

Current perspectives in stem cell research for knee cartilage repair

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Abstract: Protocols based on the delivery of stem cells are currently applied in patients, showing encouraging results for the treatment of articular cartilage lesions (focal defects, osteoarthritis). Yet, restoration of a fully functional cartilage surface (native structural organization and mechanical functions) especially in the knee joint has not been reported to date, showing the need for improved designs of clinical trials. Various sources of progenitor cells are now available, originating from adult tissues but also from embryonic or reprogrammed tissues, most of which have already been evaluated for their chondrogenic potential in culture and for their reparative properties in vivo upon implantation in relevant animal models of cartilage lesions. Nevertheless, particular attention will be needed regarding their safe clinical use and their potential to form a cartilaginous repair tissue of proper quality and functionality in the patient. Possible improvements may reside in the use of biological supplements in accordance with regulations, while some challenges remain in establishing standardized, effective procedures in the clinics.

Keywords: cartilage repair, knee, focal defects, osteoarthritis, stem cells, clinical trials

Introduction

Articular cartilage lesions, especially those affecting the knee joint, as in acute trauma or osteoarthritis, remain a major unsolved clinical problem due to the poor intrinsic repair capacity of this highly specialized tissue. While various options are available for the clinician to repair a damaged joint surface, none can reliably restore the natural articular cartilage integrity, resulting in a limited ability of the tissue to withstand mechanical stresses during physical activities throughout life.

Strategies based on the application of stem cells that can be relatively easily acquired, expanded, and selectively committed towards a cartilaginous tissue may provide effective treatments for cartilage lesions in patients. Progenitor cells of potential value to achieve this goal and already applied using experimental models in vivo include bone marrow-derived mesenchymal stem cells (MSCs) and MSCs from the adipose tissue, synovium, periosteum, umbilical cord blood, muscle, and peripheral blood. The choice of the most suitable stem cell population for cartilage repair may depend on their availability and ease of preparation, and on their potential for chondrogenic differentiation. Active experimental work is also ongoing to identify an unlimited universal source of progenitor cells, such as embryonic stem cells and induced pluripotent stem cells, but many obstacles remain regarding their clinical use due to ethical considerations and safety issues (immune rejection, tumorigenesis, teratoma formation).

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In this paper, we provide an overview of the stem cell-based treatments and surgical procedures employed in the clinic to promote and evaluate cartilage repair in focal defects and for osteoarthritis, with a depiction of biocompatible materials used for stem cell delivery in patients. We also describe innovative strategies based on possible biological supplementation of the approaches to improve healing of lesions in the future. Finally, we discuss some of the challenges for optimal clinical use of stem cells in patients in light of knowledge about natural cartilage repair and the results of reported clinical trials in terms of methodology, regulation, and quality of repair of lesions.

Principles of articular cartilage repair

Structure and function of articular cartilage

The major function of articular cartilage is to allow for smooth gliding of the articulating surfaces of a joint and to protect the subchondral bone from mechanical stress. Remarkably, adult hyaline articular cartilage is avascular, aneural, and does not have lymphatic drainage.¹ It is structured in several laminar zones and formed by chondrocytes that are surrounded by an intricate network of extracellular matrix.² Articular chondrocytes synthesize and degrade the extracellular matrix, thereby regulating its structural and functional properties according to the loads applied. This cartilaginous matrix is rich in proteoglycans and collagen fibrils composed of type II collagen, but also comprises type VI, IX, XI, and XIV collagen and a number of additional macromolecules, including cartilage oligomeric matrix protein, link protein, decorin, fibromodulin, fibronectin, and tenascin.³ Normal hyaline articular cartilage contains about 70%–80% water, which is mainly bound to proteoglycans. The basal region of the articular cartilage is characterized by increased mineral density.⁴ This layer of calcified cartilage is closely connected to the underlying subchondral bone.⁵

Deterioration of articular cartilage

Lesions of the cartilaginous joint surface may either be of limited extent in focal articular cartilage defects or generalized during osteoarthritis (Figure 1A). In focal defects, the structural integrity of the articular cartilage is disrupted in circumscribed areas, for example as a consequence of direct trauma, osteonecrosis, or osteochondritis dissecans. The resulting articular cartilage defect is of a limited two-dimensional extent and characterized as being either

chondral, involving only the cartilaginous zones, or osteochondral, reaching further into the subchondral bone.⁵

Osteoarthritis instead, is a chronic, degenerative disorder of the diarthrodial joints, characterized mainly by an activation of inflammatory and catabolic cascades at the molecular level, ultimately leading to a gradual deterioration of the articular cartilage.⁶ Under mechanical or biochemical stress (local production of proinflammatory cytokines and mediators such as interleukin-1 β , tumor necrosis factor- α , nitric oxide, prostaglandins, or matrix degradation products), the chondrocytes undergo pathological changes in their gene expression patterns that impair the cartilage homeostasis (diminished production of native matrix molecules versus enhanced production of matrix-degrading enzymes and decreased responsiveness to reparative stimuli), ultimately resulting in matrix degradation and cell senescence with apoptosis.⁶ Osteoarthritis may also be the result of a previous injury to tendons and ligaments or following intra-articular fractures, leading to joint instability and articular cartilage wear (secondary osteoarthritis). Of note, osteoarthritis affects not only the cartilage but also the subchondral bone, and (to a minor degree) the synovial lining, ligaments, tendons, and muscles.

