Comprehensive Review

Stem Cells for the Treatment of Knee Osteoarthritis: A Comprehensive Review

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Background: Knee osteoarthritis (KOA) is a very challenging condition to treat and can be resistant to medications, procedures, and even surgery. Surgery may not be an option for some patients due to obesity or comorbidities. Regenerative medicine utilizing stem cells, platelet rich plasma (PRP), amniotic fluid, and cytokine modulation is very promising in the treatment of KOA.

Objective: This is a review article to evaluate the current evidence about regenerative medicine therapies in the treatment of KOA.

Study Design: A review article.

Setting: A review of literature.

Methods: An online search of PubMed and Cochrane Library databases between January 2006 and December 2016 was performed to search related articles using the keywords of "treatment, stem cell, knee osteoarthritis," limited to the English language. The articles were then screened to make sure only articles fitting our inclusion criteria were included.

Results: Our search obtained a total of 268 articles, but only 18 articles met the inclusion criteria and were included in the current study.

Limitations: There is still limited evidence in literature about the efficacy of regenerative medicine in treating KOA. More large clinical trials are needed to confirm the evidence.

Conclusion: The present investigation demonstrates that regenerative medicine technologies provide good evidence in the treatment of osteoarthritis (OA) of the knee, but greater in-depth study to explore a more ideal way to overcome present difficulties, including standardization of sources of cells, is warranted.

Key words: Knee osteoarthritis, stem cell, treatment, platelet rich plasma, amniotic fluid, articular cartilage defect

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Osteoarthritis (OA) is a chronic joint disease characterized by articular cartilage degeneration and secondary osteogenesis. The earliest pathological changes occur in articular cartilage (1). It is usually seen in larger, weight-bearing joints, including the knee, hip, spine, and distal interphalangeal joints. Knee osteoarthritis (KOA) is the most common form of arthritis, which causes pain, stiffness, decreased function, and is one of the leading causes of disability among noninstitutionalized adults (2,3).

At present, KOA lacks a clear etiology. It has been suggested that it may be related to age, obesity, mechanical damage, joint trauma, and other factors. Symptoms

include knee pain, stiffness, swelling, and joint weakness (4). Some researchers have suggested that the pathology of KOA is related to cartilage degenerative lesions secondary to inflammation related to hyperplasia and chondrocyte apoptosis (5,6). The prevalence in women is significantly higher than that of men. With increase in age, the number of subchondral blood vessels decreases leading to cartilage-related physiological and biochemical abnormalities. In addition, chondrocytes cannot be synthesized with a long chain structure of hyaluronic acid and polyglucose, resulting in articular cartilage local softening, loss of elasticity, wear, and structural damage. This pathological process continues to cause secondary joint fibrosis, which leads to joint stiffness. Patients with KOA suffer physical debilitation, psychological damage, and poor quality of life (7,8).

The purpose of KOA treatment, therefore, is to reduce or eliminate pain, correct deformity, improve or restore joint function, and improve quality of life. Currently, there are a multitude of treatments utilized in clinical practice to treat KOA. Unfortunately, conventional treatments demonstrate only modest clinical benefits, and articular replacement by prosthesis is recommended only as a last treatment option (9,10). Furthermore, these treatments are generally intended to decrease pain, to maintain or to improve joint function, and to minimize disability. The treatments are not intended to regenerate articular cartilage. OA is characterized by degeneration of extracellular matrix, resulting in articular cartilage defect (11,12).

According to leaders in the field worldwide, regenerative medicine provides for novel medical treatments which can potentially result in repair, replacement, restoration, and regeneration of injured or diseased cells or tissues. Some tools of regenerative therapy include stem cells, platelet rich plasma (PRP), and amniotic fluid.

At present, with the widespread application of bioengineering technology, more and more researchers are exploring the feasibility of newer technical methods targeting discs, joints, ligaments, and cosmetic applications. These therapies extend to the field of OA treatment, and many of these strategies are based on complex basic science research and clinical trials. Many of these studies attempt to repair articular cartilage through regenerative therapies, and thus, provide a pathway for restoring joint structure and function. The present investigation, therefore, aims to review studies utilizing regenerative medicine therapies in KOA treatment.

Methods

A computer-based, online search of PubMed and Cochrane Library databases between January 2006 and December 2016 was performed to search related articles with the keywords of "treatment, stem cell, knee osteoarthritis" in the English language. These articles were then critically reviewed and analyzed in the present investigation.

