Stem Cell Therapies for Knee Cartilage Repair: The Current Status of Preclinical and Clinical Studies

John A. Anderson, Dianne Little, Alison P. Toth, Claude T. Moorman III, Bradford S. Tucker, Michael G. Ciccotti and Farshid Guilak

Am J Sports Med 2014 42: 2253 originally published online November 12, 2013
DOI: 10.1177/0363546513508744

Stem Cell Therapies for Knee Cartilage Repair

CME

The Current Status of Preclinical and Clinical Studies

John A. Anderson,*†‡ MD, MSc, Dianne Little,† BVSc, PhD, Alison P. Toth,† MD, Claude T. Moorman III,† MD, Bradford S. Tucker,‡ MD, Michael G. Ciccotti,‡ MD, and Farshid Guilak,† PhD

Investigation performed at Duke University Medical Center, Durham, North Carolina, and Rothman Institute, Philadelphia, Pennsylvania

Background: Articular cartilage damage of the knee is common, causing significant morbidity worldwide. Many adult tissues contain cells that are able to differentiate into multiple cell types, including chondrocytes. These stem cells have gained significant attention over the past decade and may become frontline management for cartilage defects in the very near future.

Purpose: The role of stem cells in the treatment of knee osteochondral defects was reviewed. Recent animal and clinical studies were reviewed to determine the benefits and potential outcomes of using stem cells for cartilage defects.

Study Design: Literature review.

Methods: A PubMed search was undertaken. The key phrase ''stem cells and knee'' was used. The search included reviews and original articles over an unlimited time period. From this search, articles outlining animal and clinical trials were selected. A search of current clinical trials in progress was performed on the clinicaltrials.gov website, and ''stem cells and knee'' was used as the search phrase.

Results: Stem cells have been used in many recent in vitro and animal studies. A number of cell-based approaches for cartilage repair have progressed from preclinical animal studies into clinical trials.

Conclusion: The use of stem cells for the treatment of cartilage defects is increasing in animal and clinical studies. Methods of delivery of stem cells to the knee's cartilage vary from direct injection to implantation with scaffolds. While these approaches are highly promising, there is currently limited evidence of a direct clinical benefit, and further research is required to assess the overall outcome of stem cell therapies for knee cartilage repair.

Keywords: biologic healing enhancement; biology of cartilage; knee; articular cartilage; stem cell therapy

Cartilage defects of the knee are a major cause of morbidity worldwide. About 60% of patients undergoing knee arthroscopic surgery have injuries to the articular cartilage.[46] However, few approaches are currently available for the treatment of focal cartilage lesions. Currently used techniques include microfracture or autologous cell or tissue grafting (ie, mosaicplasty, osteoarticular transfer system [OATS], or autologous chondrocyte implantation [ACI]) and minced (DeNovo NT, Zimmer Inc, Warsaw, Indiana) or micronized articular cartilage allografts (BioCartilage, Arthrex Inc, Naples, Florida). However, their long-term results may be variable or unknown.[6] Long-term follow-up after microfracture was reported by Steadman et al[81] with an improvement in clinical knee scores. However, Minas et al[68] suggested that this technique may make subsequent surgery more difficult. Mosaicplasty has limitations including donor site morbidity, limited availability, and mismatch geometry.[6] The advantages of techniques such as microfracture and mosaicplasty are the relatively low complexity of the procedure, the patient undergoing only 1 surgery, and the use of the patient's own tissue. On the other hand, ACI involves 2 operations, is technically demanding, and may result in periosteal overgrowth.[52] In a recent study, the long-term efficacy of

*Address correspondence to John A. Anderson, MD, MSc, Rothman Institute Cartilage Center, 925 Chestnut Street, Philadelphia, PA 19107 (e-mail: john.anderson@rothmaninstitute.com).

†Department of Orthopaedic Surgery, Duke University Medical Center, Durham, North Carolina.

‡Rothman Institute Cartilage Center, Rothman Institute, Philadelphia, Pennsylvania.

One or more of the authors has declared the following potential conflict of interest or source of funding: F.G. is a founder of Cytex Therapeutics. This work was supported in part by National Institutes of Health grants AR50245, AR48182, AG15768, AR48852, and AR59784.

The American Journal of Sports Medicine, Vol. 42, No. 9
DOI: 10.1177/0363546513508744

microfracture was compared with ACI, and the authors showed that ACI was not superior to microfracture, with failures in nearly a quarter of the patients in both groups.[52] However, a randomized controlled trial comparing ACI with mosaicplasty concluded that ACI resulted in superior clinical and biological outcomes.[6] Newer ACI techniques such as matrix-associated autologous chondrocyte transplantation/implantation (MACT/MACI) use biomaterials seeded with chondrocytes as a scaffold instead of a periosteal patch.[5] However, this technique still has some issues. It can require 2 operations, and the harvest of autologous chondrocytes or osteochondral plugs remains problematic because of iatrogenic damage. There may also be donor site morbidity and a potential change in the cartilage properties of the joint.[39,57]

In this regard, adult stem cells may provide a more readily accessible source of cells for the treatment of chondral or osteochondral defects. For example, bone marrow–derived mesenchymal stem cells (MSCs) are able to differentiate into many mesenchymal phenotypes, including those that form cartilage, bone, muscle, fat, and other connective tissues.[12-14,38] Other tissues contain similar but distinct populations of adult stem cells that exhibit chondrogenic capabilities, primarily adipose,[29] synovium,[22] and umbilical cord,[31] among other sources. Stem cells can maintain their multipotency during culture expansion,[50] while chondrocytes may lose their phenotype after passage.[7,37]

Another major source of adult stem cells has been adipose tissue, including subcutaneous fat or the infrapatellar fat pad of the knee. Adipose-derived stem cells (ASCs) exhibit multipotent differentiation capabilities in the mesenchymal lineage, similar to MSCs, with evidence of adipogenic, chondrogenic, myogenic, and osteogenic differentiation.[26,40,41,76] Adipose tissue is an accessible, abundant, and reliable source for the isolation of adult stem cells that may be suitable for tissue engineering and regenerative medicine applications.[36,67] The majority of peer-reviewed publications on human trials using ASCs are, at most, phase I safety and case reports.[35]

It is important to note that the method of delivery of MSCs into the knee varies and includes the following: 1-stage injection of a suspension into the joint, 1-stage implantation into the defect, and preculture in a matrix for implantation into the defect (2-stage). The aim of this report on stem cell therapy for cartilage defects of the knee is to review the most recent preclinical animal studies, provide a systematic review of clinical trials, and outline the future directions and challenges for the scientist and surgeon.