Spontaneous cartilage repair

Repair and regeneration of articular cartilage are entirely different processes and need to be distinguished. Cartilage repair leads to a tissue that shares structural similarities with hyaline articular cartilage with regard to the macroscopic aspect or cell type. However, this repair tissue manifests neither an arcade-like organization of its fibers nor a well-defined zonal stratification of its chondrocytes.⁷ Its biochemical composition is more akin to fibrous than hyaline cartilage,⁸ and its mechanical competence is significantly inferior to that of the latter.⁹ Thus, native hyaline cartilage is not re-established in this repair process.¹⁰ In contrast, cartilage regeneration is defined as the restitution ad integrum of articular cartilage at the histological, biochemical, and biomechanical levels, making it indistinguishable from the adjacent uninjured cartilage.¹¹ It is noteworthy that, in contrast with repair, regeneration of tissues readily occurs only in embryos, while it is almost absent in neonates and never noted in adults.¹¹

Focal chondral and osteochondral defects exhibit fundamental differences in the history of natural repair. Due to a lack of vascularization in the articular cartilage, access of progenitor cells to the site of a chondral lesion is limited. Thus, chondral defects are in part repopulated by cells that are migrating from the synovial membrane.^{7,12} However, filling

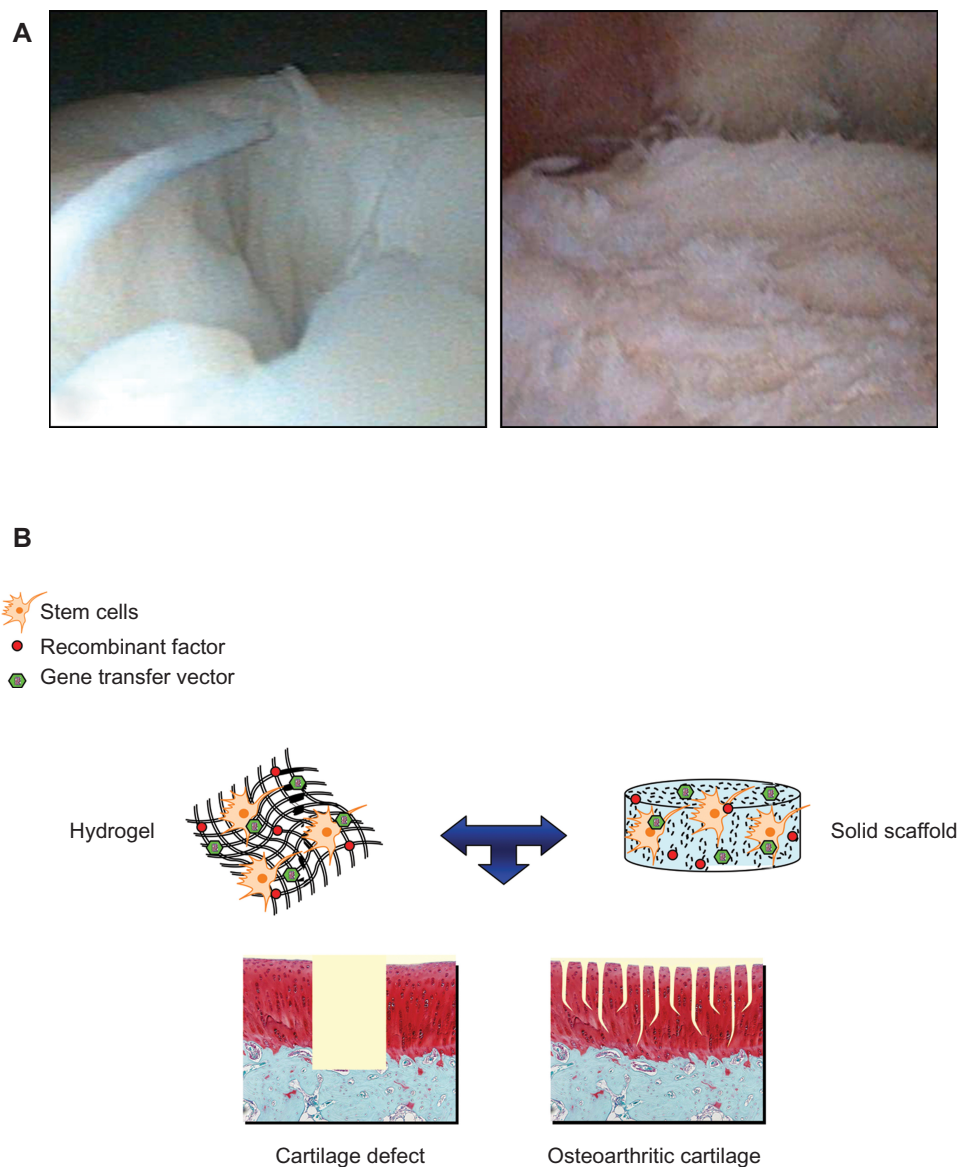


Figure 1 (A) Articular cartilage lesions. (1) Focal cartilage defect in a 28-year-old man and (2) osteoarthritic cartilage in a 49-year-old woman. **(B)** Therapeutic components of potential value to deliver stem cells for cartilage repair.

such defects is insufficient, and after some weeks or months, the repair tissue inevitably begins to degenerate.⁷ Furthermore, it integrates poorly, causing focal discontinuity and regions of hypocellularity and cluster formation within the neighboring cartilage.¹³ Ultimately, these regions of the contiguous surface become necrotic, showing neither remodeling nor resorption.¹⁴ Over time, this may lead to an increase in the size of the defect.