Results

The results of our study yielded a total of 268 articles related to the use of stem cells in treating KOA. Two-hundred forty-nine publications were excluded because they were case studies, reviews, or mechanistic studies. Our goal was to include only clinical trials in our review, and 18 controlled studies were identified, which were utilized in our investigation and are further detailed in Table 1.

Articular Cartilage Injury Mechanism in OA

Most forms of OA can be divided into 2 categories according to their main pathogenesis, e.g., primary OA and secondary OA (13). Primary OA is often spontaneous and typically presents in the form of erosive inflammation of a specific joint or multiple joints. This joint pathological state may be the result of joint biomechanical abnormalities or other hidden systemic genetic changes. In addition, the mechanical pressure within the joint may reduce the maintenance capacity of the joint tissue structure and increase the range of damage.

Secondary OA is caused by a variety of factors that affect the distribution and intensity of specific joint loads. The final pathology of both primary and secondary OA is articular cartilage injury (14,15). Normal articular cartilage is light blue and white, translucent, smooth, and shiny. Cartilage matrix and chondrocytes are the main components of a joint. Cartilage matrix is composed of proteoglycans and collagen. Collagen accounts for 50%, mostly type II collagen fibers, extending from the subchondral bone plate upward and oblique upward to the cartilage surface.

The different directions of fiber composition lead to a "mesh arch structure." Chondrocytes are arranged in the direction of collagen fibers; the cartilage surface of the collagen fibers is parallel to the articular cartilage in a tangent direction to form a line fiber membrane. Chondrocytes are related to the synthesis and decomposition of the matrix, which is the active ingredient in cartilage composition. The composition and arrange-

Table 1. *Controlled trials utilizing regenerative medicine techniques in OA of the knee.*

Study	Method	Patient Population	Intervention(s)	Outcome(s)/Result(s)	Conclusion(s)
Randsborg et al (70)	Prospective randomized controlled study	82	Compare ACI with AD and physiotherapy for the treatment of cartilage defects in the knee.	The primary outcome measure will be the difference in the KOOS knee-related quality of life subscore in the ACI group compared to the AD group at 2 yrs.	This is the first study with a high level of evidence to compare ACI with simple debridement and physiotherapy for the treatment of isolated symptomatic full thickness lesions of the knee.
Freitag et al (81)	Pilot single-center randomized controlled trial	40	Arthroscopic MFX versus arthroscopic MFX combined with postoperative MSC injections.	Primary outcome measures will include MRI assessment of cartilage volume and defects and the OOS.	The trial is in progress.
Koh et al (82)	Unblinded prospective controlled trial	80	Compare ADSCs with fibrin glue and MFX versus MFX alone for the treatment of cartilage defects in the knee.	Significantly better signal intensity was observed for the repair tissue in ADSCs with fibrin glue and MFX.	Compared with MFX alone, MFX and ADSCs with fibrin glue provided radiologic and KOOS pain and symptom subscore improvements.
Vega et al (74)	Double-blinded controlled trial	30	Allogeneic BM-MSC by intraarticular injection versus intraarticular HA.	The MSC-treated patients displayed significant improvement in functional indices versus the active controls treated with HA. Cartilage quality improvements were noted in the MSC-treated patients.	Allogeneic MSC therapy may be a valid alternative for the treatment of chronic KOA that is simple, provides pain relief, and significantly improves cartilage quality.
Akgun et al (69)	Prospective single-blinded controlled trial	14	m-AMI versus m-ACI for treatment of KOA.	The m-AMI group demonstrated significantly better functional outcomes, subjective sub-scale scores for pain, symptoms, activities of daily living and sport and recreation of the KOOS than did the m-ACI group.	For the treatment of isolated full-thickness chondral lesion of the knee, m-AMI can be used effectively and may potentially accelerate recovery.
Wong et al (71)	Prospective unblinded controlled trial	56	Intraarticular cultured autologous BM-MSCs injections in conjunction with MFX and medial opening-wedge HTO.	The effect of treatment showed an added improvement for IKDC scores, Lysholm scores, and Tegner scores. MRI scans performed one yr after surgical intervention showed significantly better MOCART scores for the cell-recipient group.	Intraarticular injection of cultured MSCs is effective in improving both short-term clinical and MOCART outcomes in patients undergoing HTO and MFX for varus knees with cartilage defects.
Koh et al (68)	Unblinded controlled trial	50	Stem cell with PRP injections combined with AD for treating KOA.	Mean Lysholm, Tegner activity scale, and VAS scores of patients in the study group improved significantly by the last follow-up visit.	Short-term results of our study demonstrate that infrapatellar fat pad-derived MSC therapy with intraarticular injections is safe and provides assistance in reducing pain and improving function in patients with KOA.
Aghdami et al (72)	Double-blinded controlled trial	46	Intraarticular injection of BM-MSC.	The BM-MSC treated group had significant clinical improvement as compared to the placebo group in all clinical end-points.	Repeated intraarticular injection of BM-MSC is safe and effective in reducing functional impairment and relieving pain in patients with moderate to severe OA of the knee.

