Current Reviews in Musculoskeletal Medicine (2020) 13:20–27
https://doi.org/10.1007/s12178-020-09598-z

STEM CELLS IN ORTHOPAEDIC SURGERY (J DRAGOO AND KJ JONES, SECTION EDITORS)

Stem Cell Treatment for Knee Articular Cartilage Defects and Osteoarthritis

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Published online: 21 January 2020

Abstract

Purpose of Review To review the current basic science and clinical literature on mesenchymal stem cell (MSC) therapy for articular cartilage defects and osteoarthritis of the knee.

Recent Findings MSCs derived from bone marrow, adipose, and umbilical tissue have the capacity for self-renewal and differentiation into the chondrocyte lineage. In theory, MSC therapy may help restore cartilage focally or diffusely where nascent regenerative potential in the intra-articular environment is limited. Over the last several years, in vitro and animal studies have elucidated the use of MSCs in isolation as injectables, in combination with biological delivery media and scaffolding, and as surgical adjuvants for cartilage regeneration and treatment of knee degenerative conditions. More recently, clinical and translational literature has grown more convincing from early descriptive case series to randomized controlled trials showing promise in efficacy and safety. Studies describing MSC for knee cartilage regeneration applications are numerous and varied in quality. Future research directions should include work on elucidating optimal cell concentration and dosing, as well as standardization in methodology and reporting in prospective trials.

Summary Backed by promise from in vitro and animal studies, preliminary clinical evidence on MSC therapy shows promise as a nonoperative therapeutic option or an adjuvant to existing surgical cartilage restoration techniques. While higher quality evidence to support MSC therapy has emerged over the last several years, further refinement of methodology will be necessary to support its routine clinical use.

Keywords Mesenchymal stem cell · Cartilage defect · Osteochondral lesion · Osteoarthritis · Stem cell therapy

Introduction

Degenerative joint disease of the knee is a common medical ailment that has a broad spectrum from isolated articular cartilage defects to end-stage osteoarthritis (OA). The development of articular cartilage lesions can increase the risk and rate of progression to end-stage disease, wherein anywhere from 5 to 30% of the general adult population is affected by OA [1, 2]. Treatment of degenerative knee conditions is dependent on initial management with conservative means that include non-steroidal anti-inflammatory (NSAID) medications and other oral analgesics, weight loss, physical therapy, and exercises [3]. In instances where such treatments fail to provide relief, non-surgical interventions such as corticosteroid injections, hyaluronate derivates, and other bioactive injectables may become an intermediary option prior to consideration of surgical cartilage restoration or arthroplasty [3, 4].

From a biologic perspective, articular cartilage defects and OA are clinically challenging entities because chondrocytes have limited native regeneration potential, particularly with age [5]. However, significant advances in regenerative medicine over the last several years have been particularly impactful for the treatment of degenerative cartilage disease. The gold standard cell therapy for cartilage restoration at this time remains autologous chondrocyte implantation (ACI), wherein autologous chondrocytes are harvested, culture-expanded

This article is part of the Topical Collection on *Stem Cells in Orthopaedic Surgery*

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in vitro, and then subsequently re-implanted into the cartilage defect in a two-stage procedure [6, 7]. However, ACI has practical limitations including the need for autologous harvest and indications limited to unifocal, pre-arthritic defects [8].

Recently, stem cell therapy has emerged as a readily accessible source for treatment with promise in both preclinical and clinical studies. Here, we discuss stem cell–based approaches, both in isolation and as adjuvants to existing surgical therapies, for the treatment of osteochondral lesions and osteoarthritis as a spectrum of degenerative disease of the knee. The literature presented here is predominantly from the last 5 years, which has shown significantly more numerous and convincing evidence in support of stem cell therapy.

Stem Cell Therapy: a Brief Scientific Overview

In the context of cartilage regeneration for the treatment of osteochondral defects and OA, "stem cell therapy" almost always describes the use of mesenchymal stem cells (MSCs). MSCs are a broad category of adult multipotent stromal cells that have the potential to self-renew and directionally differentiate into multiple lineages of cells including osteoblasts (bone), adipocytes (fat and marrow), myocytes (muscle), and chondrocytes (cartilage) [9, 10]. Commitment into each of these various lineages is dependent upon lineage-specific growth factors and signaling pathways that have been elucidated both ex vivo and in vivo. Chondrogenesis, as is the focus of this review, is dependent on chondrogenic signals such as transcription factors Sox9 and Runx2 and bone morphogenetic protein (BMP) signaling [11–13]. In vivo, MSCs are most commonly found in bone marrow, adipose tissue, periosteum, and synovium [14]. In clinical applications, they are most commonly and easily harvested from bone marrow and adipose tissue [15••,16••]. There is some evidence to suggest that adipose-derived MSCs have lower immunogenicity in addition to their easier acquisition for practical application, though neither has gained favor in clinical trials [17]. Other less clinically relevant in vivo sources include molar cells, the umbilical cord, and amniotic fluid [16••]. MSCs can be used either in a cell matrix expanded by culture or directly as a bone marrow aspirate concentrate.