In contrast, an osteochondral defect is filled with a blood clot that forms if the bone marrow communicates with the defect.^{13,14} Pluripotent mesenchymal cells present in the blood clot differentiate into chondrocytes and osteoblasts that later form the cartilaginous repair tissue and the reconstituted subchondral bone, respectively. The process of chondrogenesis

is completed after some months and indicated by the appearance of round cells and the presence of a new cartilaginous matrix. Depending on the maturation of repair tissue, this cartilaginous matrix contains proteoglycans and type I and type II collagen in different ratios.¹⁴ Specifically, expression of type I collagen, type I-associated collagen types (V, VI, XII, XV), and proliferative cell markers is upregulated in the repair tissue compared with normal articular cartilage, while transcription abundance is higher in normal cartilage for proteoglycans, noncollagenous adhesion proteins, and for biomarkers of cartilage development.¹⁵ The repair tissue has an increased water content but decreased Young's and equilibrium modulus relative to the neighboring cartilage,¹⁶ exhibiting aggrecan and type II collagen content which

gradually increases over time.^{17,18} However, this repair tissue does not integrate with the existing adjacent matrix; chondrocytes within the neighboring articular cartilage do not participate in the filling of the defect but undergo apoptosis over time, and the cartilage in this region becomes acellular. After some months, the new tissue within the defect exhibits a fibrocartilaginous phenotype and early signs of degeneration are visible. Both, the repair tissue and the cartilage at the periphery of the defect do not withstand mechanical load over time and degenerate after several years.¹³ If left untreated, the size of the defect extends into the surrounding normal cartilage, and generalized osteoarthritis of the joint may result.

In osteoarthritis, the repair capacity of articular cartilage is compromised. As the critical size of a cartilage defect to repair is 3 cm²,¹⁹ the larger lesions occurring in osteoarthritis do not allow for sufficient filling of the defect and containment of the repair tissue.⁷ Thus, osteoarthritic cartilage deterioration remains irreparable and progresses over time. Of note, mechanosensitive osteoblasts²⁰ within the subchondral bone may be activated by altered biomechanical loading following cartilage deterioration in osteoarthritis. Via humoral messengers (eg, interleukin-6, vascular endothelial growth factor) and connections between the subchondral bone and the articular cartilage, such as microcracks or invading blood vessels, activated osteoblasts may stimulate articular chondrocytes to promote chondrocyte hypertrophy and cartilage angiogenesis and mineralization, leading to pathological remodeling of the osteochondral unit in osteoarthritis.²¹

Current options to improve articular cartilage repair

For the treatment of focal articular cartilage defects, conservative approaches solely aim at reducing pain. Surgical options for chondral lesions include marrow stimulation procedures such as subchondral drilling,²² microfracture,²³ and abrasion arthroplasty.²⁴ These measures establish a communication of the cartilage defect with the bone marrow, allowing MSCs from the underlying cavity to migrate into the defect.²⁵ The transplantation of isolated and expanded autologous chondrocytes in the absence or presence of supportive biodegradable matrices (autologous chondrocyte implantation)²⁶ is another operative option for focal chondral defects. For deep osteochondral defects, established reconstructive surgical therapies include the transplantation of osteochondral cylinders from uninjured, lesser weight-bearing areas of the joint²⁷ or subchondral bone grafts combined with autologous chondrocyte implantation.²⁸

Regarding the treatment of osteoarthritis, conservative measures comprise (but are not limited to) nonpharmacological options, such as weight reduction, land-based and aquatic exercises, or physical therapy, and pharmacological approaches based on nonsteroidal anti-inflammatory drugs, opioid analgesics, or intra-articular corticosteroid or hyaluronic acid injections.^{29,30} Surgical options for osteoarthritis include osteotomies to transfer the weight load from the damaged compartment to undamaged areas, and unicompartmental or total joint replacement.

However, no conservative or operative treatment procedure for either focal or generalized articular cartilage deterioration promotes a *restitutio ad integrum*; hyaline cartilage is never obtained and the fibrocartilaginous repair tissues are incapable of withstanding mechanical stresses over time. This shortcoming in patient care urgently necessitates the quest for novel treatment options for articular cartilage defects.

Value of stem cell manipulation for knee cartilage repair

Application of progenitor cells, especially MSCs, is an attractive strategy to improve the reparative processes in sites of cartilage damage compared with the implantation of differentiated cells like articular chondrocytes.³¹ MSCs have a reliable potential for differentiation (plasticity) into cells of the mesodermal lineage (chondrocytes, osteoblasts, adipocytes).^{32,33} They also display critical homing, trophic, and immunomodulatory activities^{34–37} that may favorably influence the fate and activities of unaffected cells in the surrounding cartilage upon implantation in sites of cartilage damage or injury.

