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Mesenchymal stem cells for the treatment of cartilage lesions: from preclinical findings to clinical application in orthopaedics

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Abstract
Purpose The aim of this systematic review is to examine the available clinical evidence in the literature to support mesenchymal stem cell (MSC) treatment strategies in orthopaedics for cartilage defect regeneration.
Methods The research was performed on the PubMed database considering the English literature from 2002 and using the following key words: cartilage, cartilage repair, mesenchymal stem cells, MSCs, bone marrow concentrate (BMC), bone marrow-derived mesenchymal stem cells, bone marrow stromal cells, adipose-derived mesenchymal stem cells, and synovial-derived mesenchymal stem cells.
Results The systematic research showed an increasing number of published studies on this topic over time and identified 72 preclinical papers and 18 clinical trials. Among the 18 clinical trials identified focusing on cartilage regeneration, none were randomized, five were comparative, six were case series, and seven were case reports; two concerned the use of adipose-derived MSCs, five the use of BMC, and 11 the use of bone marrow-derived MSCs, with preliminary interesting findings ranging from focal chondral defects to articular osteoarthritis degeneration.
Conclusions Despite the growing interest in this biological approach for cartilage regeneration, knowledge on this topic is still preliminary, as shown by the prevalence of preclinical studies and the presence of low-quality clinical studies. Many aspects have to be optimized, and randomized controlled trials are needed to support the potential of this biological treatment for cartilage repair and to evaluate advantages and disadvantages with respect to the available treatments.
Level of evidence IV.

Keywords Mesenchymal stem cells · Cartilage · Injection · Surgical repair

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Introduction

Articular cartilage lesions are a debilitating disease resulting in fibrillation and subsequent degradation which can also involve the subchondral bone and lead to the development of osteoarthritis (OA). One limiting factor in the repair of these defects is the well-known low intrinsic regeneration potential of cartilage, which might be due to the difficulty encountered by progenitor cells from the blood, bone marrow, or even other compartments in entering the defect and the inability of resident articular chondrocytes that are entrapped within the surrounding matrix to migrate into the lesion to secrete a reparative matrix [79].

In recent years among the surgical techniques (such as debridement, marrow-stimulating procedures, and ACI) that can improve joint function and thus postpone the need for replacing the articular surface [43, 53], mesenchymal stem cell (MSC) strategies are emerging as a powerful tool for cartilage repair, thanks to their marked ability to

differentiate into a variety of connective tissues including cartilage, bone, fat, tendon, ligament, marrow stroma, and others [5, 52]. The regenerative effects of MSCs are due to their structural contribution to tissue repair and their immunomodulatory and anti-inflammatory action, through direct cell–cell interaction or secretion of bioactive factors [7, 10].

MSCs have a capacity for self-renewal, stemness maintenance, and a potential for differentiation into cells forming multiple mesodermal tissues (plasticity). They can migrate toward injured tissues (homing/trafficking) where they display trophic effects (synthesis of proliferative, pro-angiogenic, and regenerative molecules). Most remarkably, MSCs exert a suppressive effect on components of the immune system (immunomodulation) by inhibiting T and B lymphocyte activation and proliferation, suppressing NK cell activation, escaping CTL-mediated lysis, and modulating the secretion profiles of dendritic cells/macrophages [74], thus allowing allo- and xenotransplantation [50]. The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy has established the following minimal set of standard criteria to provide a uniform characterization of such cells [17, 32]: (1) They must be plastic-adherent when maintained in standard culture conditions; (2) they must express CD105, CD73, and CD90 and lack surface expression of CD45, CD34, CD14 (or CD11b), CD79α (or CD19), and HLA-DR; (3) and they must be capable of differentiating to chondrocytes, osteoblasts, and adipocytes in vitro.

MSCs were first identified by Friedenstein et al. [21] in 1966 in bone marrow (BMSCs). Subsequently, in 1970, Caplan's group [6] provided the first evidence of chondrogenic, osteogenic, and muscular differentiation potential of these cells and introduced the term "mesenchymal stem cells" in the early 1990s [75]. Another important study performed by Wakitani et al. [80] first showed the efficacy of autologous MSC implantation in rabbit osteochondral defect healing, and finally, in 2001 Quarto et al. [65] described the first successful clinical application of cultured MSCs by focusing on bone healing in humans.

Nowadays, MSCs can be isolated from human sources other than the bone marrow, such as adipose tissue, umbilical cord blood, synovial membrane, synovial fluid, periosteum, dermis, trabecular bone, infrapatellar fat pad, and muscle, with similar phenotypic characteristics but different propensities in proliferation and differentiation potentials [52]. Numerous studies have described the success of different MSC application modalities, through injection [15] or scaffold implantation [23], involving different biomaterials and sometimes combined with growth or transcription factors (such as recombinant molecules or even in the form of genetic sequences), such as TGF-β, BMP-7, FGF-2, or SOX9 [11, 13, 38, 78], hyaluronic acid [57], or magnetic devices [31]. Some researchers have investigated the possibility of predifferentiating MSCs into the chondrogenic lineage before implantation [9] to provide better targeted tissue regeneration. However, the optimal strategy has not yet been identified. Peripheral blood is another possible source of MSCs (PBMSCs), but current knowledge is still very limited, with poor results, low number of patients, and shorter follow-up [69]. They cannot be easily isolated, and their number is very low, especially in adult humans; moreover, a previous patient stimulation [69] is required to increase their number, making this procedure more complicated. As BMSCs, they contain heterogeneous cell populations. Lack of phenotypic markers makes the identification and the study of PBMSCs difficult [30].