In addition to their chondrogenic potential, MSCs are an ideal alternative cell source for cartilage repair for several reasons. Firstly, MSCs are easily cultured and have the ability to self-renew while undergoing differentiation into mesenchymal lineages [18, 19]. Secondly, MSCs also have significant paracrine activity wherein growth factors and cytokines nourish cartilage via angiogenesis and direct chondrocyte proliferation in a feedback loop [20]. Cytokines and growth factors such as VEGF and TGF-β also allow MSC migration into regions of cartilage ischemia [21]. Finally, MSCs are known to be immunomodulatory [22]. Though these pathways are relatively less well understood, MSC growth factor suppression of T cell proliferation and B cell antibody secretion may be important in reducing the risk of rejection and disease transmission in cases where allogeneic MSCs are used for therapeutic benefit [22–24].

Preclinical Data on Stem Cell Therapy for Knee Degeneration

Abundant literature describing the preclinical efficacy of MSCs in treating degenerative conditions of the knee has emerged in the last several years. In vivo animal models of knee degeneration can include chemical agents such as sodium iodoacetate, papain, quinolone, and collagenase to induce OA. Knee degeneration can also be induced in animals through surgical means, such as iatrogenic anterior cruciate ligament transection [25], meniscectomy [26], osteochondral fragmentation [27], or tibial plateau fracture [28]. Studies have included and ranged from small animal models such as rats and rabbits to large animal models such as sheep, pigs, and dogs [26, 29–32].

Using a surgically induced rat knee OA model, Zhou et al. found that local injection of adipose-derived MSCs alleviated histologic OA by increasing expression of transcription factors Col2 and Sox9 while also reducing proinflammatory cytokine secretion and protecting against apoptosis through autophagy induction [33]. Saulnier and colleagues showed that MSCs injected intra-articularly localize to the synovium and modulate gene expression pattern to reduce matrix metalloproteinase expression with less cartilage degeneration histologically in a rabbit model [34]. They noted no adverse local or systemic effects. Some groups have combined in vitro pretreatment of MSCs prior to injection as a means of enhancing efficacy. Sasaki et al. found that granulocyte-colony stimulating factor (G-CSF) enhanced MSC proliferation in vitro almost twofold [35]. Injection of these cultured MSCs induced partial regeneration of hyaline cartilage in trochlear osteochondral defects in a rabbit model, which occurred more quickly in the group pretreated with low-dose G-CSF medium. In light of chondrocyte sensitivity to mechanical stimulation, combination therapy using intra-articular MSC injection with temporary joint distraction has also shown to be effective in a rabbit osteochondral defect model [36]. Feng et al. reported that injectable MSCs may survive up to 14 weeks after intra-articular injection and engraft in both synovium and surface cartilage [32]. Animal studies have also elucidated that multiple rounds of MSC therapy may be necessary to have an effect on reducing OA [37•].

Injectables in animal models have often included adjuvants such as platelet-rich plasma (PRP) [31, 32, 38] and hyaluronic acid (HA) [27, 29], which theoretically may promote cell proliferation, type II collagen synthesis, and inflammatory

chemotaxis synergistically. In a dog OA model, Yun et al. found that cotreatment with injectable MSCs and PRP showed greater improvement in extracellular matrix composition, focal compression strength, and glycosaminoglycan and collagen composition than those treated with PRP or MSCs alone in comparison with sham controls [31].Similarly, Chiang et al. found that cotreatment with MSCs/HA resulted in better histological scores and cartilage content in comparison with HA alone, attributable to engraftment of allogenic MSCs in surface cartilage [27].