MSCs can be easily extracted from various tissues (eg, bone marrow, adipose tissue, synovial membrane) and expanded under specific culture conditions that allow for extensive testing prior to implantation. The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy has defined the following minimal set of standard criteria for uniform characterization of MSCs: they must be plastic-adherent cells when maintained in standard culture conditions; they must express CD105, CD73, and CD90; they must lack surface expression of CD45, CD34, CD14 (CD11b), CD79 α (CD19), and HLA-DR; and must be capable of differentiating to cells of the mesodermal lineage (chondrocytes, osteoblasts, adipocytes).^{38,39}

Bone marrow-derived MSCs

Bone marrow-derived MSCs have been a focus of stem cell research in light of their relative ease of isolation and

expansion and of their high potential for differentiation.⁴⁰ Chondrogenesis has been conveniently achieved in high-density (aggregate) cultures in the presence of a defined medium that includes dexamethasone and transforming growth factor beta (TGF- β).^{41,42} However, an inverse correlation between age and differentiation potential of bone marrow-derived MSCs has been reported, being a challenge for application in elderly patients.⁴³ Nevertheless, proof-of-concept for the use of bone marrow-derived MSCs *in vivo* has been demonstrated in animal models of articular cartilage defects and osteoarthritis (rat, rabbit, pig, sheep, horse), showing improved repair of lesions compared with conditions where cells were not provided.^{44–59}

Adipose-derived MSCs

Adipose tissue has been also an important source of MSCs. Compared with bone marrow-derived MSCs, adipose-derived MSCs from lipoaspirates are acquired using a less invasive procedure and in larger amounts.^{60,61} Adipose-derived MSCs can commit toward the chondrogenic, osteogenic, adipogenic, myogenic, and neurogenic lineages,⁶² although they display some differences from bone marrow-derived MSCs. Adipose-derived MSCs are smaller, have different gene expression and cell surface marker profiles, and can undergo a higher number of passages before senescence, showing enhanced rates of proliferation.^{61,63–67} While adipose-derived MSCs show lesser responses to TGF- β -induced chondrogenesis,⁶⁸ efficient differentiation has been nevertheless established by addition of bone morphogenetic protein 6.⁶⁴ These cells have also been successfully employed to target cartilage defects and osteoarthritis cartilage *in vivo*, revealing improved outcomes for cartilage repair.^{51,52,69–78}

Synovial-derived MSCs

Successful extraction of MSCs from the synovial membrane has been reported by harvesting of the synovial membrane via arthroscopy in a low invasive way with minimal complications at the donor site.^{79,80} Synovial-derived MSCs have higher proliferative and chondrogenic capacities than other MSCs especially when incubated with bone morphogenetic protein 2.^{80–82} Of note, administration of synovial-derived MSCs has also been performed *in vivo*, leading to enhanced cartilage repair.^{51,52,83–87} Still, many issues need to be addressed regarding the value of synovial-derived MSCs due to a certain persistence of fibroblastic features and induction of hypertrophic gene expression profiles upon chondrogenic commitment.⁸⁰

Alternative sources of progenitor cells

MSCs have also been isolated from the periosteum, trabecular bone, umbilical cord blood, amniotic fluid, Wharton's jelly, and skeletal muscle. Periosteum progenitor MSCs⁸⁸ and umbilical cord blood MSCs⁸⁹ can both be induced towards the chondrogenic lineage in the presence of TGF- β . Periosteum progenitor MSCs have been successfully applied to repair models of cartilage defects *in vivo*.^{52,57,90} However, while periosteum progenitor MSCs are phenotypically stable and easily expanded in culture,⁹¹ their use is limited by the reduced availability of donor material and the complexity of the surgical procedure of extraction.⁹¹ Umbilical cord blood, in contrast with bone marrow or adipose tissue, possesses a lower isolation efficiency but expansion is more effective.⁹² Still, administration of these cells did not allow for the proper repair of cartilage defects in animal models while triggering an inflammatory reaction in the synovium.⁵⁹ Muscle-derived stem cells exhibit a broad differentiation capacity similar to that of bone marrow-derived MSCs.⁹³ Evaluations *in vivo* revealed that muscle-derived stem cells have the potential to improve the repair of cartilage defects.^{51,52,94} However, their capability is sex-dependent (male muscle-derived stem cells have a higher potential for chondrogenic differentiation and cartilage regeneration).⁹⁵

Interestingly, peripheral blood MSCs have been also evaluated as an alternative source of cells for transplantation^{96–98} because of their ease of harvest and potential for differentiation, and implantation of such cells allowed for good repair of cartilage defects *in vivo*.⁹⁹ Of note, investigation into the value of other types of progenitor cells for cartilage repair is actively ongoing, including work on embryonic stem cells and induced pluripotent stem cells. Embryonic stem cells and induced pluripotent stem cells may provide universal, unlimited sources of cells with reparative and regenerative capabilities for cartilage lesions because they have a potential for indefinite undifferentiated proliferation and can be induced towards chondrocyte differentiation.^{100–107} Embryonic stem cells have already been used to enhance the healing of cartilage defects *in vivo*^{108–110} and to allow for the production of a cartilage matrix capable of integrating with defects in human arthritic joint cartilage.¹¹¹ However, use of embryonic stem cells remains largely controversial for ethical reasons to do with the harvesting of cells from human embryos, and due to safety issues because their use is associated with immune rejection problems and with the formation of teratomas.¹¹² Active experimental work is ongoing with induced pluripotent stem cells that can be

generated from the patient's own somatic cells, thus avoiding potential immune rejection and ethical issues related to the use of embryonic stem cells. Induced pluripotent stem cells are usually generated by reprogramming of differentiated cells such as fibroblasts by gene transfer of multiple transcription factors or using chemical methods.^{113–116} So far, induced pluripotent stem cells have been applied to cartilage defect models in vitro, leading to production and integration of a structurally and biomechanically adapted cartilage matrix.¹¹⁷ Nevertheless, there are major challenges regarding the clinical use of induced pluripotent stem cells, including the risks of teratoma formation and of tumorigenesis by possible integration (insertional mutagenesis) of retroviral vectors that deliver reprogramming genes (among which is the oncogenic Myc factor).^{118–120}

Principles of stem cell delivery for cartilage repair

Different aspects have to be considered for the development of a stem cell-based protocol that can effectively and appropriately enhance the reparative processes in sites of cartilage damage, including the selection of components to provide in the lesion and the choice of the most suitable approach for implantation.