BACKGROUND

The application of stem cells for cartilage repair and regeneration has been studied extensively in laboratory models,[§] but a review of these studies is beyond the scope of this article, which will focus on animal and clinical studies. Fundamentally, MSCs have been used to treat chondral defects in many animal models. Rodents are cost-effective and provide proof-of-concept data to serve as a bridge between in vitro experiments and more costly large animal preclinical studies.[4,17,56,70] Rabbits are easy to handle, are cost-effective, and have a reasonable joint size, but they may also spontaneously heal, have thin cartilage, and have variable loading conditions.[‖] Skeletally mature mini-pigs have been used in numerous stem cell and cartilage studies.[16,48,59,80,92] They have a stifle joint that is similar to the human knee in some respects, including relative thickness, inability to endogenously heal chondral and osteochondral defects, and similar collagen fiber arrangement.[17,49] Sheep and goats have been used frequently.[61,63,64,78,93] There are advantages and disadvantages with goat studies compared with human studies. The goat model allows the aspiration of MSCs, involves reasonably thick articular cartilage, and utilizes a relatively large stifle joint. The primary weightbearing surface, though, is the patellofemoral surface, as goats walk with the joint partially flexed.[61,73] In addition, compared with studies in humans, restricted postoperative rehabilitation is difficult and may pose ethical issues.[61] The stifle joint of the horse most closely resembles the human knee in terms of size, cartilage thickness, and the ability to extend the joint fully during gait. However, the expense, the high joint-loading conditions, and the need for elaborate facilities often make cartilage studies difficult to perform in horses.[65,66,71,85]

Before beginning clinical trials, robust manufacturing practices for the production of stem cells must be adopted. The Food and Drug Administration (FDA) and other international and national regulatory bodies have developed guidelines for adult cell production.[15] In fact, MSCs are classed as "more than minimally manipulated." All products must be evaluated for bacteria, endotoxins, mycoplasma, and a host of viral agents (cytomegalovirus, Epstein-Barr virus, hepatitis A and C, and HIV) if they are to be used for allogenic purposes. Tissue-processing devices are marketed in Europe and Asia and are under regulatory review in the United States.[36] There have been very few published reports on the application of stem cell therapy to cartilage defects in humans. Importantly, there are differences with the delivery of MSCs into the knee joint in terms of direct injection compared with implantation (1-stage vs 2-stage) into a scaffold. A study on the use of MSCs for articular cartilage repair of the patellofemoral joint in 5 knees has been performed,[87] while another group[55] reported on a single athlete.[53] There are a number of clinical trials currently being undertaken, and these are found on the clinicaltrials.gov website.

Search Strategies and Criteria

A PubMed search was performed. The key phrase "stem cells and knee" was used. The search included reviews and original articles over an unlimited time period. A search of current clinical trials in progress was performed on the clinicaltrials.gov website, and "stem cells and knee" was used as the search phrase.

[§]References 1, 2, 9, 10, 18, 19, 23, 24, 28, 30, 34, 45, 47, 60, 64, 84.

[‖]References 11, 17, 20, 42, 58, 73, 74, 79, 82, 88, 89, 91.

ANIMAL STUDIES OF STEM CELL–BASED CARTILAGE REPAIR

Small Animal Models

The effects of treating cartilage defects with stem cells have been studied in numerous recent small animal models.[56] A rabbit model compared the use of allogenic, chondrogenic, predifferentiated (supplemented with transforming growth factor–β3 [TGF-β3] and basic fibroblast growth factor) MSCs with undifferentiated MSCs in the repair of full-thickness articular cartilage defects.[20] Defects with a 5-mm diameter and 1-mm depth were created in the medial femoral condyle of both knees of each rabbit, and then each construct was implanted 3 to 4 weeks after injury into one side. One side of the knee (lateral femoral condyle) in each rabbit was left untreated, and the histological appearances of this group were compared with the 2 different MSC groups. The authors concluded that the transplantation of MSCs produced superior healing compared with intrinsic repair of the untreated cartilage defects, irrespective of their state of differentiation.[20]

Another group studied rabbits with osteochondral defects and compared those in the defect-only group to 2 groups treated with cross-linked bilayer collagen scaffolds with or without MSCs.[73] The MSC scaffold group showed the most hyaline cartilage, highest histological scores, and highest biomechanical compressive modulus at 12 weeks.[73]

In another comparison, 30 rabbits that had knee chondral defects were treated with either allogenic, undifferentiated MSCs or ACI.[82] Both groups had alginate constructs cultured for 6 weeks after creation of the defects. Both treatment groups showed similar cartilage regenerative profiles, and both resulted in superior tissue regeneration compared with untreated defects. The advantages of MSCs were highlighted, such as prolonged expansion time without phenotype transformation and the homing and engraftment of other stem cells.

The use of hydrogel scaffolds with stem cells has been a topic of recent interest. A rabbit model assessed the repair of osteochondral defects with biodegradable hydrogel composites encapsulating bone marrow–derived MSCs.[42] It was found that when compared with the hydrogel composite without MSCs, the 2 groups of hydrogels with MSCs (one with the addition of TGF-β1) facilitated subchondral bone formation but did not improve cartilage structure. Another study reviewed a biphasic osteochondral composite using a chondral phase consisting of hyaluronate and atelocollagen and an osseous phase consisting of hyaluronic acid and β–tricalcium phosphate.[3] Chondrocytes were expanded, and the authors concluded that this scaffold composite held promise for defect repair.[3]

The role of gene transfer in MSC cartilage regeneration may be important, but it is not currently well understood. A rabbit osteochondral defect model studying bone marrow–derived MSCs transduced with an adenoviral vector containing the Sox9 gene was recently reported.[11] Sox9 is a transcription factor that is essential for chondrogenesis and is a regulator for the chondrocyte phenotype.[8] Four groups were compared: (1) defect only, (2) scaffold only, (3) scaffold with MSCs, and (4) scaffold with Sox9-transduced MSCs. The fourth group had the highest (ie, best repair) International Cartilage Repair Society macroscopic scores[62] and also the highest histological scores according to Wakitani et al.[86]

