of LOE 4 (Table 2). The mean MCMS was  $49.2 \pm 16.0$ . Only 1 study (2.2%) was classified as excellent quality, whereas 33 studies (71.8%) were poor quality (Table 2). The details of LOE and MCMS are shown in Tables 3 and 4.

## Bone Marrow–Derived Mesenchymal Stem Cells (BM-MSCs)

Because BM-derived MSCs (BM-MSCs) were the first identified MSCs, they have been extensively studied and are the best characterized form of MSCs. Because BM is a relatively rich source of these cells, MSCs from BM are relatively easy to collect. Adult BM consists of blood cells in various stages of differentiation.<sup>106</sup> The adult stem cell fraction is present in the population of nucleated cells of BM, and the majority of these cells are hematopoietic stem cells (HSCs) rather than MSCs.<sup>15</sup> The MSCs are only a small percentage (0.001%-0.01%) of the total nucleated cells,<sup>93</sup> but they can expand by 100- to 10,000-fold over several weeks in culture.<sup>117</sup> As such, culture-expanded BM-MSCs have been considered for cartilage repair studies.

From our review, 19 studies have reported on transplantation or injection of BM-MSCs for treating cartilage lesions and OA of the knee joint (Table 3). Ten studies used a 2-stage implantation, which used culture-expanded BM-MSCs directly on the lesions through an arthrotomy. Seven studies used delayed injection of in vitro expanded BM-MSCs without surgery in outpatient clinics. One study used a 1-stage implantation, which employed allogenic culture-expanded BM-MSCs through an arthrotomy.<sup>21</sup> One study compared the 2-stage implantation with delayed injection as delivery methods for in vitro expanded BM-MSCs.

Two studies assessed the effect of MSCs compared with a control cell-free group. Wakitani et al<sup>109</sup> reported that transplantation of autologous BM-MSCs embedded in collagen gel covered with periosteum, in 12 patients at the time of high tibial osteotomy, showed high histological and arthroscopic scores compared with cell-free treatment for 12 patients. However, the clinical improvement was not significantly different at 16 months. Wong et al<sup>116</sup> performed a randomized controlled study to compare BM-MSC injection versus cell-free injection for treating cartilage defects in conjunction with microfracture and medial opening-wedge, high tibial osteotomy. All clinical outcomes were better in the BM-MSC group at 2 years, and MRI showed better defect filling and integration with the surrounding tissue at 1 year. Nejadnik et al,<sup>79</sup> who compared autologous BM-MSCs and autologous chondrocyte implantation for treating full-thickness cartilage defects of the knee, showed no significant differences in clinical outcomes. Lee et al<sup>65</sup> compared 2 treatment groups: (1) arthroscopic microfracture combined with outpatient intra-articular injection of autologous BM-MSCs and (2) open transplantation of autologous BM-MSCs covered with periosteum in full-thickness cartilage defect of knee. Clinical outcomes improved notably in both groups, and no adverse effects were noted. The authors concluded that the intra-articular injection technique was comparable with the open procedure. It also had the advantage of being minimally invasive and requiring only a single surgery.

### Bone Marrow Aspirate Concentrate (BMAC)

Although BM-MSCs are a promising cell type for cartilage repair, the required in vitro culture expansion of cells entails several issues. These include relatively high costs,

TABLE 1  
Details of the Harvesting Process of Cell Populations<sup>a</sup>

Cell Population	Source Tissue	Source Site	Harvesting Procedure	Culture Expansion	Advantages	Disadvantages
BM-derived MSCs	BM	Iliac crest	Aspirate BM → transfer BM to the cell separator → centrifuge for concentrate containing nucleated cells → isolate MSCs using plastic adherence → conduct expansion with culture media	Yes	High concentration of stem cells Pure population of stem cells	High cost Second surgery Sterility concerns Invasive procedure
BM aspirate concentrate	BM	Iliac crest	Aspirate BM → transfer BM to the cell separator → centrifuge for concentrate containing nucleated cells → acquire from buffy coat	No	Much used and studied Minimal manipulation	Very low concentration of stem cells Invasive procedure Heterogeneous mixture of cells
Adipose-derived MSCs	Adipose tissue	Buttock, abdomen, infrapatellar fat pad (lipoaspirate, excision)	Harvest adipose tissue → digest with collagenase → transfer to the cell separator → centrifuge for concentrate containing nucleated cells → isolate MSCs using plastic adherence → conduct expansion with culture media	Yes	High concentration of stem cells Pure population of stem cells	High cost Second surgery Sterility concerns Invasive procedure
Adipose-derived stromal vascular fraction	Adipose tissue	Buttock, abdomen, infrapatellar fat pad (lipoaspirate, excision)	Harvest adipose tissue → digest with collagenase → transfer to the cell separator → centrifuge for concentrate containing nucleated cells → acquire from pellet	No	Minimal manipulation Low cost Sterility	Low concentration of stem cells Heterogeneous mixture of cells
Synovium-derived MSCs	Synovium	Synovium of the knee	Excise synovium → digest with collagenase → centrifuge → isolate MSCs → expand with culture media	Yes	High concentration of stem cells Pure population of stem cells Superior chondrogenic potential compared with BM-MSCs High proliferation	High cost Second surgery Sterility concerns Invasive procedure Retains fibroblastic characteristics to a certain extent
Umbilical cord blood-derived MSCs	Umbilical cord	Umbilical cord blood (vein)	Harvest from umbilical cord vein → centrifuge → isolate MSCs → expand with culture media	Yes	Noninvasive procedure High concentration of stem cells Pure population of stem cells High proliferation Low immunogenicity	Lower differentiation potential than BM-MSCs Lower harvest rate than BM or adipose MSCs
Peripheral blood progenitor cells	Peripheral blood	Peripheral blood	Treat with granulocyte colony-stimulating factor to increase MSCs in peripheral blood → harvest peripheral blood → use automated cell separator (apheresis)	No	Minimally invasive Chondrogenic differentiation potential similar to that of BM-MSCs	Various isolation techniques Very low concentration of stem cells Heterogeneous mixture of cells