The aim of this review is to examine the available literature on MSC treatment strategies in clinical orthopaedics to identify their potential, pitfalls, and future trends for cartilage defect regeneration. The peripheral blood cell source was not taken into consideration due to the many limits previously indicated, and attention was focused on bone marrow, adipose, and synovial-derived MSCs (Fig. 1).

Materials and methods

The research was performed on the PubMed database considering the literature from 2002 and using the following key words: cartilage, cartilage repair, MSCs, mesenchymal stem cells, bone marrow concentrate, bone marrow-derived mesenchymal stem cells, bone marrow stromal cells, adipose-derived mesenchymal stem cells, and synovial-derived mesenchymal stem cells.

The combination used to identify the suitable papers was applied selecting for publication dates "10 years" and for language "English." All the papers found have been screened to identify clinical and preclinical studies. Papers found by screening the reference lists were also considered for the literature analysis of this review. Preclinical studies were counted per year to analyse the scientific interest on this new biological approach and the impact on the literature over time. The analysis of the research focused on clinical applications described in studies with a level of evidence between I and IV.

Results

The systematic research performed using the previously mentioned key words identified 72 preclinical papers and 18 clinical trials, with an increasing number of published studies on this topic over time (Fig. 2). Among preclinical studies two dealt with the use of bone marrow concentrate (BMC), 50 with cultured bone marrow-derived MSCs

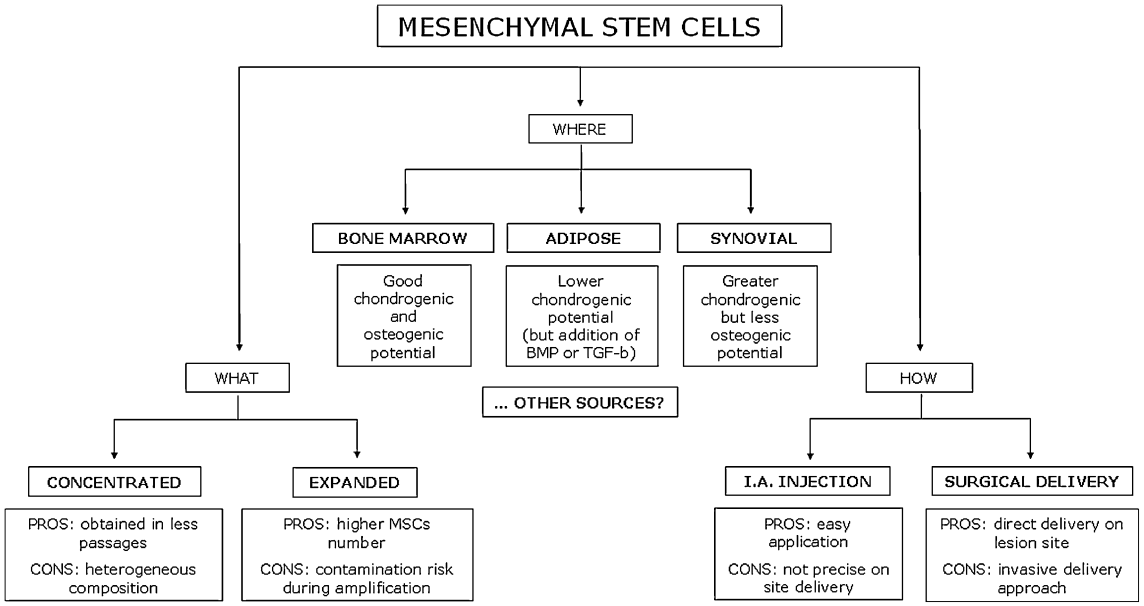

Fig. 1 Treatment strategies for the clinical application of MSCs (*i.a.* intra-articular)

(BMSCs), eight with synovial-derived MSCs, six with adipose-derived MSCs (ADMSCs), and six with comparisons between different MSC sources. Among the 18 clinical trials identified focusing on cartilage regeneration (Table 1), none were randomized, five were comparative, six were case series, and seven were case reports; only two concerned the use of ADMSCs, five the use of BMC, and 11 the use of BMSCs. Although only a few interesting preclinical studies are mentioned, all the clinical trials are described in detail, according to the specific cell and treatment categories.