To enhance regenerative potential, some have proposed the use of implantable scaffolds to enhance both chondrogenesis and incorporation into cartilage defects of MSCs. With regard to tissue engineering principles, an ideal scaffold should favor cell migration and proliferation within the in vivo biomechanical and biochemical environment of native cartilage, while also remaining biodegradable to allow for incorporation of new hyaline cartilage [39•]. Furthermore, there should be appropriate porosity for transitory incorporation and limited inflammatory reaction to prevent autophagy. Several categories of MSC scaffolds have been proposed and studied including synthetic polymers such as polyglycolic acid (PGA) [40], polylactic acid (PLA) [41], poly(lactic-co-glycolic acid) (PLGA) [42], polycaprolactone [43, 44], and natural polymers based on fibrin [45], collagen [46], heparin [47], and chitosan [48]. While these individual studies are numerous and promising, no single scaffolding design has emerged as superior and few reports directly compare scaffolding in preclinical models. As such, clinical application has been limited. One exception to this is the BST-CarGel®, a chitosan scaffold that has shown efficacy in human clinical trials for the treatment of high-grade chondral lesions of the knee in Europe [49•, 50]. BST-CarGel® is a biocompatible liquid chitosan solution prepared at physiological pH that stabilizes implanted clots, supporting hyaline cartilage rather than structurally inferior fibrocartilage that has been associated with poor outcomes in traditional marrow stimulation. In their in vitro study, Snow et al. found that retention of viable marrow-derived MSCs was observed with BST-CarGel® as a delivery vehicle [49•]. This is particularly intriguing for more durable cartilage repair given the promising clinical outcomes seen with MSCs and BST-CarGel® individually.

Despite progress made in MSC-based tissue engineering methods using these preclinical models, routine clinical applications of injectables and scaffolds have been limited because (1) the potential for graft-versus-host reactions with allogeneic MSCs, (2) difficulty in acquisition and culture of autologous MSCs, and (3) a limited milieu of scaffolding materials available through regulatory agencies. Furthermore, the extreme diversity in methodologies and therapeutics used in these studies obviates the need for higher quality study design to have reliable external validity and translation into clinical application [51].

Clinical Applications of Stem Cell Therapy

As of mid-2019, the National Institutes of Health (NIH) reports 86 studies in various stages of completion on the use of stem cell therapy for knee OA. Injectable MSC therapy may well serve the "intermediate" patient between chondromalacia and end-stage osteoarthritis, and may be added to the practitioner's armamentarium of corticosteroid, PRP, and viscosupplementation injections that are currently available for modulating clinical symptoms. If efficacious, it may also decrease the need for operative intervention such as cartilage repair and arthroplasty. We provide a brief review of clinical studies describing autologous and allogeneic MSC therapies used both as injectables and as surgical adjuvants from the last 5 years.

Injectable MSC Therapy

Autologous injectable MSCs are most commonly derived from either bone marrow or adipose-derived sources. Al-Najar et al. described a series of 13 patients with knee OA treated with two intra-articular injections of marrow-derived MSCs [52]. At 2 years, they described significant improvements in normalized Knee Injury and Osteoarthritis Outcome Score (KOOS) scores in patients. The authors also noted statistically significant improvement in mean cartilage thickness measured on T2-weighted MRI; no adverse events were described. Garay-Mendoza et al. conducted a prospective, randomized, open-label trial of stimulated autologous marrow-derived MSCs [53]. The treatment group received subcutaneous administration of G-CSF for marrow stimulation, followed by bone marrow aspiration to harvest cells for a single intra-articular injection; the control group received oral acetaminophen alone. Patients in the treatment group showed significantly greater improvement in all Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) subscales (pain, stiffness, physical functions) at 1 and 6 months. This study is among the first to demonstrate the promise of G-CSF as a biologic stimulant for MSC harvest. Drawbacks of this study included lack of study blinding as well as absence of radiographic and histologic examination of cartilage volume. Similarly in a study comparing injectable marrow-derived MSCs with HA to HA alone, another group found superior improvement in WOMAC and VAS scores at 12 months, as well as in evidence of OA on both plain radiographs and MRI [54]. The authors reported a dose-dependent relationship, wherein 100×10 [6] cells/1.5 mL had superior clinical and functional improvement compared with 10×10 [6] cells/1.5 mL. Indeed, the minimal efficacious dose of MSCs to treat OA is a topic of active research [55–57].