<sup>a</sup>BM, bone marrow; MSC, mesenchymal stem cell.

a time-delayed second surgery, and, most important, safety in terms of human application of the *in vitro* expanded cells: The safety issues include maintenance of sterility of *in vitro* culture for expanding the cells, clearance of cytokines used for the culture expansion, and genetic stability of the culture-expanded cells, all of which put the expanded cells under strict regulation for human use by regulatory authorities.<sup>107</sup> In contrast, BM aspirate concentrate (BMAC) can be easily prepared using centrifugation after BM harvest and is available for 1-stage implantation on the same day with minimal manipulation of cells. Therefore, BMAC seems to be an attractive alternative to BM-MSCs. However, such BM-derived cells in BMAC are

TABLE 2  
Level and Quality of Evidence of Clinical Studies

	No. (%) of Studies
Level of evidence	
1	6 (13.0)
2	12 (26.01)
3	7 (15.2)
4	21 (45.7)
Quality of evidence	
Excellent	1 (2.2)
Good	6 (13.0)
Fair	6 (13.0)
Poor	33 (71.8)

TABLE 3  
Details of Clinical Studies with Cell Populations Obtained from Bone Marrow<sup>a</sup>

Author (year)	Entity of Cells	No. of patients	Delivery method	Joint, pathology	F-U (mo.)	Outcomes	Brief descriptions of results
Wakitani et al <sup>110</sup> (2002)	BM-MSCs	12 MSCs 12 control	2-Stage implantation	Knee, OA	16	HSS, 2nd look	No difference in clinical outcomes at 16 months, but histologic and arthroscopic score was better in MSCs group at 6.7 weeks
Wakitani et al <sup>111</sup> (2004)	BM-MSCs	2	2-Stage implantation	Patella, CD	69	2nd look	Pain and walking were improved and maintained till 69 months with fibrocartilage repair
Adachi et al <sup>2</sup> (2005)	BM-MSCs	1	2-Stage implantation	MFC, OD	12	2nd look	Hyaline-like cartilage formation
Kuroda et al <sup>62</sup> (2007)	BM-MSCs	1	2-Stage implantation	MFC, CD	7	2nd look	Hyaline-like cartilage formation
Wakitani et al <sup>112</sup> (2007)	BM-MSCs	5	2-Stage implantation	P-F joint, CD	6	IKDC, 2nd look, MRI	Clinical improvement at 6 months till 17-27months, but fibrocartilage formation at 12 months
Adachi et al <sup>1</sup> (2007)	BM-MSCs	1	2-Stage implantation	Knee, ON	24	2nd look, MRI	Smooth cartilage-like tissue in 2nd look and MRI but weak safranin-O staining in histology
Centeno et al <sup>14</sup> (2008)	BM-MSCs	1	Delayed injection	Knee, OA	3	VAS, MRI	VAS pain scores were improved; MRI showed an increased meniscus
Centeno et al <sup>15</sup> (2008)	BM-MSCs	1	Delayed injection	Knee, OA	6	VAS, function index, MRI	MRI showed an increased meniscus and cartilage volume Range of motion and VAS pain scores were improved
Nejadnik et al <sup>79</sup> (2010)	BM-MSCs	36 MSCs 36 ACI	2-Stage implantation	Knee, CD	24	ICRS-CIEP, 2nd look	Smooth congruency, hyaline like cartilage in 2nd look No difference in clinical outcomes between two groups