Bone marrow-derived mesenchymal stem cells

BMSCs were the first type of MSCs to be identified, and the ease of collection and relatively high quantity of MSCs

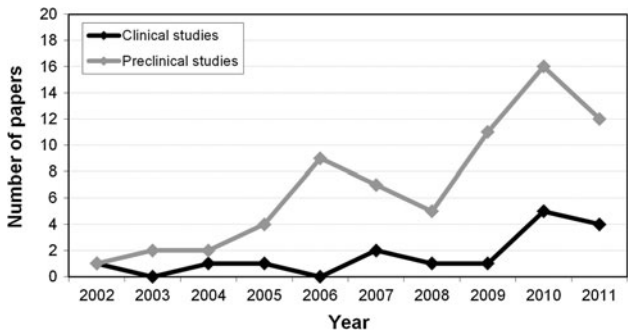

Fig. 2 Systematic research shows a growing interest in this biological treatment approach for cartilage regeneration, with an increasing number of published studies over time

still make bone marrow a commonly used source of MSCs [58]. MSCs can be used as a cell suspension expanded by culture or just as a bone marrow concentrate (Fig. 3) [26, 44]; it is known that these products differ markedly according to composition. In fact, most adult bone marrow consists of blood cells in various stages of differentiation; these components can be divided into plasma, red blood cells, platelets, and nucleated cells. Adult MSC fraction is present in the nucleated cells of the marrow, and their number is very limited compared to cultured MSCs [8], but the presence of various cell progenitor types might have a positive influence on tissue regeneration. It has to be considered that cell amplification by culture is not free from the dangers of bacterial contamination, xenogenic risk, or cellular transformation, influencing the differentiation abilities of MSCs [72]. Moreover, the problem of hypertrophy (undesirable premature terminal MSC differentiation), as reported in some experimental models of MSC-based chondrogenesis [59], needs further clarification in clinical settings. However, active laboratory research is ongoing to address these limitations, and genetic modification of MSCs via gene transfer may offer strong tools to (1) increase the yield of MSCs available for therapeutic purposes as when applying mitogenic factors (FGF-2) [13] and (2) to prevent hypertrophy of differentiated cells as achieved with cartilage- *versus* bone-specific transcription factors (SOX9) [78]. Among the many gene delivery vectors currently available, recombinant adeno-associated virus (rAAV) vectors appear to be the most promising vehicles to modify progenitor cells (or others) as they do not carry viral coding sequences (making them less immunogenic and toxic than adenoviral vectors) and do not

Table 1 Details of the 18 clinical trials identified by the systematic review focusing on cartilage regeneration with MSCs

MSCs	Publication	Study type	Treatment	Additional factors	Pathology	No. of patients	Follow-up	Results
ADMSCs	Koh et al. [42] The Knee	Comparative study	Concentrated ADMSCs injection after debridement (from infrapatellar fat pad) (1.2–2.3 × 10^6 cells)	PRP	Knee OA	25 ADMSCs 25 only PRP	16.4 months	No major adverse event with ADMSCs Similar clinical results at the last follow-up in both groups, but tendency toward greater improvement in ADMSCs group
	Pak et al. [63] J Med Case Reports	Case report	Concentrated ADMSCs injection	HA + PRP + dexamethasone	Knee OA	2	3 months	At MRI, significant increase in cartilage thickness Improvement in measured physical therapy outcomes, subjective pain, and functional status
BMC	Gigante et al. [25] Int J Immunopathol Pharmacol	Case series	BMC plus AMIC	–	Medial femoral condyle lesion	5	12 months	Nearly normal arthroscopic appearance and satisfactory repair tissue. Hyaline-like matrix in one case, a mixture of hyaline/fibrocartilage other cases
	Giannini et al. [23] Injury	Comparative study	BMC on hyaluronic acid membrane	Platelet gel (PRF)	Osteochondral talar dome lesion	10 ACI 46 ACI arthroscopic 25 BMC	36 months	Similar clinical improvement in all groups Good restoration of the cartilaginous layer with hyaline-like characteristics at MRI and histologic score
	Varma et al. [77] J Indian Med Assoc	Comparative study	BMC injection after debridement	–	Knee OA	25 BMC 25 Debridement alone		Higher improvement in symptoms, function, and quality of life
	Buda et al. [3] J Bone Joint Surg Am	Case series	BMC + hyaluronic acid membrane (arthroscopic)	Platelet gel (PRF)	Knee osteochondral lesion	20	24 months	Significant clinical improvement that increased over time Regeneration of subchondral bone and cartilage tissue
	Giannini et al. [24] Clin Orthop Rel Res	Case series	BMC + collagen powder BMC + hyaluronic acid membrane (arthroscopic)	Platelet gel (PRF)	Osteochondral talar dome lesion	48	24 months	Clinical improvement and regenerated tissue in various degree of remodelling, although none presented entirely hyaline-like cartilage