Large Animal Models

There have been multiple recent large animal studies outlining the effects of stem cells on knee osteochondral defects. Large animal models are often used to be most clinically relevant to the human condition. The rationale for using different animal types to determine different cartilage outcomes has been described previously.[17,75] While no animal model can exactly reproduce human physiology and joint loading, each model (ie, mouse, rabbit, pig, sheep, horse) provides important information to advance the field of cartilage regeneration. A study on degenerative change in an ovine model assessed perilesional changes of chronic osteochondral defects in the knees of 23 sheep.[44] The authors concluded that, like the appearance of chronic defects in humans after trauma, the area of cartilage surrounding the created defect showed signs of chronic degeneration at 1 month and 3 months.[44] The difference between acute and more clinically relevant chronic osteochondral defects was demonstrated in a goat model.[77] After creation of a 0.8 × 0.5–cm defect in the medial femoral condyle of all 21 goats, the animals were randomized to receive no treatment, early treatment, or late treatment using a periosteal graft. The authors concluded that early treatment showed significantly better cartilage repair than late or no treatment, with a concurrent decrease in the disturbance of cartilage metabolism.[77]

Following the rationale of these models, another group found that the optimal chondrogenic predifferentiation period for ovine MSCs inside collagen gel was 14 days.[93] The authors created osteochondral defects in the medial femoral condyles of merino sheep.[93] Four groups were compared: (1) chondrogenically predifferentiated ovine MSC/hydrogel constructs (preMSC gels), (2) undifferentiated ovine MSC/hydrogel constructs (unMSC gels), (3) cell-free collagen hydrogels (CF gels), and (4) untreated controls. At 6 months in vivo, the defects created with preMSC gels showed significantly better histological scores with morphological characteristics of hyaline cartilage (columnarization and type II collagen).

Furthermore, MSC-seeded triphasic constructs were compared with the OATS procedure in a merino sheep model.[63,64] The triphasic construct consisted of a chondral phase, autologous plasma as an intermediate phase, and an osseous phase. Macroscopic and biomechanical analyses showed no significant differences between groups at 12 months. The disadvantages of OATS were outlined such as morbidity at the donor site, limited size of the transplant, hemarthrosis, difficulty in shaping host tissue to fit the defect area, and inadequate bonding of the graft cartilage to surrounding tissue.

The role of growth factors in treating osteochondral defects was discussed in a recent review.[32] A team studied

16 miniature pigs and created osteochondral defects in their knees.[16] A defect-only group and a collagen gel–only group were compared with a third group that received a collagen gel containing MSCs alone and were also compared with a fourth group that received MSCs and a gel induced with TGF-β. The conclusion was that both treatments using MSCs resulted in a superior gross and histological appearance and better histological scores according to Pineda et al[72] than the non-MSC groups. In addition, using undifferentiated MSCs resulted in a superior outcome than using TGF-β–induced differentiated MSCs, especially with regard to the restoration of subchondral bone.

Moreover, MSCs have been combined with microfracture to address osteochondral defects in a horse model. In a recent study,[66] investigators hypothesized that there may be a problem with the migration and proliferation of MSCs embedded within fibrin.[51,90] They evaluated intra-articular injections of bone marrow–derived MSCs suspended in hyaluronan combined with microfracture compared with microfracture alone.[66] The conclusions were that although there was no difference clinically or histologically in the 2 groups at 12 months, the MSC group had increased aggrecan content and tissue firmness.

Another study showed that, compared with microfracture, MSC treatment was superior in terms of a short-term arthroscopic inspection and also in longer term macroscopic, histological, and quantitative magnetic resonance imaging (MRI) analyses.[33] Specifically, repair tissue in the MSC group had better type II collagen content and orientation and improved sulfated glycosaminoglycan content and also exhibited greater integration into the surrounding normal cartilage, with greater thickness and a smoother surface.

CLINICAL STUDIES OF STEM CELL–BASED CARTILAGE REPAIR

Few published clinical studies assessing outcomes after stem cell therapy for cartilage defects have been reported. Care in the interpretation of results is warranted because of small sample sizes, different delivery methods, and often ill-defined outcome measures. A systematic review was performed.

Research Question

The research aim was to determine the current clinical role of stem cells in the treatment of knee osteochondral defects. We reviewed recent clinical studies utilizing different stem cell delivery methods to determine if there were any potential benefits/outcomes of using stem cells for knee cartilage defects.

Research Protocol

The experimental design's inclusion criteria were broad because of the limited number of completed or in-progress clinical stem cell studies. Case studies, case-control studies, observational cohort studies, and randomized controlled trials were all included for review.

Literature Search

A PubMed search was undertaken. The key phrase "stem cells and knee" was used. The search included all original articles in English over an unlimited time period that specifically involved the clinical application of stem cells to the human knee. All other studies were not included. Furthermore, a search of current clinical trials in progress was performed on the clinicaltrials.gov website, and "stem cells and knee" was used as the search phrase.

Data Extraction

The data extraction items included title, outcome, institution, patient numbers, brief description, and delivery method and identifier. Table 1 highlights the studies from the PubMed search, while Table 2 summarizes the clinicaltrials.gov search.

Quality Assessment

The majority of studies found in the PubMed search were not high-level evidence (not level 1 or 2). Table 2 summarizes the clinicaltrials.gov search and reveals that a number of randomized controlled trials are currently in progress.

Data Analysis and Results

Table 1 outlines the PubMed search, and Table 2 outlines the clinicaltrials.gov search.

Interpretation of Results

Because of the low total number of clinical stem cell knee studies, and very few high-level studies, the interpretation of results is difficult.

Clinical Study Findings

Table 1 summarizes the clinical studies using stem cells for knee cartilage repair, and the different delivery methods are highlighted. In an observational cohort study, autologous MSCs were compared with ACI in 72 matched symptomatic patients with full-thickness cartilage defects, as diagnosed by clinical examination and MRI.[62,69] There was no difference between groups in terms of clinical outcomes except for physical role functioning, with a greater improvement over time in the MSC group. The International Knee Documentation Committee (IKDC),[62] Tegner, and Lysholm[83] scores were similar between groups. Of note, 5 cases in the MSC group also underwent concurrent high tibial osteotomy, and this may have acted as a confounding variable. The authors highlighted the advantages of MSCs over ACI, which include a single surgery, reduced costs, and minimal donor site morbidity.

In a case series, MSCs were transplanted on a platelet-rich fibrin glue to treat full-thickness articular cartilage defects in 5 patients.[43] Lesions ranged in size from 3 cm^2 to 12 cm^2 (mean, 5.8 cm^2), and 12-month follow-up of clinical, arthroscopic, and MRI outcomes were encouraging.