Haleem et al <sup>35</sup> (2010)	BM-MSCs	5	2-Stage implantation	MFC, CD	12	Lysholm, HSS, MRI, 2nd look	All clinical outcomes were improved, MRI showed complete congruity in 3 patients, incomplete congruity in 2 patients
Wakitani et al <sup>113</sup> (2011)	BM-MSCs	45	2-Stage implantation	Knee, OA	75	Safety	No serious complications such as tumor formation or infection
Davachi et al <sup>18</sup> (2011)	BM-MSCs	4	Delayed injection	Knee, OA	12	VAS, walking, stairs numbers	Pain, walking time and the number of stairs to climb were improved
Emadedin et al <sup>24</sup> (2012)	BM-MSCs	6	Delayed injection	Knee, OA	12	VAS, WOMAC, MRI	Pain, functional status improved, MRI showed increased cartilage thickness, repair tissue in 3 of 6 patients
Lee et al <sup>65</sup> (2012)	BM-MSCs	70 (35/35)	Delayed injection 2-Stage implantation	Knee, CD	24.5	ICRS-CIEP, MRI	No adverse effects, all clinical scores were improved Injection was comparable to surgery
Teo et al <sup>104</sup> (2013)	BM-MSCs	3 MSCs 20 ACI	2-Stage implantation	Patella, OCD	24	ICRS-CIEP, MRI	All clinical outcomes were improved Periosteal hypertrophy was observed in 2 cases of ACI
Wong et al <sup>117</sup> (2013)	BM-MSCs	28 MSCs 28 control	Delayed injection	Knee, OA	24	IKDC, Tegner, Lysholm, MRI	All clinical outcomes were better in MSCs group MRI showed better results in MSCs group
Orozco et al <sup>81</sup> (2013)	BM-MSCs	12	Delayed injection	Knee, OA	12	VAS, WOMAC, SF-36, MRI	Improvement in pain relief and WOMAC MRI showed improvement of quality of cartilage
Shetty et al <sup>102</sup> (2014)	BMAC	30	1-stage implantation	Knee, CD	30	IKDC, KOOS, MRI	All clinical outcomes were improved MRI showed good defect filling
Davachi et al <sup>19</sup> (2015)	BM-MSCs	3	Delayed injection	Knee, OA	60	VAS, walking, stairs numbers	Pain, walking time and the number of stairs to climb gradually deteriorated, but still better than baseline.
Windt et al <sup>22</sup> (2016)	BM-MSCs	10	1-Stage implantation	Knee, CD	12	VAS, KOOS, EQ5D, MRI, 2nd look	All clinical outcomes were improved MRI showed complete defect filling. Hyaline-like cartilage formation

<sup>a</sup>ACI, autologous chondrocyte implantation; BMAC, bone marrow aspirate concentrate; BM-MSCs, bone marrow-derived mesenchymal stem cells; CD, chondral defect; EQ5D, EuroQoL 5-Dimension Health Questionnaire; HSS, Hospital for Special Surgery Score; ICRS-CIEP, International Cartilage Repair Society Cartilage Injury Evaluation Package; IKDC, International Knee Documentation Committee; KOOS, Knee injury and Osteoarthritis Outcome Score; MFC, medial femoral condyle; MRI, magnetic resonance imaging; OA, osteoarthritis; OCD, osteochondritis dissecans; OD, osteochondral defect; ON, osteonecrosis; PF, patellofemoral; SF-36, 36-Item Short Form Health Survey; VAS, visual analog scale; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

TABLE 4  
Details of Clinical Studies with Cell Populations Obtained from Adipose Tissue<sup>a</sup>