Therapeutic composition

The components for optimal cartilage repair based on the delivery of stem cells include the source of cells itself,

the (recommended) presence of an instructive biomaterial for cell seeding and containment, and possible biological supplements (Figure 1B).

Cells

Among the populations of stem cells evaluated so far for their ability to enhance cartilage repair, as described above, bone marrow-derived MSCs (isolated cells or cell concentrates), adipose-derived MSCs, umbilical cord blood MSCs, and peripheral blood MSCs have been employed and tested in patients, depending on their availability and ease of preparation (Tables 1 and 2). Details of the trials are discussed later.

Biomaterials

Current approaches for knee cartilage repair focus on the use of scaffolds that provide a three-dimensional environment for guiding the cells and supporting growth of a cartilaginous repair tissue. An important advantage of using scaffolds for cell delivery (besides containment of the implanted cells inside the lesion) is that biomaterials can act as barriers for fibroblast invasion of the graft that may otherwise induce fibrous repair.^{121,122}

To date, among the biomaterials used to deliver stem cells in patients with cartilage defects or osteoarthritis, hydrogels and solid scaffolds based on natural polymers have mainly been exploited (Tables 1 and 2). Hydrogels are polymeric networks consisting of crosslinked hydrophilic polymers with

Table 1 Current stem cell-based options for knee cartilage defects

Cells	Environment	Approach	Patient follow-up	Results	Reference
BMSCs	Cells, FG	S	n=36 (24 months)	Clinical improvements, hyaline-like tissue	205
	Cells, PRFG	S	n=5 (one year)	Clinical improvements, hyaline-like tissue	202
	Cells, HA	I, S	n=70 (24 months)	Clinical improvements	195
	Cells, collagen gel	S	n=2 (5 years)	Clinical improvements, fibrocartilaginous	209
			n=1 (one year)	to hyaline-like tissue	204
			n=3 (27 months)		210
	Cells, collagen scaffold	S	n=2 (31 months)	Clinical improvements, fibrocartilaginous tissue	203
	Cells, IP-CHA	S	n=1 (none)	Hyaline-like tissue	199
	Concentrate, FG	S	n=14 (12 months)	Clinical improvements	208
	Concentrate, PRFG and HA	S	n=20 (24 months)	Clinical improvements, hyaline-like tissue	200
	Concentrate, collagen membrane	S	n=54 (5 years)	Clinical improvements, fibrocartilaginous	207
			n=21 (5 years)	to hyaline-like tissue	206
	Concentrate, AMIC	S	n=5 (12 months)	Fibrocartilaginous to hyaline-like tissue	201
PBMSCs	Cells	I	n=52 (6 years)	Clinical improvements	198
	Cells, HA	I	n=5 (3 months)	Hyaline-like tissue	196
			n=25 (24 months)		197
Cells, collagen membrane	S	n=25 (5 years)	Clinical improvements, fibrocartilaginous to hyaline-like tissue	206	

Abbreviations: BMSCs, bone marrow-derived mesenchymal stem cells; FG, fibrin glue; PRFG, platelet-rich fibrin glue; HA, hyaluronic acid; IP-CHA, interconnected porous hydroxyapatite ceramic; AMIC, autologous matrix-induced chondrogenesis (collagen type I/III matrix); I, injective treatment; S, surgical treatment; PBMSCs, peripheral blood marrow-derived mesenchymal cells.

Table 2 Current stem cell-based options for knee osteoarthritis

Cells	Environment	Approach	Patient follow-up	Results	References
BMSCs	Cells	I	n=1 (6 months)	Clinical improvements	211
			n=4 (12 months)		212
			n=6 (12 months)		213
			n=12 (12 months)		214
ASCs	Cells, collagen gel	S	n=12 (16 months)	Clinical improvements, hyaline-like tissue	219
	Concentrate	I	n=25 (6 months)		215
ASCs	Cells, PRP	I	n=25 (16 months)	Clinical improvements	216
			n=18 (24 months)		217
	Cells, PRP and HA	I	n=2 (3 months)	Clinical improvements	218

Abbreviations: ASCs, adipose-derived stem cells; BMSCs, bone marrow-derived mesenchymal stem cells; PRP, platelet-rich plasma; HA, hyaluronic acid; I, injective treatment; S, surgical treatment.