TABLE 1
Results for Searching "Stem Cells and Knee" on PubMed[a]

Authors (Year)	Outcomes	Institution	No. of Patients	Brief Description	Stem Cell Delivery Method
Nejadnik et al (2010)[69]	IKDC, ICRS, SF-36, Lysholm, Tegner	National University, Singapore	72	Observational cohort study; 36 patients underwent ACI, and 36 patients underwent BM-derived MSC implantation; concluded that BM-derived MSCs were as effective as chondrocytes in clinical outcomes	2-stage implantation; BM-derived MSCs harvested and then later arthrotomy performed to implant
Haleem et al (2010)[43]	Lysholm, revised HSS, MRI, arthroscopic ICRS	Cairo University, Egypt	5	Case series; all patients' symptoms improved at 12 mo; ICRS arthroscopic scores were 8 of 12 and 11 of 12 for 2 patients; at 12 mo, MRI showed complete congruity in 3 patients and incomplete congruity in 2 patients	2-stage implantation; autologous BM-derived MSC culture expanded, placed on PR-FG intraoperatively, and then transplanted into defects
Davatchi et al (2011)[21]	VAS, walking time to pain, stair climbing	Tehran University, Iran	4	Case series; walking time to pain improved in 3 patients; improved stair climbing and VAS scores for all	Direct delayed injection; 30 mL of BM taken and cultured for growth for 4 to 5 wk
Koh et al (2013)[54]	WOMAC, Lysholm, VAS, MRI	Yonsei Sarang Hospital, South Korea	18	Case series; infrapatellar fat pad harvested after arthroscopic debridement; clinical scores improved, and MRI scores improved; results positively related to number of stem cells injected	Direct delayed injection; after arthroscopic surgery, fat pad stem cells and PRP injected into knees

[a]ACI, autologous chondrocyte implantation; BM, bone marrow; HSS, Hospital for Special Surgery; ICRS, International Cartilage Repair Society; IKDC, International Knee Documentation Committee; MRI, magnetic resonance imaging; MSC, mesenchymal stem cell; PR-FG, platelet-rich fibrin glue; PRP, platelet-rich plasma; SF-36, Short Form–36 Health Survey; VAS, visual analog scale; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

However, the small sample size makes the interpretation of results difficult.

In another case series, 4 patients aged 55 to 65 years who had established osteoarthritis had autologous MSCs simply injected into their affected knee.[21] No standardized knee outcome scores were reported, but the number of stairs they could climb and the visual analog scale (VAS) for pain scores improved for all 4 patients. Clearly, it is difficult to make any firm conclusions from this small study, and the authors acknowledged their many limitations and aimed to determine (1) the required cellular dose, (2) the number and timing of injections, (3) the use of co-stimulators, (4) best cell subtypes, and (5) selection of the appropriate stage of disease to treat.

CLINICAL TRIALS OF STEM CELL–BASED CARTILAGE REPAIR

Ongoing or recently completed unpublished trials addressing stem cell therapy for chondral defects of the knee were reviewed on the clinicaltrials.gov website, and they are presented in Table 2. Stem cell delivery methods varied and included direct injections and both 1- and 2-stage implantations into the defect. General outcome measures included some of the following: Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), VAS, IKDC, Short Form–12 Health Survey (SF-12), Lysholm, Knee injury and Osteoarthritis Outcome Score (KOOS), histology, MRI, and arthroscopic surgery. One group is studying a 1-step procedure using expanded autologous bone marrow–derived MSCs stimulated with a protein matrix and mixed in a collagen hyaluronic acid scaffold. This paste is then transplanted into the prepared defect under arthroscopic surgery, with the addition of platelet-rich plasma (PRP). Also, MSCs are being compared with PlasmaLyte (Baxter, Deerfield, Illinois) and hyaluronan for the treatment of knee osteoarthritis, while another team is following up on patients injected with different doses of bone marrow–derived MSCs. The safety and efficacy of human umbilical cord blood-derived MSCs are being investigated in the United States. This product is also being compared with microfracture for grade 4 osteoarthritis in another trial.

Currently, ASCs are being investigated for the treatment of chondral defects. Investigators are conducting a randomized controlled trial of ASCs versus chondrocytes

TABLE 2
Results for Searching "Stem Cells and Knee" on clinicaltrials.gov[a]

Title	Outcomes	Institution	No. of Patients	Brief Description	Identifier
Transplantation of Bone Marrow Stem Cells Stimulated by Proteins Scaffold to Heal Defects Articular Cartilage of the Knee	KOOS, ICRS	University of Marseille, France	50	Fresh non–culture-expanded autologous BM-derived MSCs, stimulated with a protein matrix, are mixed in a collagen HA scaffold; this paste is transplanted into the prepared defect, under arthroscopic surgery, with an injection of PRP	NCT 01159899
Treatment of Knee Osteoarthritis With Autologous Mesenchymal Stem Cells	VAS, Oswestry, SF-36, MRI (CartiGram)	Fundacion Teknon and IBGM, University of Valladolid, Spain	12	Used 40 million BM-derived MSCs for grade 2 to 4 OA	NCT 01183728
The Effects of Intra-articular Injection of Mesenchymal Stem Cells in Knee Joint Osteoarthritis	WOMAC, VAS	Royan Institute, Iran	40	Case-control study; BM-derived MSCs will be administered at 1 mo and 4 mo after harvest; clinical and MRI follow-up to 6 mo	NCT 01504464
Autologous Stem Cells in Osteoarthritis	ROM, WOMAC, SF-36, VAS	Hospital Universitario Dr Jose E. Gonzalez, Mexico	30	One group receives acetaminophen, and the other receives BM-derived MSCs	NCT 01485198
Adult Stem Cell Therapy for Repairing Articular Cartilage in Gonarthrosis	VAS, SF-36, MRI	Centro Medico Teknon, Institut de Terapia Regenerativa Tissular, CETIR Sant Jordi, Spain	15	For grade 2 to 3 OA; at 21 d, 40 million BM-derived MSCs injected and clinical and MRI follow-up to 12 mo	NCT 01227694
Allogeneic Mesenchymal Stem Cells in Osteoarthritis	WOMAC, VAS, analgesia intake, MRI	Sanjay Gandhi Post Graduate Institute of Medical Sciences, India	60	Allogenic MSCs used in different doses	NCT 01453738
Autologous Mesenchymal Stem Cells vs Chondrocytes for the Repair of Chondral Knee Defects	SF-12, WOMAC	La Paz University Hospital, Spain	30	RCT of ASCs vs chondrocytes	NCT 01399749
Allogeneic Mesenchymal Stem Cells for Osteoarthritis	WOMAC, VAS, analgesia intake, MRI	KPJ Ampang Puteri Specialist Hospital, Malaysia	72	RCT of BM-derived MSCs vs PlasmaLyte and hyaluronan	NCT 01448434
Evaluation of Safety and Exploratory Efficacy of CARTISTEM, a Cell Therapy Product for Articular Cartilage Defects	IKDC, Lysholm, KOOS, VAS, MRI	Rush University, USA	12	Cartistem is human umbilical cord blood-derived MSCs; for grade 3 to 4 OA	NCT 01733186
ADIPOA - Clinical Study	WOMAC, ROM, SF-8, MRI	University Hospital of Montpellier, France	18	Differing concentrations of ASCs (2 million vs 10 million vs 50 million) will be injected into knees with grade 3 to 4 OA and compared	NCT 01585857
Study to Compare the Efficacy and Safety of Cartistem and Microfracture in Patients With Knee Articular Cartilage Injury or Defect	ICRS, VAS, biopsy, WOMAC, IKDC	Korea University Guro Hospital, South Korea	104	Comparison of Cartistem vs microfracture for grade 4 OA	NCT 01041001
Autologous Adipose Tissue Derived Mesenchymal Stem Cells Transplantation in Patient With Degenerative Arthritis	WOMAC, VAS, histology, MRI, arthroscopic surgery	SMG-SNU Boramae Hospital, South Korea	18	ASCs (10 million vs 50 million vs 100 million) for degenerative OA	NCT 01300598