Table 1 continued

MSCs	Publication	Study type	Treatment	Additional factors	Pathology	No. of patients	Follow-up	Results
Cultured BMSCs	Emadedin et al. [19] Arch Iran Med	Case series	Cultured BMSCs injection (20–24 × 10^6 cells)	–	Knee OA	6	12 months	No local or systemic adverse events. Decrease in pain and improvement in joint function and walking distance. Evidence of cartilage thickness increase in 3/6 at MRI
	Kasemkijwattana et al. [36] J Med Assoc Thai	Case report	Cultured BMSCs on collagen scaffold	–	Knee OA	2	31 months	Clinical improvement and good defect filling, stiffness, and incorporation to the adjacent cartilage at arthroscopic view
	Davatchi et al. [15] Int J Rheum Dis	Case series	Cultured BMSCs injection (8–9 × 10^6 cells/ml)	–	Knee OA	4	12 months	High improvement in subjective parameters No improvement at X-ray analysis
	Haleem et al. [29] Cartilage	Case series	Cultured BMSCs on platelet-rich fibrin glue scaffold (2 × 10^6 cells/cm^2)	–	Full-thickness cartilage defect	5	12 months	All patients' symptoms improvement At MRI, complete defect fill and complete surface congruity with native cartilage in 3/5
	Nejadnik et al. [61] Am J Sports Med	Comparative study	Cultured BMSCs and periosteum patch (2 × 10^6 cells/cm^2)	–	Knee cartilage defect	36 ACI 36 BMSCs	24 months	No clinical differences between ACI and BMSCs, except for physical role functioning with greater improvement in BMSCs patients
	Centeno et al. [8] Pain Physician	Case report	Cultured BMSCs injection (22.4 × 10^6 cell/cm^2)	–	Knee cartilage lesion	1	24 weeks	Improvement of range of motion and pain scores At MRI significant cartilage and meniscus growth
	Kuroda et al. [46] Osteoarthritis and Cartilage	Case report	Cultured BMSCs on collagen gel + periosteum	–	Knee full-thickness cartilage defect	1	12 months	Hyaline-like tissue regeneration improvement in clinical symptoms and return to previous activities level
	Wakitani et al. [84] J Tissue Eng Regen Med	Case report	Cultured BMSCs on collagen gel + periosteum or synovium (5 × 10^6 cell/ml)	–	Knee full-thickness cartilage defect	3	17–27 months	Improvement in all clinical symptoms maintained over time Histological findings reveal fibrocartilaginous tissue
	Adachi et al. [1] J Rheumatol	Case report	Cultured BMSCs on hydroxyapatite ceramic	–	Knee osteochondral defect	1	–	At arthroscopic view, cartilage-like and bone tissue regeneration
	Wakitani et al. [83] Cell Transplant	Case report	Cultured BMSCs on collagen gel + periosteum (5 × 10^6 cell/ml)	–	Knee full-thickness cartilage defect	2	5 years	Long-term clinical improvement At 2 years, defect fill with fibrocartilage
	Wakitani et al. [81] Osteoarthritis and Cartilage	Comparative study	Cultured BMSCs on collagen gel + periosteum (5 × 10^6 cell/ml)	–	Knee OA	12 BMSCs 12 Controls	16 months	Clinical improvement not significantly different in two groups, but better arthroscopic and histological score in cell-transplanted group

integrate in the host genome, avoiding insertional mutagenesis and oncogene activation (in contrast with lenti-/retroviral vectors) [14].

Cultured BMSCs

This treatment might be difficult to manage from a legal point of view, because these cell types might be considered as a pharmacological agent administration.

However, positive results have been shown both in animal and in human models. Among the numerous preclinical studies showing the potential of this biological approach, two recent interesting applications are reported. The first one concerns the use of predifferentiated MSCs on the cartilage lineage, which requires the addition of growth factors to culture medium to further increase the healing potential: In 2010 Zscharnack's group [88] investigated the possibility to use predifferentiated BMSCs on collagen gel