good biocompatibility, high permeability for oxygen and nutrients, and ease of cell encapsulation, which results in their uniform distribution. Fibrin, a protein involved in the clotting of blood, has been broadly employed to encapsulate cells¹²³ through ligation with integrin receptors. Fibrin is usually provided in the form of gels or glues that are biocompatible and biodegradable. Hyaluronic acid, or hyaluronan, has been widely used due to its large natural presence in the extracellular matrix and its pivotal role in cartilage homeostasis.^{124,125} Collagen-based biomaterials have also been applied extensively for cartilage regeneration¹²⁶ due to the strength and stability of this matrix protein. They can be processed as gels, membranes, sponges, or foam, being subjected to enzymatic degradation. Alternatively, three-dimensional solid porous scaffolds such as ceramics may confer mechanical stability immediately upon implantation, providing scaffolding to support the growth of cartilaginous repair tissue and filling of the lesions.^{127,128} Still, the relatively poor integration of the different biomaterials with the surrounding cartilage remains a key problem that must be solved to permit continuity between the newly formed cartilage and the native one, long-term healing, and biomechanical competence.^{129–134} For a more substantial analysis of the most currently used scaffolds for cartilage tissue engineering, we refer to several comprehensive reviews of the literature.^{135–137}

Biological supplementation

Although there is no specific clinical information on the benefits of supplementing stem cell-based therapies with biological factors as yet, active experimental research is ongoing to determine the potential benefits of various molecules for repair or regeneration of damaged cartilage.

Candidate factors with therapeutic value for the remodeling of articular cartilage can be provided to stem cells seeded in scaffolds in a recombinant form or via gene transfer using nonviral or viral (adenoviral, retroviral,

lentiviral, recombinant adeno-associated viral) vectors.^{2,31,138} Growth factors are among the most studied agents, including TGF- β ^{80,129,139–152} the bone morphogenetic proteins (2, 4, 7; also as a BMP-4/sFlt1 combination to block angiogenesis),^{129,141,153–159} cartilage-derived morphogenetic protein 1,^{152,160} insulin-like growth factor I,¹⁴⁶ platelet-derived growth factor,¹⁴³ and connective tissue growth factor.¹⁶¹ Other classes of molecules have also been evaluated, including specific transcription factors (SOX5, SOX6, SOX9, ZNF145)^{162–165} and antiapoptotic proteins (Bcl-xL).¹⁶⁶ It is interesting to note that most of this laboratory work so far has been performed in models of cartilage defects^{129,139–154,158–167} and relatively few in experimental osteoarthritis,^{155–157,164} possibly because of the availability of very distinct animal models of osteoarthritis (reflecting the complexity of this disorder) that are in general more arduous to generate and monitor than those for focal defects.^{168,169} It remains to be evaluated, however, whether such biological strategies will be feasible in the operating room and applicable in patients. In light of the report of a patient who died after being enrolled in an arthritis gene therapy trial,¹⁷⁰ the use of gene transfer vectors remains a critical issue for clinical translation to treat nonlethal disorders such as cartilage defects and osteoarthritis. Nevertheless, while first placed on clinical hold, the study was cleared and allowed to proceed with minor changes by the US Food and Drug Administration because the death was not attributed to the gene treatment, showing that such an approach may still be considered as part of the clinical tools for cartilage repair.

Treatment approaches

Stem cell-based treatments for focal defects or for generalized osteoarthritis can be performed by intra-articular injection or via surgical arthrotomy with cell transplantation at the site of the lesion in conjunction (or not) with a periosteal or synovial flap.

Injective treatment

This technique involves administration of a suspension containing therapeutically active stem cells by intra-articular injection. The procedure of intra-articular injection itself is abundantly described and has been established for decades,^{171–178} is technically easy to perform because it is less invasive, and is suitable for outpatients. Also, the risks associated with stem cell injections are less severe compared with an open surgical treatment. On the other side, delivery of cells using this approach cannot be achieved precisely within the lesion and cells might engraft and populate other nontarget tissues. Therefore, injections may have more value to treat generalized articular cartilage degeneration, as in osteoarthritis.

Surgical treatment

Surgical cell transplantation necessitates an arthrotomy with exposure of the joint surface. Numerous operative approaches to the knee joint and various indications have been described for this purpose.^{179–186} These may be adapted for surgical transplantation of stem cells into cartilage lesions. However, this procedure is prone to significant neurovascular complications and a higher postoperative infection rate compared with injective treatments, and usually requires several days of hospital stay. Still, it allows for very precise delivery of cells to the site of injury. Supplementation of the procedure with an instructive biomaterial that can further contain the implanted seeded cells is feasible with this technique. For these reasons, surgical delivery of stem cells is more adapted to treat focal defects of the joint surface.

Also noteworthy is that both types of procedures can be combined with surgical options currently employed for focal defects or generalized cartilage lesions such as arthroscopic debridement, marrow stimulation procedures, or osteotomies to unload injured joint compartments. Importantly, and in contrast with the conventional two-step autologous chondrocyte implantation procedure, transplantation of stem cells can be designed as a single-step protocol, although under specific preparative conditions (see below).