Author (year)	Entity of Cells	No. of cases	Delivery method	Joint disease	F-U	Outcomes	Brief descriptions of results
Pak <sup>82</sup> (2011)	ADSVF	4	Direct injection 4 additional injections; CaCl <sub>2</sub> -PRP	Hip, ON; Knee, OA	3	VAS, functional rating index, MRI	Restore bone in osteonecrosis, and cartilage in OA. All clinical outcomes improved
Pak <sup>83</sup> (2012)	ADSVF	2	Direct injection 4 additional injections; CaCl <sub>2</sub> -PRP	Hip, ON	12	VAS, Harris hip score, functional rating index, MRI	All clinical outcomes improved MRI showed repair of medullary bone-like tissue in necrotic femoral head
Koh et al <sup>54</sup> (2012)	ADSVF	25 with cells 25 with control	Direct injection	Knee, OA	12	VAS, Lysholm, Tegner	Clinical outcomes improved More improvement in SVF group
Koh et al <sup>58</sup> (2013)	ADSVF	18	Direct injection	Knee, OA	24.3	VAS, Lysholm, WOMAC, MRI	Clinical outcomes improved WORMS improved
Kim et al <sup>51</sup> (2013)	ADSVF	31 with cells 37 with control	Direct injection	Ankle, OD	21.8	VAS, AOFAS, Roles and Maudsley score, Tegner	Clinical outcomes improved, with greater improvement in SVF group
Pak et al <sup>84</sup> (2013)	ADSVF	100	Direct injection 4 additional injections; CaCl <sub>2</sub> -PRP	Knee OA, hip ON, ankle	26	VAS, MRI	Pain improved. No tumor formation
Pak et al <sup>85</sup> (2013)	ADSVF	3	Direct injection 3 additional injections; CaCl <sub>2</sub> -PRP with HA, or dexamethasone	CP	12	VAS, MRI	Pain improved by 80-90%. MRI at 3 months showed improvement of cartilage (softened cartilage).
Jo et al <sup>39</sup> (2014)	Ad-MSCs	18	1-stage injection	Knee, OA	6	VAS, KSS, WOMAC, MRI, 2nd look	All clinical outcomes improved Cartilage defect decreased, ICRS grade improved.
Koh et al <sup>56</sup> (2014)	ADSVF	37	Direct injection	Knee, OA	24	VAS, KOOS, Lysholm 2nd look	All clinical outcomes improved Cartilage maintained in 14 of 16 patients
Bui et al <sup>13</sup> (2014)	ADSVF	21	Direct injection	Knee, OA	8.5	Pain, Lysholm, MRI	All clinical outcomes improved Cartilage thickness increased in MRI
Koh et al <sup>59</sup> (2014)	ADSVF	23 with cells 21 with control	Direct injection	Knee, OA	24.4	VAS, KOOS, Lysholm 2nd look	All clinical outcomes improved, with greater improvement in SVF group
Kim et al <sup>45</sup> (2014)	ADSVF	39 no scaffold 17 with scaffold	Injection under arthrosomy	Knee, OA	28.6	IKDC, Tegner, ICRS, 2nd look	All clinical outcomes improved, with better ICRS grades in fibrin glue scaffold
Kim et al <sup>49</sup> (2014)	ADSVF	24 with cells 26 with control	Injection under arthrosomy	Ankle, OD	27.4	VAS, AOFAS, Tegner, MRI	All clinical outcomes improved. All clinical outcomes and MOCART score better in SVF
Kim et al <sup>43</sup> (2015)	ADSVF	55	Injection under arthrosomy	Knee, OA	26.5	IKDC, Tegner, satisfaction	All clinical outcomes improved. Age and size are important for outcomes
Koh et al <sup>57</sup> (2015)	ADSVF	30	Injection under arthrosomy	Knee, OA	25.0	VAS, KOOS, Lysholm 2nd look	All clinical outcomes improved. Cartilage maintained in 26 of 30 patients
Kim et al <sup>48</sup> (2015)	ADSVF	20 PRP 20 Fibrin glue	Direct injection Injection under arthrosomy	Knee, OA	28.6	IKDC, Tegner, ICRS, 2nd look	IKDC and ICRS were better in Fibrin glue group
Pers et al <sup>93</sup> (2016)	Ad-MSCs	18	1-stage injection	Knee, OA	6	VAS, WOMAC, KOOS, MRI, histology	All clinical outcomes improved. MRI showed limited possible improvement.
Koh et al <sup>60</sup> (2016)	ADSVF	40 with cells 40 with control	Injection under arthrosomy	Knee, CD	24.3	VAS, Lysholm. KOOS, MRI	All clinical outcomes improved, with better KOOS pain and symptoms in SVF
Kim et al <sup>44</sup> (2016)	ADSVF	24	Injection under arthrosomy	Knee, OA	24	IKDC, Tegner, MRI	Better cartilage repair in SVF All clinical outcomes improved. MOAKS was significantly improved.
Kim et al <sup>47</sup> (2016)	ADSVF	26 with cells 23 with control	Injection under arthrosomy	Ankle, OA	12	VAS, AOFAS, 2nd look	All clinical outcomes improved with greater improvement and better ICRS grades in SVF
Kim et al <sup>50</sup> (2016)	ADSVF	31 with cells 33 with control	Injection under arthrosomy	Ankle, OA	12.8	VAS, AOFAS, 2nd look	All clinical outcomes improved with greater improvement and better ICRS grades in SVF

<sup>a</sup>ADSVF, adipose tissue-derived stromal vascular fraction; Ad-MSCs, adipose tissue-derived mesenchymal stem cells; AOFAS, American Orthopaedic Foot and Ankle Society score; CD, cartilage defect; CP, chondromalacia of patella; HA, hyaluronic acid; ICRS, International Cartilage Repair Society; IKDC, International Knee Documentation Committee; KOOS, Knee injury and Osteoarthritis Outcome Score; KSS, Knee Society Score; MOAKS, MRI Osteoarthritis Knee Score; MOCART, magnetic resonance observation of cartilage repair tissue; MRI, magnetic resonance imaging; OA, osteoarthritis; OD, osteochondral defect; ON, osteonecrosis; PRP, platelet-rich plasma; VAS, visual analog scale; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index; WORMS, whole organ MRI score.