Table 1 (cont.). *Controlled trials utilizing regenerative medicine techniques in OA of the knee.*

Study	Method	Patient Population	Intervention(s)	Outcome(s)/Result(s)	Conclusion(s)
Pham et al (80)	Unblinded controlled trial	60	Endocscopic surgery versus endoscopic surgery with ADSC injection.	All patients in the treated groups significantly reduced pain, reduced the WOMAC score, and clearly increased the Lyshom scores and VAS scores compared to the control group after 18 mos.	Injection of autologous mixture of stem cell-enriched stromal efficiently improved the OA after 6 mos.
Gupta et al (75)	Double-blinded controlled trial	60	The in vitro differentiation potential of adult human bone marrow-derived, cultured, pooled, allogeneic MSCs (Stempeucel, Stempeutics, Malaysia) injected into knee joint with different doses of cells (25, 50, 75, or 150 million cells) or placebo.	Intraarticular administration of Stempeucel was safe, and a trend towards improvement was seen in the 25-million-cell dose group in all subjective parameters Adverse events were predominant in the higher dose groups.	Intraarticular administration of Stempeucel is safe. A 25-million-cell dose may be the most effective among the doses tested for pain reduction.
Bhattacharyaet al (84)	Double-blinded controlled trial	52	Treated with freshly collected amniotic fluid (10 mL), as a source of cell therapy, compared with 40 mg triamcinolone.	The results demonstrated a significant improvement in VAS at the third month, which was sustained at the sixth month interval assessment in both groups, but more so in the cell therapy group. Again, a better and more positive improvement trend was noted in assessments of WD.	The result of the therapy strongly supports the potential of this new form of cell therapy in case of advanced OA. The present treatment proved to be much superior to and longer lasting than the conventional widely practiced therapy.
Vangsness et al (76)	Double-blinded controlled trial	55	Allogeneic MSCs via intraarticular injection to the knee following partial medial meniscectomy.	There was significantly increased meniscal volume determined by quantitative MRI in 24% of patients in group A and 6% in group B at 12 mos post-meniscectomy. Patients experienced a significant reduction in pain compared to control.	There was evidence of meniscus regeneration and improvement in knee pain following treatment with allogeneic human MSCs.
March et al (77)	Double-blinded controlled trial	40	Injection of autologous adipocyte-derived MSCs after arthroscopy for relief of pain in patients with KOA.	Both treatment and placebo groups experienced a significant decrease in ICOAP total pain score from baseline.	The treatment process was well-tolerated and there were no major medium term safety concerns. The effect on symptom modification at 6 mos was similar in both groups.
Lamo-Espinosa et al (73)	Multicenter unblinded controlled trial	30	BM-MSCs in combination with HA injection for treatment of KOA.	No adverse effects were reported after BM-MSCs administration or during follow-up, BM-MSC administered patients improved according to the VAS during all follow-up evaluations and median value for control.	The single intraarticular injection of in vitro expanded autologous BM-MSCs in combination with HA is a safe and feasible procedure that results in a clinical and functional improvement of KOA, especially when 100 x 10⁶ cells are administered.

