REVIEW ARTICLE

Regenerative Medicine Therapies for Osteoarthritis and Cartilage

Majid Alhomrani

Department of Clinical Laboratories Science, The Faculty of Applied Medical Sciences, Taif University, Taif, Saudi Arabia

Abstract

Osteoarthritis (OA) has a high burden and impact on society as it affects the quality of life of both young and older patients. OA is a degenerative joint disease characterized by the degeneration of articular cartilage. This cartilage is an avascular, unique matrix composed of chondrocyte cells, which can resist compression and redistribute loads but have poor self-regenerative capacity. Numerous types of treatment are available, such as non-pharmacology treatments involving diet, physiotherapy, exercise, and pharmacological which include different types of drugs. None of these two types has proven to be the ideal treatment, only symptomatic treatment. Total knee replacement is the final and only treatment available and used only when the other types of treatment fails. The intra-articular injection is an alternative treatment for OA, due to the localized nature of the disease. Various types of blood products are currently used, including platelet-rich plasma and orthokine to alert the inflammation response and enhance the healing process. Recently, regenerative treatments have widely been introduced to overcome the limitations of current treatments. Mesenchymal Stromal Cells (MSCs), which can differentiate into chondrocytes, are used to regenerate articular cartilage. In addition, the improvements in tissue engineering technology such as the use of different types of the scaffold as well as blood product and growth factors with MSC have had a great impact in treating OA and regenerating cartilage. This review will discuss the pathogenesis of OA and describes the current clinical management to treat the OA.

Keywords: Blood component therapy, Cartilage, Cell therapy, Growth factors, Mesenchymal stromal cell, Osteoarthritis, Synovitis

INTRODUCTION

Osteoarthritis (OA) is a commonly occurring form of arthritis affecting diarthrodial joints, but most common involved the knee; hip and hand, foot and spinal joints and can cause severe long-term pain, reduced functionality, decreased quality of life and lower life satisfaction.[1] It is the most prevalent cause of mobility, disability and chronic musculoskeletal pain in the ageing population affecting millions of people worldwide and causing the World Health Organization to designate the year 2000–2010 the bone and joint decade.[2,3] Common OA risk factors include previous joint surgery, joint injury, obesity, occupational bending, and lifting injuries.[4]

OA has been considered a wear and tear non-inflammatory disease leading to the loss of articular cartilage.[5] While this condition is considered non-inflammatory, there is still strong evidence supporting the presence of inflammation in the synovium of OA patients leading to synovitis.[4,6] In addition, OA is mainly characterized by loss of articular cartilage but also involves the entire joint structure change including the synovial membrane, ligaments, subchondral bone, and calcified cartilage.[7,8]

Pathological changes occurring with OA include the destruction of cartilage, which is observed at the articular surface in the form of fibrillation. There is also a hypertrophic reaction (sclerosis) in subchondral bone, subchondral bone cysts, newborn formation (osteophyte) at joint margins, synovial membrane alterations, increased synovial fluid volume with decreased viscosity, and degeneration of ligaments. Along with these changes, the knee joint may suffer from menisci destruction [Figure 1].[9]

Address for correspondence:
Dr. Majid Alhomrani, Department of Clinical Laboratories Science, The Faculty of Applied Medical Sciences, Taif University, Taif, Saudi Arabia.
E-mail: sasdaq@gmail.com