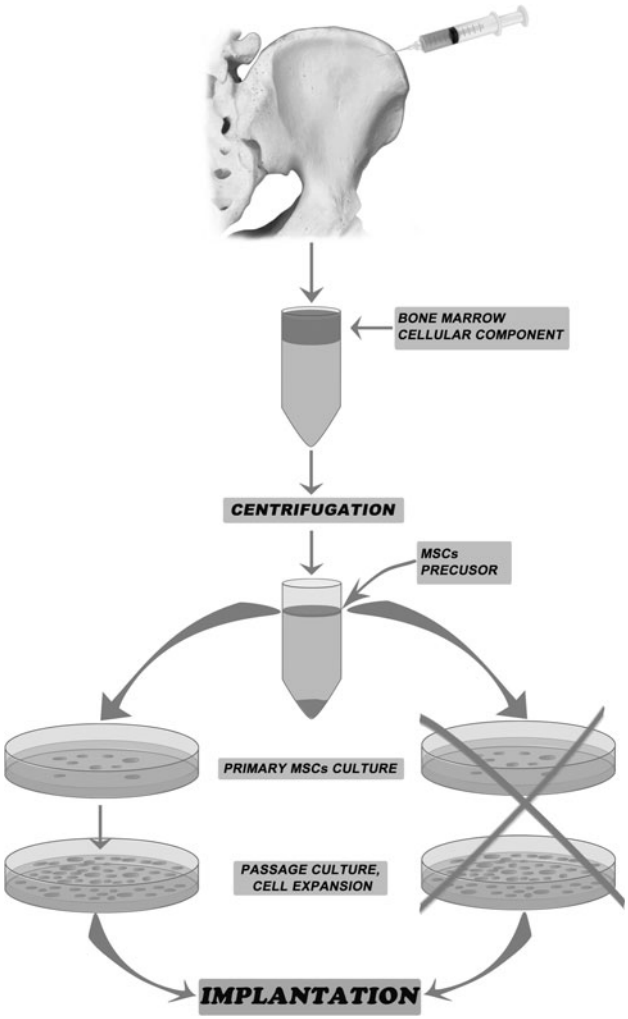

Fig. 3 MSCs can be used as cell suspension expanded by culture or just as bone marrow concentrate

for the repair of chronic, full-thickness chondral defects in the medial femoral condyles of sheep and showed significantly better histological scores with some morphologic characteristics of hyaline cartilage at 6 months postoperatively. The second one is the use of BMSCs as an improvement of a classic technique for cartilage treatment, as shown by McIlwraith et al. [55] in ten horses with chondral defects: Both medial femorotibial joints were treated with the microfracture repair technique, but only one received a single injection of MSCs and hyaluronan. At 12 months arthroscopic and gross evaluation highlighted a significant increase in repair tissue firmness and a trend for better overall repair tissue quality, thus suggesting a possible application modality of these cells in clinics.

Surgical treatment

In 2002 Wakitani et al. [81] described the use of BMSCs embedded in collagen gel covered with periosteum in 12 osteoarthritic knees after high tibial osteotomy, whereas 12 more patients were used as controls. At 16 months, results revealed a similar clinical improvement in both groups, but in the cell-treated group better arthroscopic and histological scores were found. The same research group two years later described the successful results of two patients with full-thickness knee cartilage defects treated using the previously reported technique: After 6 months they found a clinical improvement, which remained stable 4 and 5 years after treatment [83].

In 2005 Adachi et al. [1] presented a case report of a 21-year-old man affected by a large osteochondral knee defect treated with cultured BMSCs on hydroxyapatite ceramic. The biopsy of repaired tissue revealed cartilage and bone regeneration.

Two years later, Wakitani et al. [84] described results after the treatment of three patello-femoral cartilage defects with BMSCs on collagen gel covered with autologous periosteum or synovium and showed an improvement in clinical symptoms at 6 months, which was maintained over the follow-up period (17–27 months). Histology evaluations were performed at 12 months, which revealed that the defect had been repaired with fibrocartilaginous tissue. In the same year Kuroda et al. [46] described good clinical improvement using the same construct on a 31-year-old male judo player with a full-thickness knee cartilage defect, who returned to his previous activity level 1 year after surgery. Histological evidence showed hyaline-like tissue.

In 2010 Nejadnik et al. [61] compared the first-generation ACI technique with BMSC implantation in a cohort study (evidence level III): 72 matched (lesion site and age) patients affected by full-thickness knee cartilage defects underwent chondral repair using chondrocytes (36 patients)

or BMSCs (36 patients). Clinical outcomes were measured before surgery and 3, 6, 9, 12, 18, and 24 months of follow-up. They showed a similar pattern of clinical and subjective improvement up to 2 years postoperatively. Interestingly, men showed a significantly better improvement than women, and patients younger than 45 years scored significantly better than patients older than 45 years in the autologous chondrocyte implantation group, but age did not make a difference in outcomes in the BMSC group. The authors concluded this comparative evaluation by reporting the advantages of the BMSC technique that was as effective as using chondrocytes for cartilage repair but required one less knee operation, cost less, and minimized donor-site morbidity. In the same year, Haalem et al. [29] used BMSCs on platelet fibrin glue scaffold for the treatment of articular knee cartilage defects in five patients: All patients' symptoms improved at 12 months, and MRI revealed complete defect filling and complete surface congruity with native cartilage in three patients, but two showed incomplete congruity.

Finally, in 2011 Kasemkijwattana et al. [36] showed good defect filling and repair tissue stiffness with cultured BMSCs on collagen scaffold in two patients with knee OA and reported good incorporation with the adjacent cartilage and a significant clinical improvement.

Injective treatment

A case report by Centeno et al. [8] in 2008 showed encouraging results after treating a knee cartilage lesion by intra-articular injection of BMSCs. At 6 months, MRI showed an increase in cartilage and meniscus volume, and an improvement in range of motion and pain score was reported.

In 2011, Davatchi et al. [15] performed a single intra-articular BMSC injection in four osteoarthritic knees. They described a marked clinical improvement in subjective parameters, although physical parameters (such as number of stairs to climb, walking time, and resting time) improved much less. Good results were also reported more recently by Emadedin et al. [19]: Six patients with radiological evidence of knee OA underwent a single injection of BMSCs. No adverse events were described, and pain, functional status of the knee, and walking distance tended to improve up to 6 months after the injection. MRI analysis before and 6 months after treatment showed an increase in cartilage thickness and a considerable decrease in the size of oedematous subchondral bone in three out of six patients.