[a]ASC, adipose-derived stem cell; BM, bone marrow; HA, hyaluronic acid; ICRS, International Cartilage Repair Society; IKDC, International Knee Documentation Committee; KOOS, Knee injury and Osteoarthritis Outcome Score; MRI, magnetic resonance imaging; MSC, mesenchymal stem cell; OA, osteoarthritis; PRP, platelet-rich plasma; RCT, randomized controlled trial; ROM, range of motion; SF-8, Short Form–8 Health Survey; SF-12, Short Form–12 Health Survey; SF-36, Short Form–36 Health Survey; VAS, visual analog scale; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

for the repair of chondral knee defects. The ADIPOA trial is examining the effects of differing concentrations of ASCs injected into the knees of patients with grade 3 to 4 osteoarthritis. A recently published case series highlighted the effects of ASCs on moderate to severe knee osteoarthritis.[54] After arthroscopic surgery, the investigators harvested the infrapatellar fat pad, and ASCs were derived and counted with a hemocytometer. A mean of 1.18 million stem cells (range, 0.3 million to 2.7 million stem cells) were then prepared with 3.0 mL of PRP and injected back into the knee. At a minimum 2-year follow-up, the 18-patient study showed improved clinical and MRI results. The authors concluded that these improved results were positively related to the number of stem cells injected.

DISCUSSION

The treatment of articular cartilage defects still remains a great challenge for the surgeon and scientist alike. Stem cells have been used with promise in animal studies and also recently in clinical studies. The success of translation from the laboratory to the patient remains to be seen. The purpose of this review was to outline the current role of stem cells in both animal and clinical cartilage defect models; to report structural, functional, and clinical benefits; and to highlight their role in the future. A systematic review was performed on the clinical studies.

There are many animal studies that report the effects of stem cells on cartilage repair in terms of structural, biomechanical, and functional outcomes. The results in small animals treated with MSCs, either alone or with varying combinations of growth factors, scaffolds, or gene transfer agents, have been promising in terms of structural and biomechanical benefits. Large animal models may be more relevant to human knee anatomy, biomechanics, and clinical outcomes. Sheep, pig, goat, and horse models using MSCs, with and without growth factors or scaffolds, highlight the potential for cartilage repair.

The clinical benefits of MSCs in cartilage repair are still being evaluated. There have been few published large clinical studies utilizing standardized, established outcome scores, so the interpretation of results is difficult. A number of studies involved direct injections of cell suspension into the knee but showed no evidence that the cells were responsible for the repair of joint tissues.[25,27] There is an increase in the number of groups around the world that are studying bone marrow–derived MSCs, ASCs, and human umbilical cord blood-derived stem cells and their effects on cartilage repair. The combination of MSCs with scaffolds, growth factors, PRP, and gene therapy is also being investigated. In other studies, the direct injection of these stem cells into the knee joint is being investigated as a therapy for arthritis,[25,27] independent of the osteochondral repair techniques outlined in this article. The field of stem cells and cartilage repair is certainly an exciting one and will continue to expand rapidly.

FUTURE DIRECTIONS AND CHALLENGES

The regulation of stem cell treatment of cartilage defects is a major challenge. Discussion between regulatory agencies and individual companies or university laboratories often remains confidential because of intellectual property issues. It is important that future trials remain safe and efficacious. It has been suggested that a joint committee of representative basic scientists, bioethicists, biostatisticians, clinicians, and manufacturing/biotechnology representatives should be established to develop a minimum set of safety and efficacy parameters.[35] These data could then be posted to an online registry for long-term follow-up.

ACKNOWLEDGMENT

The authors thank Ms Virginia Carden, MSLS, AHIP, for her kind help with the references in this article.

REFERENCES

1. Abrahamsson CK, Yang F, Park H, et al. Chondrogenesis and mineralization during in vitro culture of human mesenchymal stem cells on three-dimensional woven scaffolds. *Tissue Eng Part A*. 2010; 16(12):3709-3718.
2. Acharya C, Adesida A, Zajac P, et al. Enhanced chondrocyte proliferation and mesenchymal stromal cells chondrogenesis in coculture pellets mediate improved cartilage formation. *J Cell Physiol*. 2012;227(1):88-97.
3. Ahn JH, Lee TH, Oh JS, et al. Novel hyaluronate-atelocollagen/beta-TCP-hydroxyapatite biphasic scaffold for the repair of osteochondral defects in rabbits. *Tissue Eng Part A*. 2009;15(9):2595-2604.
4. Anraku Y, Mizuta H, Sei A, et al. The chondrogenic repair response of undifferentiated mesenchymal cells in rat full-thickness articular cartilage defects. *Osteoarthritis Cartilage*. 2008;16(8):961-964.
5. Behrens P, Bitter T, Kurz B, Russlies M. Matrix-associated autologous chondrocyte transplantation/implantation (MACT/MACI): 5-year follow-up. *Knee*. 2006;13(3):194-202.
6. Bentley G, Biant LC, Carrington RW, et al. A prospective, randomised comparison of autologous chondrocyte implantation versus mosaicplasty for osteochondral defects in the knee. *J Bone Joint Surg Br*. 2003;85(2):223-230.
7. Benya PD, Padilla SR, Nimni ME. Independent regulation of collagen types by chondrocytes during the loss of differentiated function in culture. *Cell*. 1978;15(4):1313-1321.
8. Bi W, Deng JM, Zhang Z, Behringer RR, de Crombrugghe B. Sox9 is required for cartilage formation. *Nat Genet*. 1999;22(1):85-89.
9. Bian L, Zhai DY, Mauck RL, Burdick JA. Coculture of human mesenchymal stem cells and articular chondrocytes reduces hypertrophy and enhances functional properties of engineered cartilage. *Tissue Eng Part A*. 2011;17(7-8):1137-1145.
10. Bouffi C, Thomas O, Bony C, et al. The role of pharmacologically active microcarriers releasing TGF-beta3 in cartilage formation in vivo by mesenchymal stem cells. *Biomaterials*. 2010;31(25):6485-6493.
11. Cao L, Yang F, Liu G, et al. The promotion of cartilage defect repair using adenovirus mediated Sox9 gene transfer of rabbit bone marrow mesenchymal stem cells. *Biomaterials*. 2011;32(16):3910-3920.
12. Caplan AI. Mesenchymal stem cells: the past, the present, the future. *Cartilage*. 2010;1(1):6-9.
13. Caplan AI. New era of cell-based orthopedic therapies. *Tissue Eng Part B Rev*. 2009;15(2):195-200.