Table 1 (cont.). *Controlled trials utilizing regenerative medicine techniques in OA of the knee.*

Study	Method	Patient Population	Intervention(s)	Outcome(s)/Result(s)	Conclusion(s)
Lu et al (79)	Double-blinded controlled trial	18	Intraarticular injection of autologous haMSCs in OA patients.	Injection of haMSCs was associated with significant reduction of NRS-11, WOMAC, the MOS item short form health survey SF-36 score, increased thickness of articular cartilage, and reduction of edema.	Intraarticular injection of haMSCs is effective for reducing pain, improving knee function, and cartilage regeneration in patients with KOA.
Koh et al (66)	Single-blinded controlled trial	44	HTO with PRP injection. HTO in conjunction with MSC therapy and PRP injection.	The patients in the MSC-PRP group showed significantly greater improvements in the KOOS subscales for pain and symptoms. The MSC-PRP group showed a significantly greater improvement in the VAS pain score.	MSC therapy, in conjunction with HTO, mildly improved cartilage healing and showed good clinical results in some KOOS subscores and the VAS pain score, compared with PRP only.
Varma et al (83)	Double-blinded controlled trial	50	AD buffy coat (MSC concentrate) injection in combination with the AD.	The results suggest that the technique used in the study considerably improved the overall OA outcome score, especially the quality of life within the studied follow-up period and at the end of the follow-up.	Buffy coat (MSC concentrate) injection along with the AD of the knee is safe and results in a clinical and functional improvement.
Saw et al (86)	Unblinded controlled trial	50	Intraarticular injections of HA with and without PBSC.	The total ICRS II histologic scores for the control group averaged 957, and they averaged 1,066 for the intervention group. On evaluation of the MRI morphologic scores, the control group averaged 8.5 and the intervention group averaged 9.9.	After arthroscopic subchondral drilling into grade 3 and 4 chondral lesions, postoperative intraarticular injections of autologous PBSC in combination with HA resulted in an improvement of the quality of articular cartilage repair over the same treatment without PBSC.

ment of the cartilage matrix determines biomechanical properties of the cartilage that possesses high viscosity of the viscoelastic biomass with good stress adaptability.

The cartilage deformation and arched fiber structure bear the direction of collagen fibers in the direction of conduction and dispersion to the subchondral bone when the cartilage bears normal weight. The pressure disappears and the fiber returns to its original state after unloading. However, in load conduction disorders, the cartilage matrix arch structure will be destroyed and chondrocytes will be damaged. In addition, when the articular cartilage load increases, this results in increased intra-articular pressure, which in turn affects the secretion of synovial fluid and decreases nutrition to chondrocytes, leading to dehydration, condensation, fragmentation, and necrosis.

OA can result from cartilage focal injuries, and these injuries are gradually extended to involve a particular interphalangeal chamber, which causes changes in other joint surfaces (16-18). The fracture is initially parallel to the articular surface and then penetrates the damaged articular cartilage vertically and finally reaches the subchondral bone. Early cell proliferation has been observed around the cracks, but is confined to the superficial region. Over time, the thickness of cartilage gradually decreases (19-20). Therefore, the key to treating OA is to prevent the development of this process and to repair damaged chondrocytes.

Current Status of Cell Therapy Research

Articular cartilage tissue itself can be regenerated to a certain extent, including chondrocytes, cartilage matrix, and elastic fibers; however, the regeneration

process is slow and difficult to bear the heavy pressure placed on the joint. In fact, mesenchymal stem cells (MSCs) in the synovial fluid have cartilage-regenerating potential, but their differentiated cartilage tissue is very fragile, even if the joint is under minimal pressure. In addition, the number of MSCs with articular cartilage potential in the articular cavity is small, and the process of differentiation into cartilage is slow (21,22).

Some researchers believe that early treatment of OA can be achieved by chondrocyte transplantation and introduction of ectopic MSCs or progenitor cells and other methods to repair damaged cartilage. The principle of autologous cartilage transplantation, as well as autologous chondrocyte transplantation, is to promote cartilage formation by supplementing new chondrocytes in situ.

MSCs can continue to differentiate into chondrocytes. In this regard, Friedenstein et al (23) and Chamberlain et al (24) were the first to use MSCs to treat OA. Pittenger and colleagues (25) have reported that MSCs can be cultured and amplified without loss of pluripotent differentiation potential.

Both in vivo and in vitro, MSCs have cartilage differentiation potential. MSCs are widely distributed in several tissues as bone marrow, periosteum, trabecular bone, fat pad tissue, synovial membrane, skeletal muscle, and deciduous teeth (26). Regardless of the tissue origin of these cells, they have the ability to differentiate into a variety of cell lines, including connective tissue cells such as bone, fat, cartilage, and muscle (27). On the basis of this understanding, scientists have created a growing number of cellular techniques for the treatment of KOA.