Bone marrow concentrate

The potential of using BMC instead of BMSCs has been described in some preclinical studies where good results were reported: Saw et al. [70] investigated the use of BMC in combination with hyaluronic acid after full-thickness chondral defects and subchondral drilling in the goat model and found complete coverage of the defect with evidence of hyaline cartilage regeneration after 24 weeks. In the same year, Fortier et al. [20] reported satisfactory results by combining BMC injections with the microfracture technique: Treatment with BMC showed histological and macroscopic improvements in the repair tissue. MRI revealed an increase in defect filling and an improvement in repair tissue integration with normal surrounding cartilage.

Surgical treatment

A case series by Giannini et al. [24] in 2009 presented the treatment of osteochondral talar dome lesions with BMC and collagen powder or hyaluronic acid membrane: At 24 months, 48 patients showed newly formed tissue well integrated with the surrounding tissue, and only two had cartilage hypertrophies. Clinical scores improved in all patients. One year later, the same research group performed two further clinical studies: The first showed good subchondral bone and cartilage tissue regeneration after arthroscopic implantation of BMC on hyaluronic acid membrane and platelet-rich fibrin (PRF) in 20 osteochondral knee defects [3]. These good findings were confirmed in the second study that compared three different techniques for osteochondral talar dome tissue regeneration: 10 patients underwent open ACI, 46 arthroscopic ACI, and 25 one-step BMC transplantation. A similar clinical improvement was detected in all groups, with good restoration of the cartilaginous layer, as seen by MRI and histological analysis, which resembled hyaline cartilage [23].

More recently, in 2011 Gigante et al. [25] presented an augmentation of the AMIC technique with BMC in five patients with medial femoral condyle lesions; the result was nearly normal arthroscopic appearance, although evidence of hyaline-like matrix was found only in one case.

Injective treatment

Varma et al. [77] reported good results using BMC injection in 50 patients with mild to moderate knee OA: 25 subjects underwent debridement and subsequent BMC injection, whereas other 25 underwent debridement alone. During the follow-up, authors observed an improvement in symptoms, with shortened hospital stay and better quality of life after BMC injection.

Adipose-derived mesenchymal stem cells

Adipose-derived MSCs (ADMSCs) obtained from lipoaspirates offer a great advantage as a cell source for cartilage

tissue engineering, due to their abundance, easy availability, and their potential to differentiate into cartilage, besides bone, tendons, skeletal muscle, and fat [71]. Their potential has been shown in the preclinical field in combination with different scaffold types. In 2006 Masuoka et al. [54] used ADMSCs on atelocollagen scaffold for the healing of rabbit osteochondral defects: Histological analysis showed that the defects were filled with hyaline-like cartilage, expressing high levels of type II collagen. Similar good results were obtained by Dragoo et al. [18] 1 year later, using ADMSCs on fibrin glue scaffold for the treatment of full-thickness rabbit articular cartilage defects: Complete healing of subchondral bone and hyaline-like tissue regeneration were found.

A lower chondrogenic potential has been reported when compared with BMSCs, but this disadvantage might be overcome by using a combination of transforming growth factor beta 2 (TGF-β2) and bone morphogenetic proteins (BMPs) or high doses of TGF-β2 and IGF-I in combination [38]. In 2010, Im et al. [33] evaluated the efficacy of ADMSCs enriched with TGF-ß2 and BMP-7 on polycaprolactone (PCL) scaffold for rabbit cartilage defect healing: Interestingly, augmentation with growth factors improved the gross appearance of the osteochondral defects while not actually leading to better histological results and induced a greater degree of foreign body reaction, thus underling the limits and risks of this procedure and the need for more studies to understand better the potential of this combined approach before applying it to clinical practice.

Injective treatment

In 2011 Pak et al. [63] reported good results after treatment of two patients affected by knee OA with the injection of concentrated ADMSCs together with HA, dexamethasone and PRP. Concentrated ADMSCs were obtained by double centrifugation of lipoaspirates and digestion with collagenase. After 3 months, subjective pain and functional status improved, and MRI revealed a significant increase in cartilage thickness.

More recently, Koh et al. [42] described the use of concentrated ADMSCs, isolated from the infrapatellar fat pad, for the treatment of knee OA in a case–control study: 25 patients were enrolled, and after debridement, they received an injection of concentrated ADMSCs and PRP; subsequently, another two injections of PRP were performed weekly. The control group received only debridement and PRP injections. No major adverse events were reported using ADMSCs. Clinical results at the last follow-up (average: 16 months) were similar in both groups, although the study group tended to have a greater degree of improvement.