14. Caplan AI. Why are MSCs therapeutic? New data: new insight. *J Pathol*. 2009;217(2):318-324.
15. Center for Biologics Evaluation and Research, Food and Drug Administration, US Department of Health and Human Services. Guidance for industry regulation of human cells, tissues, and cellular and tissue-based products (HCT/Ps): small entity compliance guide. April 19, 2012. Available at: http://purl.access.gpo.gov/GPO/LPS112358. Accessed February 25, 2013.
16. Chang CH, Kuo TF, Lin FH, et al. Tissue engineering-based cartilage repair with mesenchymal stem cells in a porcine model. *J Orthop Res*. 2011;29(12):1874-1880.
17. Chu CR, Szczodry M, Bruno S. Animal models for cartilage regeneration and repair. *Tissue Eng Part B Rev*. 2010;16(1):105-115.
18. Chung C, Beecham M, Mauck RL, Burdick JA. The influence of degradation characteristics of hyaluronic acid hydrogels on in vitro neocartilage formation by mesenchymal stem cells. *Biomaterials*. 2009;30(26):4287-4296.
19. Cournil-Henrionnet C, Huselstein C, Wang Y, et al. Phenotypic analysis of cell surface markers and gene expression of human mesenchymal stem cells and chondrocytes during monolayer expansion. *Biorheology*. 2008;45(3-4):513-526.
20. Dashtdar H, Rothan HA, Tay T, et al. A preliminary study comparing the use of allogenic chondrogenic pre-differentiated and undifferentiated mesenchymal stem cells for the repair of full thickness articular cartilage defects in rabbits. *J Orthop Res*. 2011;29(9):1336-1342.
21. Davatchi F, Abdollahi BS, Mohyeddin M, Shahram F, Nikbin B. Mesenchymal stem cell therapy for knee osteoarthritis: preliminary report of four patients. *Int J Rheum Dis*. 2011;14(2):211-215.
22. De Bari C, Dell'Accio F, Tylzanowski P, Luyten FP. Multipotent mesenchymal stem cells from adult human synovial membrane. *Arthritis Rheum*. 2001;44(8):1928-1942.
23. Diao H, Wang J, Shen C, et al. Improved cartilage regeneration utilizing mesenchymal stem cells in TGF-beta1 gene-activated scaffolds. *Tissue Eng Part A*. 2009;15(9):2687-2698.
24. Diekman BO, Christoforou N, Willard VP, et al. Cartilage tissue engineering using differentiated and purified induced pluripotent stem cells. *Proc Natl Acad Sci U S A*. 2012;109(47):19172-19177.
25. Diekman BO, Guilak F. Stem cell-based therapies for osteoarthritis: challenges and opportunities. *Curr Opin Rheumatol*. 2013;25(1):119-126.
26. Diekman BO, Rowland CR, Lennon DP, Caplan AI, Guilak F. Chondrogenesis of adult stem cells from adipose tissue and bone marrow: induction by growth factors and cartilage-derived matrix. *Tissue Eng Part A*. 2010;16(2):523-533.
27. Diekman BO, Wu CL, Louer CR, et al. Intra-articular delivery of purified mesenchymal stem cells from C57BL/6 or MRL/MpJ superhealer mice prevents post-traumatic arthritis. *Cell Transplant*. 2013;22(8):1395-1408.
28. Erickson IE, van Veen SC, Sengupta S, Kestle SR, Mauck RL. Cartilage matrix formation by bovine mesenchymal stem cells in three-dimensional culture is age-dependent. *Clin Orthop*. 2011;469(10):2744-2753.
29. Estes BT, Diekman BO, Gimble JM, Guilak F. Isolation of adipose-derived stem cells and their induction to a chondrogenic phenotype. *Nat Protoc*. 2010;5(7):1294-1311.
30. Fink T, Rasmussen JG, Emmersen J, et al. Adipose-derived stem cells from the brown bear (Ursus arctos) spontaneously undergo chondrogenic and osteogenic differentiation in vitro. *Stem Cell Res*. 2011;7(1):89-95.
31. Fong CY, Subramanian A, Gauthaman K, et al. Human umbilical cord Wharton's jelly stem cells undergo enhanced chondrogenic differentiation when grown on nanofibrous scaffolds and in a sequential two-stage culture medium environment. *Stem Cell Rev*. 2012;8(1):195-209.
32. Fortier LA, Barker JU, Strauss EJ, McCarrel TM, Cole BJ. The role of growth factors in cartilage repair. *Clin Orthop*. 2011;469(10):2706-2715.
33. Fortier LA, Potter HG, Rickey EJ, et al. Concentrated bone marrow aspirate improves full-thickness cartilage repair compared with microfracture in the equine model. *J Bone Joint Surg Am*. 2010;92(10):1927-1937.