Received: 22-04-2022
Revised: 18-05-2022
Accepted: 25-05-2022

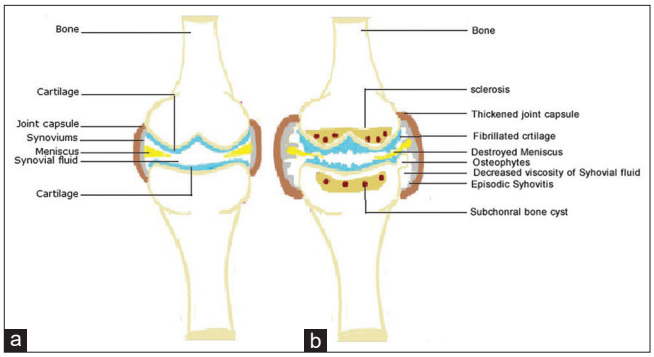

Figure 1: Comparison between healthy (a) and osteoarthritis (b) joint

The articular cartilage is a unique and unusual network composed of a single cell type named chondrocytes, and extracellular matrix (ECM) consist of proteoglycans (aggrecan), collagen type II, and other components which is mainly synthesized by chondrocytes.[10-12] The cartilage usually has difficulty in healing or reproducing spontaneously after degeneration or damage. This difficulty is because the cartilage has a unique complex structure resulting from interactions between cells, fluid, framework, and aggrecan and avascular surroundings.[13,14] In this review, different aspects of OA therapies were summarized that are available and provide an overview of the regenerative medicine that is currently used.

OA treatment strategies

The current treatment options for OA are aimed to improve health-related quality of life, reduce joint pain, physical disability and handicap, improve and maintain joint mobility, limit the progression of the disorder, and finally educate the patient.[15]

Non-pharmacological treatment is an important option used for OA. Information and education should be given to all OA patients to unload the damaged joint as well as reduce pain. The patient may benefit from lifestyle changes, weight reduction, and exercises such as aerobics, range of motion exercises, aquatic exercises, walking, and muscle strengthening. It is also important to advise patients about their footwear and the shoe and insoles to benefit the patient. Physical therapy can also be a benefit to patients.[15,16]

Pharmacological therapies such as the use of acetaminophen (paracetamol) an analgesic that inhibits the cyclooxygenase (COX) enzyme.[15] Nonsteroidal anti-inflammatory drugs (NSAIDs), commonly administered to patients who show no benefit with paracetamol, are a method of inhibiting COXs (both COX-1 and COX-2 isoenzymes).[16,17] Both paracetamol and NSAIDs are used to reduce the symptoms of OA without having any effects on cartilage.[16]

Concomitantly, during the early stage of OA, glucosamine and/or chondroitin sulfate can be used to slow the process of cartilage degeneration or to reverse it. However, evidence is lacking for their therapeutic effects and this has caused the Osteoarthritis Research Society International to recommend discontinuation of these treatments if no response appears in 6 months.[15,18,19] On the other hand, The National Institute for Health and Clinical Excellence (NICE) in the UK does not recommend the use of glucosamine or chondroitin products for the treatment of OA.[20]

Interestingly, the use of injected drugs is increasing due to the monoarticular or oligoarticular nature of OA. These types of treatment avoid the risk of untoward side effects and several types of injected drugs are used. Corticosteroids are anti-inflammatory agents that interrupt the inflammatory and immune cascade at several levels.[21] These agents should be considered when the patient does not respond to oral anti-inflammatory drugs and has severe pain.[15]

Hyaluronic acid (HA) is a natural component of cartilage and is essential for joint lubrication, shock absorbency and the formation of the ECM.[22] Viscosupplementation is a procedure that involves the injection of HA into the joint space, to restore the viscosity and elasticity of the synovial fluid.[22] Viscosupplementation can improve the patient's condition and has long-term benefits, especially in moderate-grade knee OA patients.[21,22] However, the NICE does not recommend the use of intra-articular HA injections for the treatment of OA.[20]

If patients are not benefitting from the use of these pharmacological agents, then the use of weak opioids and narcotic analgesics should be considered. For the management of severe pain, the use of strong opioids can be considered, and non-pharmacological therapies in such patients should be continued.[15]

When combinations of non-pharmacological and pharmacological therapies do not reduce pain, do not improve functionality and quality of life, and the disease has reached the end stage, then joint replacement surgery should be considered. This is the only treatment that is considered a curative for OA. The surgical treatment for OA is excision and replacement of the entire joint, commonly referred to as a total joint replacement. However, in some patients, the replacement may be partial. In young and physically active patients with symptomatic hip OA, osteotomy and joint preserving surgery should be considered, while a high tibial osteotomy for a young OA patient's knee could delay the need for knee replacement.[15,20,23] It is obvious that the current types of treatments are symptomatic relief for a short period without an improvement in the condition and the only treatment is the total joints replacement. These drawbacks of treatment options increase the demand for the development of new therapeutic options.