Synovial-derived mesenchymal stem cells

Synovial-derived stem cells are a promising source of stem cells for cartilage tissue engineering because they display greater chondrogenic and less osteogenic potential than MSCs derived from bone marrow or periosteum [16], but the evidence of their potential is still limited to preclinical studies. Among these, in 2006 Koga et al. [39] implanted a collagen/synovial cultured MSC construct plus periosteum flap for the healing of full-thickness knee articular cartilage defects in adult rabbits. Cartilage defects appeared to be glossy, smooth, and similar to neighbouring cartilage, and the margin of the repaired tissue seemed to integrate into the surrounding native cartilage. Histological scores improved continuously over the follow-up period. Subsequently, in 2010 Shimomura et al. [73] reported similar good results using a scaffold-free three-dimensional tissue-engineered construct: This novel construct was derived from synovial MSCs and extracellular matrix synthesized by the cells after culturing in vitro. Complete repair of chondral lesions was seen in all MSC-treated pigs, with repair tissue similar to normal cartilage. Finally, an interesting approach was showed by Hori et al. [31] in 2011 using a permanent magnet implantation at the bottom of the defect in the rat knee and a subsequent injection of magnetic-labelled synovial MSCs: Complete regeneration of articular cartilage with high histological scores was seen at 12 weeks, thus showing both the usefulness of this cell-based approach and the effectiveness of this cell delivery method.

Comparative studies

No clinical comparative studies focusing on different MSC sources are currently available in the literature, and the optimal MSC source has not yet been identified. Some preclinical evaluations suggest various options with different chondrogenic potential. For example, synovium-derived MSCs have the best potential for chondrogenesis, followed by bone marrow-derived and periosteum-derived MSCs, whereas adipose- and synovium tissue-derived MSCs are superior in terms of adipogenesis, and bone marrow-, synovium-, and periosteum-derived MSCs are superior in terms of osteogenesis [10, 68]. An important drawback of these studies is that the evaluation of in vitro chondrogenesis may not represent the chondrogenic potential of MSCs transplanted into cartilage defects [40]. Some more robust indications are available from preclinical studies in the animal model. In 2002 Wakitani et al. [82] compared periosteum MSCs and BMSCs on collagen gel for the treatment of articular cartilage defect in rabbits. Histological scores of the two cell-transplanted groups were similar and improved at 4 weeks compared with those at 2 weeks, but the cartilage

that was formed by the periosteum MSCs became more irregular compared with that from BMSCs. One year later, Nathan et al. [60] described their results after comparing periosteum MSCs and ADMSCs in a fibrin carrier for the treatment of rabbit knee chondral defects. Histologically and biomechanically, the defects repaired by cells derived from adipose tissue were better healed than those repaired by periosteum-derived cells. In 2006, Park et al. [64] used MSCs from perichondrium/periosteum, bone marrow, or fat of adult rats to regenerate knee cartilage defects. Before implantation, MSCs were predifferentiated to chondrogenic lineage through adenoviral vectors carrying BMP-2 cDNA. Results suggested that MSCs from periosteum and bone marrow were superior to cells isolated from fat in forming hyaline cartilaginous tissue. In 2008, Koga et al. [41] showed the superiority of synovial MSCs and BMSCs for the repair of full-thickness rabbit knee chondral defects. They compared synovial MSCs, BMSCs, ADMSCs, and muscle MSCs seeded on collagen gel and successively transplanted into the defect site with a periosteal patch: At 4 weeks synovial and bone marrow MSCs showed more cartilage matrix in the defect site than ADMSCs and muscle-derived MSCs. When synovial MSCs were transplanted, the border between regenerated cartilage-like tissue and subchondral bone moved upward and close to the native height at 12 weeks. Subsequently, in 2009 Frisbie et al. [22] compared the use of ADMSC and BMSC injections in horses with osteoarthritic carpal joints. Histological, radiological, and clinical evidence showed no significant differences in both treatment groups 70 days after operation. More recently, in 2011 Zhang et al. [87] compared the healing potential of bone mononuclear cells (BNCs) and BMSCs embedded in a collagen hydrogel and injected into full-thickness rabbit knee chondral defects: 8 weeks after surgery, no significant differences were detected in repair tissue quality or integration with surrounding native cartilage between BNCs and BMSCs groups.

Finally, Xie et al. [86] compared in vitro BMSCs and ADMSCs seeded onto a three-dimensional PRP-derived scaffold. BMSCs showed a higher proliferation rate and a higher expression of cartilage-specific genes and proteins than did ADMSCs. Moreover, besides the good results obtained with both cell sources, in an osteochondral defect model in rabbits, implanted BMSCs also exhibited better gross appearance and histological and immunohistochemical characteristics, higher cartilage-specific gene and protein expression, as well as subchondral bone regeneration.

Discussion

This systematic research showed a growing interest in this biological treatment approach for cartilage regeneration, producing an increasing number of published studies over time. However, knowledge about this topic is still preliminary, as shown by the prevalence of preclinical studies and, among the clinical findings, by the presence of studies of low quality due to weak methodology, small number of patients, and short-term follow-up. Nonetheless, the studies available suggest a potential for these cell-based treatments to be developed in many directions, with different available cell sources, the possibility to use them concentrated or expand them in vitro, to apply them as a simple minimally invasive injective approach, or to be delivered surgically, alone or augmented with growth factors or scaffolds, and many other improvements are being developed.