34. Gadjanski I, Spiller K, Vunjak-Novakovic G. Time-dependent processes in stem cell-based tissue engineering of articular cartilage. *Stem Cell Rev*. 2012;8(3):863-881.
35. Gimble JM, Bunnell BA, Chiu ES, Guilak F. Taking stem cells beyond discovery: a milestone in the reporting of regulatory requirements for cell therapy. *Stem Cells Dev*. 2011;20(8):1295-1296.
36. Gimble JM, Guilak F, Bunnell BA. Clinical and preclinical translation of cell-based therapies using adipose tissue-derived cells. *Stem Cell Res Ther*. 2010;1(2):19.
37. Goldring MB, Sandell LJ, Stephenson ML, Krane SM. Immune interferon suppresses levels of procollagen mRNA and type II collagen synthesis in cultured human articular and costal chondrocytes. *J Biol Chem*. 1986;261(19):9049-9055.
38. Goldring MB, Tsuchimochi K, Ijiri K. The control of chondrogenesis. *J Cell Biochem*. 2006;97(1):33-44.
39. Guilak F. The deformation behavior and viscoelastic properties of chondrocytes in articular cartilage. *Biorheology*. 2000;37(1-2):27-44.
40. Guilak F, Estes BT, Diekman BO, Moutos FT, Gimble JM. 2010 Nicolas Andry Award: multipotent adult stem cells from adipose tissue for musculoskeletal tissue engineering. *Clin Orthop*. 2010;468(9):2530-2540.
41. Guilak F, Lott KE, Awad HA, et al. Clonal analysis of the differentiation potential of human adipose-derived adult stem cells. *J Cell Physiol*. 2006;206(1):229-237.
42. Guo X, Park H, Young S, et al. Repair of osteochondral defects with biodegradable hydrogel composites encapsulating marrow mesenchymal stem cells in a rabbit model. *Acta Biomater*. 2010;6(1):39-47.
43. Haleem AM, Singergy AA, Sabry D, et al. The clinical use of human culture-expanded autologous bone marrow mesenchymal stem cells transplanted on platelet-rich fibrin glue in the treatment of articular cartilage defects: a pilot study and preliminary results. *Cartilage*. 2010;1(4):253-261.
44. Hepp P, Osterhoff G, Niederhagen M, et al. Perilesional changes of focal osteochondral defects in an ovine model and their relevance to human osteochondral injuries. *J Bone Joint Surg Br*. 2009;91(8):1110-1119.
45. Hermida-Gomez T, Fuentes-Boquete I, Gimeno-Longas MJ, et al. Quantification of cells expressing mesenchymal stem cell markers in healthy and osteoarthritic synovial membranes. *J Rheumatol*. 2011;38(2):339-349.
46. Hjelle K, Solheim E, Strand T, Muri R, Brittberg M. Articular cartilage defects in 1,000 knee arthroscopies. *Arthroscopy*. 2002;18(7):730-734.
47. Hui JH, Chen F, Thambyah A, Lee EH. Treatment of chondral lesions in advanced osteochondritis dissecans: a comparative study of the efficacy of chondrocytes, mesenchymal stem cells, periosteal graft, and mosaicplasty (osteochondral autograft) in animal models. *J Pediatr Orthop*. 2004;24(4):427-433.
48. Jung M, Kaszap B, Redohl A, et al. Enhanced early tissue regeneration after matrix-assisted autologous mesenchymal stem cell transplantation in full thickness chondral defects in a minipig model. *Cell Transplant*. 2009;18(8):923-932.
49. Kaab MJ, Gwynn IA, Notzli HP. Collagen fibre arrangement in the tibial plateau articular cartilage of man and other mammalian species. *J Anat*. 1998;193(Pt 1):23-34.
50. Kim J, Kang JW, Park JH, et al. Biological characterization of long-term cultured human mesenchymal stem cells. *Arch Pharm Res*. 2009;32(1):117-126.
51. Kisiday JD, Hale BW, Almodovar JL, et al. Expansion of mesenchymal stem cells on fibrinogen-rich protein surfaces derived from blood plasma. *J Tissue Eng Regen Med*. 2011;5(8):600-611.
52. Knutsen G, Engebretsen L, Ludvigsen TC, et al. Autologous chondrocyte implantation compared with microfracture in the knee: a randomized trial. *J Bone Joint Surg Am*. 2004;86(3):455-464.
53. Koga H, Engebretsen L, Brinchmann JE, Muneta T, Sekiya I. Mesenchymal stem cell-based therapy for cartilage repair: a review. *Knee Surg Sports Traumatol Arthrosc*. 2009;17(11):1289-1297.
54. Koh YG, Jo SB, Kwon OR, et al. Mesenchymal stem cell injections improve symptoms of knee osteoarthritis. *Arthroscopy*. 2013;29(4):748-755.