Autologous Chondrocytes Implanted

The implementation of autologous chondrocyte implantation (ACI) requires acquisition of cartilage tissue from healthy joints, separation of chondrocytes from them and the expansion of culture, and then injection into the defect of the cartilage tissue. This technology was approved by the Food and Drug Administration in 1997 and at present has developed into a third generation, which is termed matrix-induced ACI.

The acquired autologous chondrocytes are inoculated into a 3-dimensional histocompatibility scaffold system. After amplification, the cells are introduced into joints by means of arthroscopy or open surgery (28). It has been found that the improvement of the articular structure after ACI is better than autologous osteochondral graft mosaic angioplasty (29). There is no significant difference in the effects of these techniques on structural or clinical outcomes in the short term compared with microfractures. However, the effects of ACI in the long term appear to be better (30).

A long-term follow-up study over 10 years has confirmed that ACI has a lower rate of failure and better recovery of joint function compared with autologous osteochondral mosaic (31). Another study involving greater than 20 years of follow-up has also demonstrated that ACI technology is more positive than autologous bone cartilage transplantation, mosaic angioplasty, and microfracture technology in the knee joint and other large joint injury (32).

At present, ACI faces many challenges. Because chondrocytes are obtained by culturing chondrocytes in the same joint low-weight region, additional surgical procedures are required. The number of chondrocytes from healthy cartilage is limited, and with the growth of chondrocyte, the proliferative capacity gradually decreases, so the number of chondrocytes which can be used for transplantation is limited, especially in elderly patients. Cartilage repair and further reconstitution takes a long time after autologous chondrocytes have been implanted (29).

In Vitro Culture of Expanded MSCs for Repair of Articular Cartilage

Bone marrow stem cells, in particular, have been shown to differentiate in the presence of appropriate growth stimuli, along specific pathways for production of cartilage tissue. MSCs have been isolated first from bone marrow and subsequently from a variety of other tissues such as adipose tissue, placenta, umbilical cord and cord blood, dental pulp, and amniotic fluid. However, the ability of MSCs isolated from these tissues to form cartilage is currently being evaluated (33).

MSCs or MSC-like cells are believed to replace cells lost related to aging or tissue injury. MSCs are usually isolated by their plastic adherence and can be expanded in large-scale culture for clinical use (21). Animal experiments have revealed that MSCs cultured after expansion can repair cartilage and subchondral bone and can control the progress of secondary OA. Wakitani et al (34), using rabbit models, have found that MSC injection into the full-thickness injury of femoral condyles resulted in quick formation of a glass-like repair tissue. In comparison, injection with collagen gel that did not contain MSCs formed fibrous tissue. Soler et al (35) developed a series of clinical trials in which autologous MSCs were injected into the body after in vitro

expansion, resulting in regenerative cartilage in the injured area and therefore, concluded that the therapy is safe and viable. Existing studies have revealed that MSCs' cultured and expanded cartilage repair effect is equally effective as other therapies (36). Autologous chondrocytes and MSCs have their own advantages and disadvantages. Overall, at present, adult stem cells, which are typically represented by MSCs, are being favored by the majority of researchers (37).

The Source of MSCs and MSC-Induced Differentiation of Cartilage

MSCs are derived from mesoderm mesenchyme and are widely distributed in the bone marrow, bone membrane, muscle, synovium, synovial fluid, liver, peripheral tissue, umbilical cord blood, fat, placenta, fetal lung, fetal kidney, umbilical cord, and other tissues (38,39). Bone marrow and adipose tissue are the main sources for therapeutic MSCs, with bone marrow being the gold standard source for musculoskeletal tissue-engineering approaches. Many studies have revealed that MSCs from different tissue sources can differentiate to cartilage (40).

When comparing different sources of MSCs, each source has its advantages and disadvantages. Regarding the content of MSCs in tissues, umbilical cord content is undoubtedly the highest, followed by amniotic membrane and fat, with umbilical cord blood content being very small (41). Umbilical cord and amniotic membrane derived MSCs have the highest potential for differentiation. MSCs from umbilical cord, amniotic membrane, and fat sources have a better immune regulation than other sources.

In terms of secreting cytokines, the total amount of secreting cell growth factor in umbilical cord MSCs has been found to be significantly higher than that in bone marrow MSCs (40). MSCs have the potential of multi-directional differentiation, and help with both osteogenesis and chondrogenesis. It is a very complicated process to induce MSCs to differentiate into cartilage and apply for treatment of cartilage, as their differentiation is affected by many factors, including cytokines, hormones, and growth factors.