With the advent of rapid in vitro expansion protocols and the relative ease of harvest, autologous adipose-derived MSCs have also shown promise in recent years [56–58]. A phase I

study from a European consortium demonstrated in a preliminary series of 18 patients that adipose-derived MSCs reduced pain and function subscales of the WOMAC at 6 months' follow-up [58]. With small and likely underpowered sample sizes in mind, they found no significant differences in clinical outcome based on dose escalation from 2×10 [6] to 50×10 [6] cells/injection. Enrollment for a placebo-controlled, double-blind phase II study is now underway. In a double-blinded randomized controlled trial, Song et al. reported similar positive clinical outcomes in a dose-dependent matter [57]. A single dose of 5×10 [7] cells/injection exhibited the highest improvement in clinical outcome scores (WOMAC, SF-36) and radiographic cartilage volume in their series.

Allogeneic MSCs derived and expanded from human umbilical cord and bone marrow have been described in recent years with the potential for more logistic convenience than autologous options. Gupta and colleagues in India recently described the use of Stempeucel®, an off-the-shelf ex vivo suspension of marrow-derived allogeneic MSCs, in preclinical and phase II clinical trial [59•]. In a randomized trial of 60 patients, they demonstrated a non-statistically significant trend towards improvement in VAS and WOMAC scores compared with placebo at 6 and 12 months. In higher doses ($\geq 50 \times 10$ [6] cells/injection), they did note knee pain and swelling in a minority of patients. Vega and colleagues reported similar results in comparing allogeneic marrow-derived MSCs to HA injections alone [60]. A handful of small clinical studies have described the use of umbilical-derived MSCs [61, 62••]. Matas et al. performed a triple-blind randomized trial comparing patients treated with single-dose umbilical cord MSCs, multiple-dose umbilical cord MSCs, and HA injections [62••]. Only patients receiving MSCs had improvement in WOMAC scores at 1 year compared with baseline while those treated with HA did not. Furthermore, patients receiving multiple doses of MSCs had greater improvements in WOMAC, VAS, and SF36 scores than those who received a single dose.

Intra-articular MSC injections have also been combined with various growth factors, cytokines, and drug delivery materials in an attempt to improve clinical efficacy. Serum platelet-rich plasma (PRP) is perhaps the most commonly described injectable adjuvant. Autologous PRP has been shown to be rich in several chondrogenic growth factors such as TGF-β and platelet-derived growth factor (PDGF) and as such may increase the chondrogenic differentiation yield of injected MSCs [63]. In several sequential case series, Koh et al. demonstrated significant improvement in pain and functional improvement in patients receiving combined MSC/PRP injections compared with patients receiving PRP alone [64–67]. Their group demonstrated improvement in patient-reported outcome scores with MSC/PRP injections in isolation for knee OA, as well as an adjuvant for patients undergoing arthroscopic debridement and high tibial osteotomy (HTO) for less advanced degenerative disease. It is important to note that data on combined MSC/PRP therapy, while consistently positive, is limited to individual case series without consistent comparison groups [68, 69]. Further study ascertaining the relative contributions of MSC and PRP, as well as optimal dosing, is warranted.

Surgical Adjuvants

In addition to the evidence for injectable MSC therapy, there is extensive literature describing the use of MSC therapy as an intraoperative adjuvant for cartilage regeneration in procedures such as arthroscopic drilling, microfracture, and HTO. It is theorized that MSCs may serve as a chondrocyte source where endogenous regeneration and ex vivo culture may be limited. In a randomized trial of 56 patients with varus unicompartmental knee OA, Wong et al. studied the use of allogeneic marrow-derived MSCs for patients undergoing HTO and microfracture [70]. Cell recipients showed significant improvement in Tegner, Lysholm, and IKDC scores at 2 years; postoperative Magnetic Resonance Observation of Cartilage Repair Tissue (MOCART) scores were also improved. Koh et al. demonstrated that infrapatellar fat pad–derived MSC therapy was effective as an adjuvant for arthroscopic debridement [67]. Using MSC/PRP therapy as an adjuvant in HTO, the same group later reported significant improvement in MR appearance of cartilage as well as KOOS and VAS subscores at short term [65]. Using autologous peripheral blood stem cells as an adjuvant, Saw and colleagues reported superior outcomes following arthroscopic subchondral drilling. Another randomized controlled trial comparing arthroscopic microfracture with or without postoperative adipose-derived MSC injections is underway in Australia with early results expected in the coming years [71]. All of these described techniques may serve as a potential alternative to ACI, which has drawbacks of two-stage surgery and high costs to expand chondrocytes with comparatively limited growth potential. However, at this time, the evidence to support ACI is higher in quality and has consistently demonstrated success at long-term follow-up.