55. Kuroda R, Ishida K, Matsumoto T, et al. Treatment of a full-thickness articular cartilage defect in the femoral condyle of an athlete with autologous bone-marrow stromal cells. *Osteoarthritis Cartilage*. 2007;15(2):226-231.
56. Kurth TB, Dell'accio F, Crouch V, Augello A, Sharpe PT, De Bari C. Functional mesenchymal stem cell niches in adult mouse knee joint synovium in vivo. *Arthritis Rheum*. 2011;63(5):1289-1300.
57. LaPrade RF, Botker JC. Donor-site morbidity after osteochondral autograft transfer procedures. *Arthroscopy*. 2004;20(7):e69-e73.
58. Lee CH, Cook JL, Mendelson A, Moioli EK, Yao H, Mao JJ. Regeneration of the articular surface of the rabbit synovial joint by cell homing: a proof of concept study. *Lancet*. 2010;376(9739):440-448.
59. Lee KB, Hui JH, Song IC, Ardany L, Lee EH. Injectable mesenchymal stem cell therapy for large cartilage defects: a porcine model. *Stem Cells*. 2007;25(11):2964-2971.
60. Li WJ, Chiang H, Kuo TF, Lee HS, Jiang CC, Tuan RS. Evaluation of articular cartilage repair using biodegradable nanofibrous scaffolds in a swine model: a pilot study. *J Tissue Eng Regen Med*. 2009;3(1):1-10.
61. Lind M, Larsen A, Clausen C, Osther K, Everland H. Cartilage repair with chondrocytes in fibrin hydrogel and MPEG polylactide scaffold: an in vivo study in goats. *Knee Surg Sports Traumatol Arthrosc*. 2008;16(7):690-698.
62. Mainil-Varlet P, Aigner T, Brittberg M, et al. Histological assessment of cartilage repair: a report by the Histology Endpoint Committee of the International Cartilage Repair Society (ICRS). *J Bone Joint Surg Am*. 2003;85 Suppl 2:45-57.
63. Marquass B, Schulz R, Hepp P, et al. Matrix-associated implantation of predifferentiated mesenchymal stem cells versus articular chondrocytes: in vivo results of cartilage repair after 1 year. *Am J Sports Med*. 2011;39(7):1401-1412.
64. Marquass B, Somerson JS, Hepp P, et al. A novel MSC-seeded triphasic construct for the repair of osteochondral defects. *J Orthop Res*. 2010;28(12):1586-1599.
65. McCarthy HE, Bara JJ, Brakspear K, Singhrao SK, Archer CW. The comparison of equine articular cartilage progenitor cells and bone marrow–derived stromal cells as potential cell sources for cartilage repair in the horse. *Vet J*. 2012;192(3):345-351.
66. McIlwraith CW, Frisbie DD, Rodkey WG, et al. Evaluation of intra-articular mesenchymal stem cells to augment healing of microfractured chondral defects. *Arthroscopy*. 2011;27(11):1552-1561.
67. Merceron C, Portron S, Masson M, et al. The effect of two and three dimensional cell culture on the chondrogenic potential of human adipose-derived mesenchymal stem cells after subcutaneous transplantation with an injectable hydrogel. *Cell Transplant*. 2011; 20(10):1575-1588.
68. Minas T, Gomoll AH, Rosenberger R, Royce RO, Bryant T. Increased failure rate of autologous chondrocyte implantation after previous treatment with marrow stimulation techniques. *Am J Sports Med*. 2009;37(5):902-908.
69. Nejadnik H, Hui JH, Feng Choong EP, Tai BC, Lee EH. Autologous bone marrow–derived mesenchymal stem cells versus autologous chondrocyte implantation: an observational cohort study. *Am J Sports Med*. 2010;38(6):1110-1116.
70. Nishimori M, Deie M, Kanaya A, Exham H, Adachi N, Ochi M. Repair of chronic osteochondral defects in the rat: a bone marrow–stimulating procedure enhanced by cultured allogenic bone marrow mesenchymal stromal cells. *J Bone Joint Surg Br*. 2006;88(9):1236-1244.
71. Nixon AJ, Fortier LA, Goodrich LR, Ducharme NG. Arthroscopic reattachment of osteochondritis dissecans lesions using resorbable polydioxanone pins. *Equine Vet J*. 2004;36(5):376-383.
72. Pineda S, Pollack A, Stevenson S, Goldberg V, Caplan A. A semiquantitative scale for histologic grading of articular cartilage repair. *Acta Anat (Basel)*. 1992;143(4):335-340.
73. Qi Y, Zhao T, Xu K, Dai T, Yan W. The restoration of full-thickness cartilage defects with mesenchymal stem cells (MSCs) loaded and cross-linked bilayer collagen scaffolds on rabbit model. *Mol Biol Rep*. 2012;39(2):1231-1237.
74. Rasanen T, Messner K. Regional variations of indentation stiffness and thickness of normal rabbit knee articular cartilage. *J Biomed Mater Res*. 1996;31(4):519-524.
75. Reinholz GG, Lu L, Saris DB, Yaszemski MJ, O'Driscoll SW. Animal models for cartilage reconstruction. *Biomaterials*. 2004;25(9):1511-1521.
76. Ronziere MC, Perrier E, Mallein-Gerin F, Freyria AM. Chondrogenic potential of bone marrow– and adipose tissue–derived adult human mesenchymal stem cells. *Biomed Mater Eng*. 2010;20(3):145-158.
77. Saris DB, Dhert WJ, Verbout AJ. Joint homeostasis: the discrepancy between old and fresh defects in cartilage repair. *J Bone Joint Surg Br*. 2003;85(7):1067-1076.
78. Saw KY, Hussin P, Loke SC, et al. Articular cartilage regeneration with autologous marrow aspirate and hyaluronic acid: an experimental study in a goat model. *Arthroscopy*. 2009;25(12):1391-1400.
79. Shapiro F, Koide S, Glimcher MJ. Cell origin and differentiation in the repair of full-thickness defects of articular cartilage. *J Bone Joint Surg Am*. 1993;75(4):532-553.
80. Shimomura K, Ando W, Tateishi K, et al. The influence of skeletal maturity on allogenic synovial mesenchymal stem cell-based repair of cartilage in a large animal model. *Biomaterials*. 2010;31(31):8004-8011.
81. Steadman JR, Briggs KK, Rodrigo JJ, Kocher MS, Gill TJ, Rodkey WG. Outcomes of microfracture for traumatic chondral defects of the knee: average 11-year follow-up. *Arthroscopy*. 2003;19(5):477-484.
82. Tay LX, Ahmad RE, Dashtdar H, et al. Treatment outcomes of alginate-embedded allogenic mesenchymal stem cells versus autologous chondrocytes for the repair of focal articular cartilage defects in a rabbit model. *Am J Sports Med*. 2012;40(1):83-90.
83. Tegner Y, Lysholm J. Rating systems in the evaluation of knee ligament injuries. *Clin Orthop*. 1985;198:43-49.
84. Vickers SM, Gotterbarm T, Spector M. Cross-linking affects cellular condensation and chondrogenesis in type II collagen-GAG scaffolds seeded with bone marrow–derived mesenchymal stem cells. *J Orthop Res*. 2010;28(9):1184-1192.
85. Vidal MA, Robinson SO, Lopez MJ, et al. Comparison of chondrogenic potential in equine mesenchymal stromal cells derived from adipose tissue and bone marrow. *Vet Surg*. 2008;37(8):713-724.
86. Wakitani S, Goto T, Pineda SJ, et al. Mesenchymal cell-based repair of large, full-thickness defects of articular cartilage. *J Bone Joint Surg Am*. 1994;76(4):579-592.
87. Wakitani S, Nawata M, Tensho K, Okabe T, Machida H, Ohgushi H. Repair of articular cartilage defects in the patello-femoral joint with autologous bone marrow mesenchymal cell transplantation: three case reports involving nine defects in five knees. *J Tissue Eng Regen Med*. 2007;1(1):74-79.
88. Wang W, Li B, Li Y, Jiang Y, Ouyang H, Gao C. In vivo restoration of full-thickness cartilage defects by poly(lactide-co-glycolide) sponges filled with fibrin gel, bone marrow mesenchymal stem cells and DNA complexes. *Biomaterials*. 2010;31(23):5953-5965.
89. Wei X, Gao J, Messner K. Maturation-dependent repair of untreated osteochondral defects in the rabbit knee joint. *J Biomed Mater Res*. 1997;34(1):63-72.
90. Wilke MM, Nydam DV, Nixon AJ. Enhanced early chondrogenesis in articular defects following arthroscopic mesenchymal stem cell implantation in an equine model. *J Orthop Res*. 2007;25(7):913-925.
91. Yan H, Yu C. Repair of full-thickness cartilage defects with cells of different origin in a rabbit model. *Arthroscopy*. 2007;23(2):178-187.
92. Zhang Y, Wang F, Chen J, Ning Z, Yang L. Bone marrow–derived mesenchymal stem cells versus bone marrow nucleated cells in the treatment of chondral defects. *Int Orthop*. 2012;36(5):1079-1086.
93. Zscharnack M, Hepp P, Richter R, et al. Repair of chronic osteochondral defects using predifferentiated mesenchymal stem cells in an ovine model. *Am J Sports Med*. 2010;38(9):1857-1869.