Transforming Growth Factor-β (TGF-β)

TGF-β is one of the earliest biological activity factors which can induce cartilage generation. It plays an important role in the proliferation and differentiation of chondrocytes. It stimulates expression of type II collagen and proteoglycans in MSCs and promotes chondrocyte proliferation, the ability to regulate MSCs differentiation, and extracellular matrix synthesis. It has also been demonstrated that TGF-β pretreatment of periosteum in adult rabbit injured joints can improve the quality of osteogenesis in osteochondral tissue (42). Zhang et al (43) have also demonstrated that TGF-β can induce chondrogenic differentiation of stem cells, stem cell chondrogenesis, and overgrowth.

Basic Fibroblast Growth Factor (bFGF)

bFGF is a protein isolated from the bovine pituitary and has been found to be widely present in human tissues, including bone and cartilage. It plays an important role in embryonic development and cartilage repair. It has been reported that bFGF combined with TGF-β action on MSCs can induce chondrocytes. bFGF can inhibit the expression of COL2A1 and COL10A1 and the production of alkaline phosphatase, enhancing differentiation to hypertrophic chondrocytes (44). Park and Na (45) have reported that combining bFGF and TGF-in vitro experiments can accelerate formation of chondrocytes and increase chondrocyte yield.

Insulin-Like Growth Factors (IGF)

IGF and TGF-β play a very important role in cartilage tissue engineering (46). Exogenous IGF-1 can promote chondrocyte mitosis and type II collagen and proteoglycan of extracellular matrix synthesis (42). Uebersax et al (47) have planted human MSCs on IGF-1 silk fibroin in stents. Each stent was placed in TGF-β1 containing supplemental medium. Investigators demonstrated chondrogenic differentiation in human MSC stents after 2 weeks. There was no cartilage differentiation in the control group, which did not have IGF-1. In this regard, it has also been found that when IGF-1 has been added into medium alone, human MSC differentiation into chondrocytes was not observed (48). Therefore, in the process of MSC differentiation into chondrocytes, IGF-1 plays a critical role in promoting induction of TGF-β1 and/or other growth factors.

Bone Morphogenetic Proteins (BMPs)

BMPs are related to TGF-β, which can promote the differentiation of MSCs into chondrocytes, especially BMP-7 (49). BMPs interact with certain membrane receptors in the mediation or modulation of the development of bone and cartilage. It has been found that BMP-7 has the role of promoting growth and maturation of high-density monolayer cells cultured in serum-free cells and cells suspended in agarose (50). Kuroda

et al (51) have demonstrated that BMP-4 gene-derived stem cells have more potent cartilage properties than experimental controls to produce stronger cartilage repair. Additionally, they can increase alkaline phosphatase activity of cells and improve the synthesis of messenger ribonucleic acid and type II collagen. BMP-2 and IGF-1 combined or BMP-2 and TGF-β in combination can enhance the ability of BMPs through action on receptors, triggering the Smads pathway-related signal conduction cascade, thereby enhancing the development of bone and cartilage and enhancing MSCs to differentiate into chondrocytes (52,53). BMP-2 combined with TGF-β act through the Wnt signaling pathway, up-regulation of Wnt3a lead to β-catenin protein aggregation, followed by induction of Sox-9 production and cartilage formation (54).

Platelet-Derived Growth Factor (PDGF)

Mishima et al (55) have demonstrated that in chondrocytes and MSCs, directional migration is inducible. PDGF produces the most effective increase in cell migration of cytokines and enhances cartilage repair or tissue engineering tissue fusion.

Dexamethasone (DEX)

DEX is a long-acting glucocorticoid required for the differentiation of human MSCs into chondrocytes in vitro. DEX can induce MSCs to differentiate into cartilage, osteocytes, and adipocytes. Further, DEX plays a very important role in the regulation of stem cell cartilage formation to reduce fibrous cartilage tissue growth and hyperplasia. Regulation of chondrocyte overgrowth involves many mechanisms and pathways, including parathyroid hormone-related protein/Indian hedgehog, Wnt/b-catenin, TGF-b/sma, and mad-related family (SMA) pathway (56).

Stem Cell Implantation Method

Successful stem cell therapy requires the selection of appropriate implantation methods into the joint to maximize therapeutic effect of stem cells. Current approaches in clinical trials utilize direct implantation of MSCs and MSC stent implantation (57).