not MSCs but are a heterogeneous cell population containing a small fraction of MSCs. BMAC may have a high potency for cartilage and osseous defect healing, because it contains not only stem cells and precursor cells as a repair source but also accessory cells that support angiogenesis and vasculogenesis by producing several growth factors.<sup>28</sup>

Only 1 study using BMAC was found by means of a comprehensive search during this systematic review (Table 3).<sup>101</sup> Application of BMAC with hyaluronic acid gel after microfracture in 30 patients with chondral defects showed improved clinical outcome as well as good defect filling and hyaline-like cartilage repair on MRI. In this study, the phrase “bone marrow mesenchymal cell” was erroneously used interchangeably with “bone marrow aspirate concentrate.”

### Adipose Tissue–Derived Mesenchymal Stem Cells (Ad-MSCs)

Adipose precursors were first isolated from human adipose tissue by plastic adherence in 1976.<sup>105</sup> Adipose tissue–derived MSCs (Ad-MSCs) may be more suitable than BM-MSCs for clinical applications because the adipose tissue can be easily and repeatedly obtained by a minimally invasive and well-established procedure,<sup>12</sup> and the amount of MSCs from adipose tissue is approximately 500-fold greater than that from BM when isolated from an equivalent amount of aspirate.<sup>89</sup> Ad-MSCs were identified from stromal vascular fraction of human lipoaspirates and acquired by culture expansion.<sup>122</sup> However, Ad-MSCs have been shown to have inferior chondrogenic potential compared with BM-MSCs *in vitro*,<sup>37</sup> although the chondrogenic differentiation of Ad-MSCs can be enhanced by modulation with *in vitro* added factors.<sup>26,102</sup>

Only 2 recent studies have reported the possibility of intra-articular injection of Ad-MSCs as an alternative option for the treatment of knee OA in elderly patients (Table 4).<sup>39,92</sup> One study evaluated safety and efficacy of intra-articular injection of Ad-MSCs under diagnostic arthroscopy for a phase 1 study consisting of 3 dose-escalation cohorts (low-dose, medium-dose, and high-dose groups with 3 patients in each) and also for a phase 2 study that included 9 patients receiving a high dose of cells.<sup>39</sup> Pain and function were improved without adverse effects, and the patients' cartilage defects decreased with hyaline-like cartilage repair. The other study evaluated safety and efficacy of intra-articular injection of Ad-MSCs for a phase 1 clinical trial consisting of 3 dose-escalation cohorts (low-dose, medium-dose, and high-dose groups with 6 patients in each).<sup>92</sup> Pain and function were improved without serious adverse events. The cells used in these studies were isolated from abdominal fat aspirates, processed by enzyme digestion, centrifugation, and then culture expansion.

### Adipose Tissue–Derived Stromal Vascular Fraction (ADSVF)

Adipose tissue–derived stromal vascular fraction cells (ADSVFs) are the portion of cell pellet after centrifugation

of adipose tissue or lipoaspirate, which is a mononuclear cellular fraction of the adipose tissue.<sup>69</sup> Whereas Ad-MSCs are relatively homogeneous as a result of culture expansion,<sup>30</sup> ADSVFs are not homogeneous but are a heterogeneous cell population, composed mostly of pericytes, endothelial cells, smooth muscle cells, fibroblasts, macrophages, and MSCs.<sup>69</sup> ADSVFs have been known to have 1% to 10% fraction of Ad-MSCs.<sup>6,12</sup> However, researchers and authors of many previous reports have erroneously used the term *MSCs* to refer to ADSVFs, adding more confusion to an already confusing set of information in terms of the clinical results for various stem cell–based therapies.

We found 19 clinical studies using ADSVF to treat cartilage lesions, osteoarthritis, or osteonecrosis. They reported the clinical results of administering ADSVF by intra-articular injection (Table 4). For all of the studies except one, the authors reported that they injected “Ad-MSCs” but the injected cells were in fact ADSVFs, according to the entity of the cell source described for their studies; Ad-MSCs and ADSVFs are actually quite different entities, as we described above. In this review, we classified those studies as in Appendix Table A1 (available in the online version of this article).