Current clinical applications of stem cells for knee cartilage repair

Stem cell therapy is actually widely employed in the clinic to treat focal cartilage defects and osteoarthritis of the knee using both injective and surgical treatments. Various scale-based methods are available to monitor the outcomes of articular cartilage repair in patients. The Short Form (SF-36) health survey monitors health status and compares disease

burdens regarding vitality, physical functioning, bodily pain, general health perceptions, physical role functioning, emotional role functioning, social role functioning, and mental health.¹⁸⁷ The visual analog scale is a subjective linear psychometric response scale on which patients specify their level of pain intensity by indicating a position along a continuous (analog) line between two endpoints. Results are given as percentages where higher values indicate more severe pain.¹⁸⁸ The International Knee Documentation Committee (IKDC) developed an objective scale (effusion, motion, ligament laxity, crepitus, harvest site pathology, one leg hop test, radiographic findings)¹⁸⁹ and a subjective score (severity of symptoms, limitations in sports activities, and joint function).¹⁹⁰ The Lysholm knee scoring scale evaluates limping, the use of a support, joint locking and instability, pain, swelling, stair climbing, and squatting.¹⁹¹ The Knee Injury and Osteoarthritis Outcome Score (KOOS) assesses symptoms, pain, function in daily living, sports, recreational activities, and quality of life.¹⁹² The cartilage injury evaluation package of the International Cartilage Repair Society (ICRS, cartilage.org) includes the ICRS injury questionnaire and subjective knee evaluation form (both patient-reported), and the ICRS knee surgery history registration, knee examination form, articular cartilage injury mapping system, articular cartilage injury classification, osteochondritis dissecans classification, and the cartilage repair assessment system (all surgeon-reported). Of note, the ICRS subjective knee evaluation form and objective knee examination form correspond to the subjective and objective IKDC scores, respectively. The Hospital for Special Surgery knee rating scale is based on the individual criteria of pain, function, range of motion, muscle strength, flexion deformity, and instability.¹⁹³ The Western Ontario and McMaster Universities Osteoarthritis Index is the most commonly used instrument for patients with knee osteoarthritis, and includes questions related to difficulties during activities of daily living, pain, and stiffness.¹⁹⁴

Applications for articular cartilage defects

Injective treatments

Intra-articular stem cell injections for the clinical treatment of focal lesions so far have only been investigated in conjunction with marrow stimulation procedures, based on the use of bone marrow-derived MSCs¹⁹⁵ and peripheral blood MSCs^{196–198} (Table 1).

Injection of bone marrow-derived MSCs during microfracture treatment yielded significant improvements in the SF-36, IKDC subjective knee evaluation form, and Lysholm

knee scale in patients after 2 years, while magnetic resonance imaging (MRI) revealed good defect filling and integration of the repair tissue.¹⁹⁵

Injection of peripheral blood MSCs allowed improvement in the KOOS, Lysholm score, visual analog scale, and KOOS pain scale in patients with ICRS grade 3 or 4 lesions for up to 6 years.¹⁹⁸ Moreover, second-look arthroscopies of subchondral drilling of ICRS grade 3 and 4 lesions in patients combined with five weekly injections of peripheral blood MSCs in hyaluronic acid starting one week postoperatively revealed a well-integrated repair tissue of fibrocartilaginous and hyaline-like cartilaginous aspect without delamination or hypertrophy after 3 months compared with hyaluronic acid treatment alone.^{196,197} Evaluations with core biopsies and MRI scans after 18 months further revealed improved cartilage repair in the presence of peripheral blood MSCs.

Surgical treatments

Surgical stem cell transplantation in cartilage defects has been developed in association with the use of matrices or biomaterials to deliver bone marrow-derived MSCs (fibrin glue, hyaluronic acid, collagen matrices and scaffolds, hydroxyapatite ceramic)^{199–210} and peripheral blood MSCs (collagen matrices and scaffolds²⁰⁶) (Table 1).

Transplantation of bone marrow-derived MSCs as isolated cells or marrow concentrates using fibrin glue^{205,208} or platelet-rich fibrin gel²⁰² revealed clinical and subjective improvements in patients for 1–2 years postoperatively using the ICRS cartilage injury evaluation package, IKDC subjective knee examination form, Lysholm knee scale, revised Hospital for Special Surgery knee grading scale, and ICRS arthroscopic scores. This was accompanied by formation of a hyaline-like repair tissue similar to first-generation autologous chondrocyte implantation²⁰⁵ and with MRI findings showing surfaces with good defect filling²⁰² and correct contours and continuity with the native cartilage,^{202,208} and without changes in the subchondral bone.²⁰⁸ Similar approaches using isolated bone marrow-derived MSCs or marrow concentrates in hyaluronic acid yielded clinical and subjective improvements in patients 2 years postoperatively using the SF-36, IKDC subjective knee examination form, KOOS, and Lysholm knee scale.^{195,200} Good subchondral and cartilage repair was also documented by scoring of cartilage repair using MRI evaluation.²⁰⁰ Alternatively, transplantation of isolated bone marrow-derived MSCs or marrow concentrates in collagen-derived elements (gel, scaffold, membrane, matrix) led to improved clinical outcomes in patients between 6 months and 5 years postoperatively

using the KOOS functional and pain scale, visual analog scale, IKDC, and Lysholm score.^{203,206,207} Second-look arthroscopy revealed good defect filling with incorporation in the adjacent cartilage,²⁰³ and formation of a repair tissue of fibrocartilaginous^{209,210} or even hyaline-like nature.²⁰⁴ MRI evaluations also showed reconstruction of the cartilaginous surface and good integration of the repair tissue,^{206,207} while core biopsies yielded hyaline-like matrix or a mixture of hyaline and fibrocartilage.²⁰¹ Finally, implantation of bone marrow-derived MSCs in an interconnected porous hydroxyapatite ceramic allowed for cartilage and bone regeneration in patients at second-look arthroscopy.¹⁹⁹

Similarly, transplantation of peripheral blood MSCs in a collagen membrane²⁰⁶ in patients with ICRS grade 3 and 4 lesions yielded significant clinical improvements at one and 5 years postoperatively using the KOOS and Lysholm functional scores, the visual analog scale, and the KOOS pain scale. MRI evaluations also showed satisfactory reconstruction of the cartilaginous surface and good integration of the repair tissue.