Mesenchymal stem cells in cartilage regeneration represent a promising new approach with preliminary interesting findings ranging from focal chondral defects to articular OA degeneration. However, many aspects are still controversial, and they have to be clarified. Firstly, the optimal MSC source has not yet been identified: MSCs can be isolated from various human sources, such as adipose tissue, umbilical cord blood, synovial membrane, synovial fluid, periosteum, dermis, trabecular bone, infrapatellar fat pad, and muscle [52], but they present various differentiation abilities [10, 22, 40, 41, 60, 64, 68, 82, 86, 87]. Besides these cell properties, the yield of cells obtained by extraction might also be a limiting factor and contributes to the choice of cell source for clinical application. The continually emerging field of experimental stem cell research and cartilage repair, particularly with the help of preclinical large animal models, will provide some new solutions to this issue.

The most appropriate cell source is not the only controversial aspect. For example, many important biological pathways that determine the fate of transplanted MSCs in cartilage defects, particularly with the view to hypertrophic differentiation, are unknown. Controlling the chondrogenesis of MSCs in this environment is not understood. The interplay of MSCs with the adjacent osteochondral unit has not yet been clarified.

Another aspect that has to be considered is the potential risks in MSCs use: One possibility is—besides cancer or immunological disease—the differentiation of these cells into unwanted tissue, as reported by Breitbach et al. [2] who described the calcification of MSCs injected into infarcted rat hearts. For the treatment of articular cartilage defects, this implies in theory the risk of such MSC-mediated endochondral ossification to occur at least in some parts of the repair tissue, thus jeopardizing the formation of good-quality tissue and the clinical outcome. Improvement in noninvasive imaging of the cartilaginous repair tissue will help to detect with a high-resolution early signs of such unwanted ossifications and to understand the real dimension of this problematic aspect.

Recent concerns have been expressed about the potential transformation of MSCs during the culture process [67]. Conversely, Wakitani et al. [85] demonstrated the safeness of using BMSCs in cartilage repair in 41 patients followed up 5–137 months after transplantation: Neither tumours nor infection were observed. The debate is still ongoing and warrants close scrutiny, since such stem cell therapies are far from being accepted in the field of clinical articular cartilage repair, nor has their long-term safety been convincingly proven. Moreover, reliable clinical data based on long-term, randomized, double-blind, controlled, multicenter studies with systematic follow-up are largely lacking. Such information is needed, as it might determine the true value of MSC therapy for articular cartilage defects and help to identify the best indications for it.

This cell-based treatment for cartilage regeneration is still in its infancy and many aspects remain to be clarified and optimized. Among these, one of the most clearly missing elements is the knowledge of the proper cell dosage to be administered. The dose–response relationship of MSC transplantation for clinical cartilage repair has not yet been established. The current literature shows the variety in quantity of transplanted cells into the defect site, making clinical outcome comparison very difficult. As the number of cells per defect volume that would be required for successful articular cartilage regeneration remains unclear, identification of such an effective quantity of MSCs represents another key point.

Lastly, improvement in the effect of MSCs by using therapeutic agents (growth, transcription, or signalling factors) provided as peptides or genetic sequences is under active investigation to evaluate the optimal conditions for cartilage tissue regeneration (optimal factor or combination of factors, most effective delivery system, best suited biomaterial for cell containment). The transplantation of activated MSCs in experimental cartilage defects has been tested by using various scaffolds, in the presence of recombinant peptides (TGF-β, BMP-2 or BMP-4, and PDGF) [48, 56, 66, 76] or via administration of gene vectors (TGF-β, BM-2, BM-4, BM-7, SOX5, SOX6, SOX9, Shh, CDMP1, and ZNF145) [4, 12, 27, 28, 34, 35, 37, 45, 47, 49, 51, 62] and has led to improved healing of the treated defects. Nevertheless, so far a complete regeneration of the cartilage lesions has not yet been achieved with these systems, thus showing the need for further development probably based on the use of the most potent rAAV [21, 22] and for a better understanding of the intrinsic repair processes in this highly specialized tissue.

The technical challenge here will be to assemble these components into a clinically valid and useful system. However, as found in the field of ACI over the past two decades, such technical improvements are possible, effective, and will very likely also translate into improved systems for MSC delivery.

Conclusion

This systematic research showed a growing interest in this biological treatment approach for cartilage regeneration, which has produced an increasing number of published studies over time. However, knowledge on this topic is still preliminary, as shown by the prevalence of preclinical studies and, among the clinical findings, the presence of low-quality studies. Many aspects have to be optimized, such as the best cell source and the most appropriate delivery method, the most effective dose and augmentation procedure, and the correct treatment indication, and contraindications and risks have to be investigated. Randomized controlled trials are needed to support the potential of this biological approach for cartilage treatment and to evaluate advantages and disadvantages with respect to the available treatments.

Conflict of interest The authors declare that they have no conflict of interest.

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