Several studies compared the effect of ADSVFs with a cell-free group. One study described OA of the knee treated with arthroscopic debridement, followed by the injection of ADSVFs with platelet-rich plasma (PRP), compared with injection of PRP without the cells. The clinical improvements were not significantly different, although the ADSVF group showed a tendency for greater improvement.<sup>54</sup> Another study described the use of ADSVFs for osteochondral lesion of talus in a case-control study. The study group was treated with arthroscopic microfracture and ADSVF injection, and the control group was treated with only arthroscopic microfracture. All clinical results were improved at final follow-up, with a significantly greater improvement in the study group.<sup>51</sup> The other study described OA of the knee treated by high tibial osteotomy combined with injection of ADSVFs and PRP compared with high tibial osteotomy combined with injection of PRP only. All clinical results were improved at final follow-up, with a significantly greater improvement in the study group. The investigators also reported that all clinical outcomes and MRI were better in the ADSVF group.<sup>49</sup>

### Synovium-Derived Mesenchymal Stem Cells (Sy-MSCs)

Synovium-derived MSCs (Sy-MSCs) were first isolated and characterized from synovial membrane surrounding the knee joint in 2001.<sup>20</sup> Several studies have reported that Sy-MSCs have greater chondrogenic potential *in vitro* than MSCs from other stem cell sources.<sup>53,95,120</sup> Moreover, Sy-MSCs have been shown to have a greater chondrogenic differentiation than MSCs from other stem cell sources such as adipose tissue or muscle.<sup>95</sup> On the basis of the favorable results from *in vitro* studies, investigators have conducted animal studies to evaluate the effect of Sy-MSCs *in vivo*. Koga et al<sup>52</sup> demonstrated that implantation of Sy-

MSCs for healing of full-thickness osteochondral defects of femur in rabbits resulted in extensive cartilage matrix formation with good integration into the surrounding native cartilage. Using a rabbit model, Lee et al<sup>63</sup> reported that implantation of Sy-MSCs with PRP gel resulted in successful resurfacing of defects in the cartilage and restoration of the subchondral bone in osteochondral defect of femur.

A recent study reported that implantation of matrix-induced autologous MSCs was a favorable alternative treatment for knee chondral defects in human.<sup>4</sup> This study compared autologous Sy-MSC implantation with autologous chondrocyte implantation. In both groups, the clinical outcomes were improved at 24-month follow-up. However, at all follow-up intervals, Sy-MSC implantation showed better functional outcomes and subjective Knee injury and Osteoarthritis Outcome Score (KOOS) than chondrocyte implantation. This study showed LOE 1 and good quality of evidence (MCMS: 73).

### Human Umbilical Cord Blood-Derived Mesenchymal Stem Cells (hUCB-MSCs)

Human umbilical cord blood (hUCB) has several advantages as a source for therapeutic cells. The hUCB has been used for more than a decade in reconstitution of hematopoietic tissue to treat hematological disorders and other diseases.<sup>108</sup> The extracorporeal nature of hUCB avoids the ethical concerns associated with using embryonic-derived stem cells. It can also avoid the donor site morbidity of other sources such as BM or adipose tissue. In 1994, Ye et al<sup>119</sup> first reported on mesenchymal-like cells from hUCB that adhered to plastic. Erices et al<sup>25</sup> concluded that the adhesive cells in hUCB were MSCs, and since then many studies have reported the isolation, proliferation, and differentiation potential of hUCB-MSCs.<sup>11,41,99,118</sup> The hUCB-MSCs have exhibited a higher proliferation rate, have shown karyotype stability after prolonged expansion, and could be more readily induced to differentiate into chondrocytes, more so than BM-MSCs and Ad-MSCs.<sup>121</sup> Some studies also described hUCB-MSCs as having more chondrogenic potential than BM-MSCs.<sup>9,67</sup> The hUCB-MSCs have clearly shown an immunomodulatory capacity equivalent to BM-MSCs and Ad-MSCs,<sup>113,121</sup> and these cells are known not to require tissue matching for allogeneic transplantation.<sup>114</sup> In addition, the hUCB-MSCs have low immunogenicity and are immunomodulatory in vitro and in vivo.<sup>3,16,66</sup>

The hUCB-MSCs can be used as off-the-shelf stem cell products; however, until recently, most studies examining the chondrogenic potential of hUCB-MSCs have been limited to in vitro studies.<sup>9,21,38,67,73,121</sup> One recent study suggested that hUCB-MSCs can stimulate the differentiation of locally presented endogenous chondroprogenitor cells by thrombospondin 2, which ultimately leads to cartilage repair.<sup>38</sup> Some in vivo animal studies have reported that hUCB-MSC transplantation showed hyaline-like cartilage repair, subchondral bone remodeling, and integration with the surrounding cartilage.<sup>34,86,88</sup>

Only one recent study has reported the possibility of hUCB-MSC transplantation as an alternative option for the

treatment of knee OA.<sup>87</sup> This phase 1/2 clinical trial evaluated safety and efficacy of transplantation of hUCB-MSCs and hyaluronic acid hydrogel composite to treat osteoarthritic cartilage defects in 7 patients with knee OA. Pain and function were improved without adverse effects and were maintained over 7 years without significant deterioration. Persistent regenerated hyaline-like cartilage was observed through histological evaluation and MRI. This study showed LOE 2 and good quality of evidence (MCMS: 79).