Complications and Safety

Data specifically on the safety profile of MSC therapy is limited and non-systematic. Autologous harvest of MSCs from either bone marrow or adipose tissue has theoretical risks for donor site morbidity and infection, while allogeneic sources pose the potential for disease transmission and host-graft interactions with immunologic response. In a 2013 systematic review, Peeters et al. reported on adverse events in a total of 844 subjects treated with autologous marrow-derived MSC therapy [72••]. Two serious, intervention-related adverse events were noted including one pulmonary embolism and one infection successfully treated with antibiotics following

bone marrow aspiration: twenty-two other procedure-related and seven other stem cell–related adverse events. The majority of these adverse events included local transient pain and swelling at either the harvest or injection site. It is possible that immunomodulation (e.g., PDGF, VEGF, or TGF-β stimulation), either intrinsically from MSCs or as a result of PRP or other delivery media, may contribute to this pain and swelling. Low-grade fevers and transient laboratory abnormalities were also reported. In their more recent systematic review of RCTs on both autologous and allogeneic MSC injections, Pas et al. identified no serious adverse events, with local adverse events of transient pain and swelling in the treated joint in a small minority of patients [15••]. Another recent meta-analysis described only infrequent local adverse events with MSC therapies [73]. They found that the adverse event rate was not significantly different between study and control groups. Given the continued interest in clinical application of MSC therapy, further studies to describe safety and adverse events in a standardized way are necessary.

Future Directions

While the extensive literature reviewed here shows promise for MSC therapy as a treatment option for degenerative cartilage disease, many questions are simultaneously raised. Firstly, the consistency in reporting and study design in MSC and orthobiologic therapies in general is a central issue. While it is understandable that early literature is descriptive case series without a structured control group, future research endeavors should seek higher quality evidence to ensure efficacy and safety for patients [74••]. In many instances, commercial promotion of therapeutic uses of stem cell therapy greatly exceeds the supporting body of evidence and has proven resistant to regulatory efforts [74••, 75]. To the degree that concern exists regarding misinformation in the direct-to-consumer marketing of biologic treatments such as stem cell and PRP, the American Academy of Orthopedic Surgeons and NIH have made a consensus statement on minimal standards for product development and clinical research to allay concerns for safety and ethical responsibility to patients [76•]. Secondly and in this same vein, RCTs describing MSC are extremely limited. A recently published systematic review identified only five studies with level I or II evidence on injectable MSC therapy up to 2017 [15••]. Data comparing MSC therapies with reliable cell-free controls, as well as with established cartilage restoration procedures such as ACI, microfracture, and osteochondral grafting, are necessary to support routine clinical use. Finally, future studies are necessary to ascertain the role and limitations of serial injections, as well as dose dependence in terms of response to MSC therapy. While some animal studies have demonstrated that repeat intra-articular injections may be more effective in OA, clinical studies have yet to replicate this finding [37•, 77].

Conclusions

With potential for chondrogenesis from a rich and readily available cell source, MSC therapy is a potential area wherein novel nonoperative and operative approaches for the treatment of knee cartilage defects and osteoarthritis may be feasible. In vitro and animal studies have shown potential for these cells to promote chondrogenesis in vivo with advances in cell sources, delivery media, and scaffolding. In the last several years, preliminary clinical evidence on MSC therapy shows promise both as a nonoperative therapeutic option and as an adjuvant to existing cartilage restoration techniques with improved short-term clinical outcomes and radiographic health of cartilage. This evidence has been primarily case series without well-defined control groups and standardization in methodology, though RCTs have begun to emerge. Future directions that may help realize clinical potential should include standardized study methodology and reporting, further study on dosing, and comparison with other gold standard therapies to demonstrate clinical efficacy and safety.

Compliance with Ethical Standards

Conflict of Interest Dr. Arshi has no conflicts of interest to declare.

Dr. Petrigliano declares the following potential conflicts of interest: paid consultant for Biomet and Stryker.

Dr. Williams declares the following potential conflicts of interest: paid consultant for Arthrex, JRF Ortho, and Lipogems; stock or stock options from Cymedica, Gramercy Extremity Orthopedics, Pristine Surgical, and RecoverX; research support from Histogenics.

Dr. Jones declares the following potential conflicts of interest: paid consult for Vericel, JRF Ortho, Arthrex, and CONMED Linvaetec; research support from Aesculap/B.Braun, Musculoskeletal Transplant Foundation, and Zimmer.

Human and Animal Rights and Informed Consent This article does not contatin any studies with human participants or animals performed by any of the authors.

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- •• Of major importance

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