MSC Stent Implantation

A stent is needed to transplant cells in the microenvironment and to provide a carrier for nutrients. According to their different materials, they can be divided into collagen, fiber, hyaluronic acid, and other types. Kayakabe et al (58) have reported that autologous bone marrow MSCs can be transplanted into the rabbit joint with hyaluronic acid gel sponge as a carrier, which can effectively repair damaged articular cartilage. After 12 weeks of transplantation, similarly repaired articular cartilage was observed around the injured articular cartilage. The authors believe that hyaluronic acid gel sponge can affect cartilage differentiation of MSCs. Wakitani et al (59) reported loading the MSCs into type I collagen hydrogels to repair full-thickness cartilage injury. Guo et al (60) have also utilized autologous MSCs bioceramic β-triphosphate scaffold to treat OA.

Local Injection of MSCs

Local application of MSCs has many advantages, not only to strengthen joint repair, but also to reduce OA-induced degeneration. It is the simplest method for treating OA (61). Murphy et al (62) found that bone marrow MSCs transplanted to treat OA in goats can stimulate meniscus tissue regeneration and reduce damage to the injured area. Centeno et al (63), through monitoring 24 weeks of magnetic resonance imaging (MRI) tracking, found that implantation of autologous MSCs can stimulate cartilage growth and reduce the pain of degenerative joints.

Mixed Injection

Mixed injection enhances efficacy by implanting a scaffold mixed with cytokines or growth factors. Mrugala et al (64) used fibrin gel containing sheep MSCs with or without the addition of chitosan and TGF-β3 to treat OA in joints. The authors reported that the addition of chitosan and TGF-β3 sheep MSCs can produce therapeutic effects. PRP is an autologous tissue of cartilage growth factor rich in TGF-β and platelet-derived growth factor, which can be used as a source of tissue for the treatment of cartilage injury (65). Koh et al (66) have demonstrated that combining MSCs and PRP when injecting into the joint cavity in the treatment of OA achieves significant improvement. Haleem et al (67) have suggested that implantation of a platelet-rich fibrous gel cell scaffold containing autologous bone marrow MSCs in the joint may be a more effective treatment for repairing articular cartilage injury. Seo et al (65) have found that the combined use of double gel/beta-tricalcium phosphate with stem cells, chondrocytes, BMP-2, and PRP in the treatment of malocclavian cartilage injury can stimulate cartilage regeneration. Koh et al (68) conducted another study which administered injections of patients' stem cells prepared with PRP as a novel biological scaffold. These studies have

demonstrated safety, efficacy in reducing pain, and improvement in function in patients with KOA.

Clinical Study of Cell Therapy

With the increasing application of cell technology in animal models and in vitro experiments, the use of cartilage cells and MSCs based on tissue-engineering technology to repair articular cartilage injury and to achieve cartilage regeneration has demonstrated good results in recent years. Cartilage tissue without lymph, blood vessels, nerves, and containing only a cell composition of chondrocytes is ideal for tissue engineering and regeneration repair.

The indication for cell technology in the treatment of cartilage defects is to repair degenerative injury of the articular surface, in order to withstand joint weight and confrontation pressure. Clinical application of ACI technology has been around for greater than 20 years. Akgun et al (69) conducted a single-center, randomized, controlled, single-blind study, and the authors reported that MSC treatment technology can effectively accelerate repair of cartilage defects (69). Randsborg et al (70) have conducted a 2-year randomized controlled trial to compare ACI with simple arthroscopic debridement (AD) and physiotherapy for the treatment of cartilage lesions in the knee. The primary end-point results of the study have demonstrated a significant difference in the Knee Injury and Osteoarthritis Outcome Score (KOOS) knee-related quality of life subscore in the ACI group when compared to the AD group at 2 years. A combination of self-explanatory questionnaires, clinical parameters, clinical tests, radiographs, and MRI are being used as secondary end-points. This clinical trial is ongoing.

Wong et al (71) obtained bone marrow MSCs from patients with knee cartilage injury *in vitro* and then injected into the articular cavity undergoing high tibial osteotomy and microfracture, following-up for 2 years. The cell-recipient group demonstrated significantly better Tegner, Lysholm, and International Knee Documentation Committee scores.