### Peripheral Blood-Derived Progenitor Cells (PBPCs)

Peripheral blood has been considered an alternative cell source for regenerative medicine because peripheral blood was known to contain progenitor cells.<sup>104</sup> However, concern arose regarding the peripheral blood progenitor cells (PBPCs) due to very small numbers of MSCs in the PBPC population and limitation of repeated extraction of MSCs from peripheral blood.<sup>104,115</sup> Roufosse et al<sup>94</sup> demonstrated that progenitor cells from BM were transported to the damage site via the circulating peripheral blood for tissue regeneration. On the basis of this principle, researchers found that progenitor cells can be increased in the peripheral blood by blood mobilization technique.<sup>40</sup> PB-MSCs have been shown to have chondrogenic differentiation similar to that of BM-MSCs.<sup>17,29</sup> In addition, some animal studies reported that PB-MSCs were an effective therapeutic option for cartilage repair.<sup>29,36</sup>

Only 3 studies have reported the possibility of intra-articular injection of PBPCs as an alternative option for cartilage repair.<sup>96-98</sup> One study reported that autologous PBPCs with hyaluronic acid after multiple subchondral drilling showed hyaline-like cartilage repair.<sup>97</sup> Another study compared the effect of PBPCs on cartilage repair after multiple subchondral drilling.<sup>98</sup> In this study, addition of intra-articular injection of PBPCs showed better cartilage repair than subchondral drilling alone. However, International Knee Documentation Committee (IKDC) scores were similar. The other study reported that intra-articular injection of PBPCs with hyaluronic acid after multiple subchondral drilling concomitant with high tibial osteotomy showed cartilage repair histologically resembling the native articular cartilage.<sup>96</sup> The LOEs for these studies were 4, 1, and 4 and quality of evidence was poor (MCMS: 36), good (MCMS: 79), and poor (MCMS: 36), respectively.

Only 1 of the 3 studies adequately described the cell entity as "peripheral blood progenitor cells." The other 2 studies inadequately reported that they used "peripheral blood stem cells," but the injected cells were in fact PBPCs according to the entity of the cells described in their papers.<sup>96,98</sup> In this review, we classified those studies in Appendix Table A1.

## DISCUSSION

Stem cell therapy is emerging as an alternative strategy for articular cartilage repair in humans based on successful preclinical studies for cartilage repair. Therefore, clinical application of stem cells for cartilage lesion or osteoarthritis in humans is increasing. Currently, due to advancements in



cell isolation techniques, human MSCs are also isolated from various tissues like BM, adipose tissue, synovial membrane, umbilical cord blood, synovial fluid, bone, and muscle, with similar phenotypic characteristics but different propensities in proliferation and differentiation potential. Therefore, the entity of cells used for the treatment of cartilage lesions is important in order to evaluate and compare the results of various stem cell-based therapies.

Currently, many clinical studies with MSCs from various cell sources have been conducted for cartilage repair. Most clinical studies included in this review have reported improved clinical outcomes. In comparative studies, clinical benefits of MSCs were unclear. Some studies reported that there were no differences in clinical outcomes,<sup>79,109</sup> whereas other studies reported that clinical outcomes in MSCs were better than those in control or other treatment.<sup>51,54,81</sup> It was difficult to conclude whether MSCs were effective in cartilage repair because studies had different cell sources, delivery methods, and evaluation methods. Cell sources for stem cell therapy in cartilage repair were BM-MSCs, BMAC, Ad-MSCs, ADSVFs, PBPCs, Sy-MSCs, and hUCB-MSCs. The different entity of cell population in stem cell therapy could show different outcomes. Regarding delivery methods, surgical transplantation and intra-articular injection have been used for cell delivery. Several outcome assessment tools, MRI, and histological analysis were used for clinical evaluation with various follow-up periods. Most studies included in this systematic review showed low level of evidence and low quality of evidence. Although the number of LOE 1 and 2 studies has increased in recent years, more than 60% of clinical studies are LOE 3 and 4. In addition, more than 80% of clinical studies show low quality of evidence. Therefore, until now, limited evidence was available regarding clinical benefit of stem cell therapy for cartilage repair. However, any benefit to human subjects still requires extensive evaluation. Many aspects of stem cell-based therapy remain to be optimized and evaluated, such as the cell sources, delivery methods, and risks involved in such trials. Durability and quality of regenerated cartilage also require further evaluation.