Other protocols are ongoing (clinicaltrials.gov), such as those based on the transplantation of isolated bone marrow-derived MSCs or marrow concentrates (NCT00885729, NCT00891501, NCT00850187^{211–213} with a collagen I scaffold, NCT01159899²¹⁴ with a protein matrix and a collagen hydroxyapatite scaffold), adipose-derived stem cells (NCT01399749)²¹⁵, and umbilical cord blood MSCs (NCT01041001, NCT01626677, NCT01733186^{216–218} with CARTISTEM[®] and sodium hyaluronate), although the outcomes have yet to be published.

Applications for osteoarthritis Injective treatments

Intra-articular stem cell injections for osteoarthritis have been investigated so far using bone marrow-derived MSCs^{219–223} and adipose-derived stem cells^{224–226} (Table 2).

Injection of isolated bone marrow-derived MSCs^{219–222} or of marrow aspirates via arthroscopic debridement²²³ allowed improvement in visual analog scale pain scores and range of motion^{219–223} as well as osteoarthritis outcome scores²²³ in patients at 6–12 months postoperatively. Furthermore, increases in cartilage growth and thickness with decreases in the size of poor cartilage and edematous subchondral bone were documented on MRI and by T2 relaxation measurements.^{219,221,222}

Injection of adipose-derived stem cells using platelet-rich plasma and arthroscopic debridement^{224,225} or platelet-rich plasma with hyaluronic acid²²⁶ yielded improved

clinical outcomes using the Western Ontario and McMaster Universities Osteoarthritis Index, Lysholm, and visual analog scale pain score in patients between 16 months and 2 years, with an enhanced whole-organ cartilage MRI score^{224,225} and improved subjective pain score and functional status in patients 3 months postoperatively, along with increased cartilage thickness on MRI.²²⁶

Surgical treatments

In line with the fact that surgical stem cell transplantation is more suitable for focal defects than for osteoarthritis, only one study by Wakitani et al²²⁷ has addressed this approach to date (Table 2). This group evaluated the benefits of transplanting bone marrow-derived MSCs with a collagen gel in patients after high tibial osteotomy. Clinical evaluations prior to and after surgery (up to 16 months) using the Hospital for Special Surgery knee rating scale revealed no difference between the cell-treated and cell-free group. However, arthroscopic and histological grading of the repair tissues on core biopsies performed at 7 and 42 weeks after treatment showed improved scores in the cell-treated group at both time points.

Other protocols are ongoing (clinicaltrials.gov), and include those based on transplantation of isolated bone marrow-derived MSCs or marrow concentrates (NCT01152125, NCT01485198, NCT01895413, NCT01931007, NCT01879046^{228–231} by arthroplasty, ie, ARTHROSTEM, NCT01448434²³² and NCT01453738²³³ with Plasmalyte-A and hyaluronan, NCT01459640²³⁴ with hyaluronic acid, ie, Orthovisc® [Anika Therapeutics, Inc, Bedford, MA, USA]), adipose-derived stem cells (NCT01300598, NCT01585857, NCT01739504, NCT01809769, NCT01885832, NCT01947348,^{235–241} NCT01879046 by arthroplasty, ie, ARTHROSTEM), and peripheral blood MSCs (NCT01879046 by arthroplasty, ie, ARTHROSTEM), although the outcomes have not as yet been published.

Conclusion and perspectives

Stem cell implantation is a promising approach for cartilage repair in the knee and is already in clinical use for focal defects and generalized osteoarthritis. However, more controlled studies are needed to achieve both efficacy (appropriate biological and biomechanical properties) and safety in patients, given that cartilage lesions are not life-threatening disorders. There are still some issues regarding the effective use of stem cells, including their reduced potentiality with age and disease, like in osteoarthritis with an inflammatory environment, the effects of cellular aging

upon sequential expansion, and the critical questions of production of fibrocartilage instead of hyaline cartilage in the lesion and of terminal differentiation with cell hypertrophy and mineralization leading to the replacement of cartilage by bone. Regarding the safe administration of stem cells, there is a potential risk of colonization of nontarget tissues, possible induction or stimulation of tumorigenesis, and transmission of infection, as well as the use of human (allogeneic) or animal serum-derived agents during cell expansion.

Further, standardization of the implantation procedure needs to be addressed from the clinical point of view, depending on the age and background of patients with possible associated pathologies, on the type, size, and localization of the lesion(s), on the length of follow-up, and on the methods used for assessment of cartilage repair. From a laboratory point of view, standardization is also necessary regarding the optimal source and amount of cells requested, the number of injections, the benefits of isolated cells versus culture-expanded cells versus cell concentrates (ie, one-step versus two-step procedure) with specified conditions of preparation and maintenance, and the use of autologous versus allogeneic samples.

Nevertheless, despite these considerations, the clinical outcomes of the ongoing and available trials in patients are encouraging, showing the potential of stem cell therapy for cartilage repair upon further elaboration and appropriate optimization in line with the regulatory standards and legal requirements for production/manufacture, use, and application of biologics of the international drug regulatory frameworks, particularly the European Medicines Agency (ema.europa.eu) and the US Food and Drug Administration (fda.gov).

Disclosure

The authors have no conflicts of interest to report in this work.

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