Aghdami et al (72) investigated the effects of intra articular injection of autologous bone marrow derived MSCs (BM-MSC) on the symptoms of moderate to severe KOA. The patients were followed-up for 9 months. The BM-MSC treated group had significant clinical improvement as compared to the placebo group in the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) total score, WOMAC physical function subscore, WOMAC-pain sub score, and pain-free walking distance. Primary radiologic data have indicated that subchondral edema decreased in some patients, and the thickness of cartilage increased in the MSC group. Lamo-Espinosa et al (73) have conducted a phase I/II multicenter randomized clinical trial in which they used patients' autologous bone marrow after culture, then randomly assigned them to either intraarticularly administered hyaluronic acid alone (control) or together with 10 × 106 or 100 × 106 cultured autologous BM-MSCs and followed-up the patients for 12 months. The BM-MSC treatment group achieved good results, especially in the high-dose group, in both visual analog scale and WOMAC scores. In addition, x-rays revealed a reduction of the knee joint space width in the control group, which was not seen in the BM-MSC high-dose group. The MRI results showed that joint damage decreased only in the BM-MSC high-dose group.

Vega et al (74) conducted a study of 30 patients with KOA who did not obtain significant effects through traditional treatment. The patients were randomly divided into 2 groups: intraarticular injection of allogeneic BM-MSCs (each 40 * 106 cells) or hyaluronic acid (single-dose 60 mg), respectively. At the one-year follow-up, the MSC treatment group demonstrated more obvious improvement in symptoms as compared to the hyaluronic acid treatment group. Assessment by MRI found that the cartilage lesion area was significantly reduced and the cartilage quality was improved in a significant manner. They concluded that MSCs are an effective treatment for chronic KOA, can improve clinical symptoms and cartilage quality, and the use of allogeneic MSCs is easier than the use of autologous MSCs.

Gupta et al (75) also conducted a similar study. They tried different doses of cells (25, 50, 75, or 150 million cells) to be injected into the knee joint, followed by 2 mL hyaluronic acid (20 mg). The 25-million-cell dose demonstrated the most effective pain reduction. However, there was no significant change in MRI findings compared with the placebo group. In addition, Vangsness et al (76) found that allogeneic human MSCs can induce meniscus regeneration and relieve pain caused by KOA.

March et al (77) conducted the first randomized controlled trial evaluating the effect of autologous, non-expanded, adipocyte-derived MSCs on reducing pain in human KOA. They demonstrated that patient symptoms significantly improved. In 2014, Jo et al (78) used 3 different doses of autologous adipose-derived MSCs (ADMSCs) to treat early OA; post-injection MRI,

arthroscopy, and tissue examination of high-dose (1.0 × 108) cells group revealed that articular cartilage regeneration was obvious and clinical symptoms were significantly improved. Lu et al (79) performed a similar study and also demonstrated that intraarticular injection of high-dose ADMSCs into the OA knee can improve function and relieve pain.

Some trials have compared clinical and radiologic efficacy of adipose-derived stem cells (ADSCs) and endoscopic surgery and microfractures in the treatment of OA (80-83). The ADSCs group has achieved good results. Bhattacharya et al (84) conducted a study which confirmed amniotic fluid is a cocktail of MSCs with antibacterial properties, which may be utilized as a cell-therapy source for repair of damaged cartilage in the setting of OA.

Sekiya et al (85) used patient's synovium-derived stem cells to treat OA and achieved significant therapeutic effects. Saw et al (86) demonstrated after arthroscopic subchondral drilling into grade 3 and 4 chondral lesions, postoperative intraarticular injections of autologous peripheral blood stem cells in combination with hyaluronic acid resulted in an improvement of the quality of articular cartilage repair.

Tucker and colleagues (87) indicated that future research in cellular therapies should focus on what they called an "outcome triad" which includes: a) molecular and cellular responses both intraarticularly and systemically, b) clinical outcome (pain and function), c) structural outcome.

Contamination of Cell Lines

It is very important to maintain strict sterility when dealing with cells until they are used for therapeutic purpose. Contamination of cell lines has been reported before. If contaminated cells are used for therapy, this may result in immune reaction and/or rejection of cells (88).

Conclusion

Regenerative medicine therapies, including stem cells, PRP, and amniotic fluid cells, have demonstrated good evidence in the treatment of OA of the knee. At present, a large number of basic and clinical science-focused studies have been completed to help clarify the critical regenerative medicine components and appropriate methodological considerations needed in the mediation or modulation of significant KOA repair. There are several approaches and cell lines used for treating KOA; well-designed randomized controlled trials are needed to evaluate the most effective approach.

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