With a rapidly increasing interest in MSCs in cartilage repair, there appear some problems in use of the term *mesenchymal stem cells*. Because stem cell therapy for cartilage repair in humans is at an early phase, researchers need to be alert to and observant of proper terms in describing the entity of cell population used for a given clinical study to prevent confusion in understanding the results of the given stem cell-based therapy. However, some studies have erroneously used the term *MSCs* to also describe a heterogeneous cell population containing only a small amount of MSCs. We believe that it is a serious oversight to use the term *MSCs* in lieu of *cell concentrate*, as these are different entities, and using these terms interchangeably adds more confusion to the already confusing set of information that currently exists for stem cell-based therapies. Cells that are termed *MSCs* need to have been isolated from a pellet of concentrate, followed by culture expansion, and subsequently characterized for the following: self-renewal, expression of specific cell surface markers, and multilineage

differentiation capacity. Thus, the obtained cell population would be relatively homogeneous and can be designated as *MSCs*.<sup>30</sup> Cell populations collected by centrifugation with or without collagenase treatment can only be referred to as cell concentrates, stromal vascular fraction cells, or cell source-derived cells, as these are heterogeneous populations of cells that may contain only a small portion of *MSCs*.

The clinical application of culture-expanded *MSCs* is generally under strict regulation by governmental regulatory authorities in most countries in the world.<sup>71</sup> In contrast, cells prepared with minimal manipulation and without culture expansion, such as cell concentrates, cell source-derived cells, or cell source-derived stromal vascular fraction cells, may be considered as part of the “practice of medicine” and are easier to use in patients because regulation is less strict around the world. The US Food and Drug Administration (FDA) routinely permits use of human cells and tissue-based products meeting the following 4 conditions stated in Section 1271.10 of the Code of Federal Regulations for Food and Drugs: (1) minimally manipulated; (2) intended only for homologous use; (3) not combined with another substance except water or sterilizing, preservation, or storage agents; and (4) either “having no systematic or metabolic effect” or “being for autologous use, allogenic use in first- or second-degree blood relative, or reproductive use.” To apply *MSCs* with culture expansion in humans, the details of the entire procedure for cell preparation have to be approved by the FDA or other governmental regulatory authority for use in clinical trials. Hence, one must be accurate in differentiating and using the terms *MSCs*, *cell concentrate*, *cell source-derived cells*, and *cell source-derived stromal vascular fraction cells*.

Recently, several studies used the term *ADSVFs* mixed with *Ad-MSCs* (Appendix Table A1). Most studies have been performed with *ADSVFs*, with delivery via direct intra-articular injection. All the cited studies in the present review described using *Ad-MSCs* for treatment of cartilage lesions; however, they did not use *MSCs* with culture expansion but instead were using *ADSVFs*. Indeed, none of the studies were approved by the FDA or other governmental regulatory authority, according to the content of the reports. Some of the investigators noted that the governmental regulatory authority had allowed the use of *Ad-MSCs* in autologous cell transplantation as long as they were obtained and processed within the same medical facility with minimal processing.<sup>84,85</sup> We believe that referring to such cellular preparations with minimal manipulation as *Ad-MSCs* is a significant error. *ADSVFs* are a pellet of cells from centrifugation of lipoaspirates, enriched for mononuclear cells.<sup>68</sup> Composition of *ADSVFs* is not as homogeneous as that of culture-expanded *Ad-MSCs* but is heterogeneous, containing pericytes, endothelial cells, smooth muscle cells, fibroblasts, and macrophages along with a small fraction containing *Ad-MSCs*. Similarly, *BMACs* are not *MSCs* but are heterogeneous cell populations containing a small portion of *BM-MSCs*. A *BM-MSC* population can be obtained only by isolation from a pellet of concentrate of *BM aspirates*, followed by culture expansion and profiling of the cells for various parameters

of MSCs. Thus, the cells obtained by minimal manipulation for these studies were ADSVFs or BMAC. Several authors wrote letters to the editor of the journals that published the studies, pointing out the mixed usage and incorrect terms regarding the entity of cell populations. These authors noted that incorrect titles or invalid use of important terms may result in misinterpretation of study results and may lead to tolerance for the incorrect use of such terms in the future.<sup>7,32,33,64</sup> Some authors did not accept our advice and adhered to using their incorrect terms.<sup>47,55</sup> Therefore, we believe that revealing erroneous use of terms in stem cell therapy for the treatment of cartilage lesions is essential to gauge the relevant potential of stem cell therapy and to correctly assess the scientific rationale for stem cell-based therapy.

## CONCLUSION

On the basis of the current literature review, we conclude that limited evidence is available regarding clinical benefit of stem cell therapy for articular cartilage repair. Because the literature contains substantial errors in describing the therapeutic cells used, researchers need to be mindful of the terms used (ie, whether the cells used were stem cells or a cell population containing a small portion of stem cells) to prevent confusion regarding the results of a given stem cell-based therapy. High-level studies with appropriate terms to describe the entity of cell population used are required to gauge the relevant potential of specific stem cell therapies for articular cartilage repair.

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