It has been discovered an important role for Rac1 in OA development. Hence, the inhibition activity of Rac1 by

controlled release of Rac1 inhibitor therapy may consider a good OA treatment strategy.[24]

BLOOD COMPONENTS-BASED THERAPY

In OA patients, both chondrocytes and synovial cells produce high levels of pro-inflammatory cytokine interleukin-1 (IL-1) and tumor necrosis factor α (TNFα) which destroy articular cartilage through reduced collagen synthesis and increased catabolic activity. IL-1 has two isoforms, α and β, β is the major isoform produced in human tissue. IL-1 can activate the cell through two receptors: cell-surface receptors type I (IL-1RI) and type II (IL-1RII). Chondrocytes and synovial fibroblasts are very sensitive to IL-1 due to the high number of IL-1RI on them. In addition, IL-1β and TNFα cause chondrocytes to be active and secrete matrix metalloproteinases (MMPs) which can destroy cartilage. Furthermore, a high number of anti-inflammatory cytokines are found in high levels in OA synovial fluid such as upregulation of IL-I receptor antagonists (IL-1Ra) which inhibit the IL-1R and have the ability to block several catabolic pathways involved in OA but the IL-1Ra cannot compete with the high level of IL-1β.[7,25,26] Hence, there is a great interest in using IL-1Ra as a therapeutic option as this treatment aims to inhibit IL-1 action. Since the early 1980s, there were several trials through different methods[27-35] leading to the development of Autologous Conditioned Serum (ACS) marketed as Orthokine which is a syringe, that contains ACS rich with anti-inflammatory factors produced by a physicochemical treatment of the whole blood.[36] Orthokine causes an increase in many anti-inflammatory agents such as IL-1Ra, which increased 140-fold, IL-4, and IL-10 with no increase in pro-inflammatory cytokines.[36,37] However, other studies have shown an increase in pro-inflammatory cytokines such as IL-1β and TNFα.[37] The injection of Orthokine has excellent benefits on the patient including safety profile, reducing pain, and increasing their functionality.[38] The mechanisms of ACS are still not completely understood, and more research needs to be done to determine its effects whether on symptoms or regeneration of cartilage.

Platelet-rich plasma (PRP) is another blood product. Platelets are non-nucleated small bodies in the blood that contain cytokines, bioactive factors, and proteins and play a major role in hemostasis. Plasma is the liquid component of blood and contains proteins, ions, and clotting factors.[39] Autologous PRP therapy has been attracting worldwide attention because it is simple to isolate and prepare, inexpensive, lacks an immune reaction or disease transmission. Natural concentrations of autologous growth factors (GFs) are obtained by minimally invasive methods and the platelets have a physiological role in the natural healing process.[40,41]

This therapy has been widely experimented with in the field of medicine including wound healing, plastic surgery, cartilage degeneration, and tendon injuries with the hope of enhancing the healing process by increased differentiation, recruitment, and proliferation of cells involved in tissue regeneration.[39,42] This enhancement results from the high fold reservoir of important GFs, cytokines, and other bioactive molecules associated with PRP and platelets α and dense granules [Table 1].[43-46] Furthermore, GFs are released by platelet activation. They include insulin-like growth factor (IGF), transforming growth factor β (TGF-β), platelet-derived growth factor, vascular endothelial growth factor, fibroblast growth factor (FGF), hepatocyte growth factor, platelet factor 4, and epithelial growth factor. These may play a major role in cellular processes including chemotaxis, cell proliferation and differentiation, mitogenesis, angiogenesis, and metabolism.[39,43]

Preparation of PRP begins with the collection of autologous whole blood from the patient with the addition of an anticoagulant to prevent the activation of the coagulation cascade and clot formation. The citrate anticoagulant is usually used. It is available in various forms including calcium citrate, acid citrate dextrose, and sodium citrate. These bind to the calcium and prevent the clot from occurring. This is followed by the centrifugation steps, which distribute, isolate, and concentrate various blood components. Two different preparation systems exist; they differ based on centrifugation spin parameters. The first is a buffy-coat-based system that requires high and long spins to isolate platelets' poor plasma and a buffy coat, which has both white and red blood cells. The second is the plasma-based system which requires slow and short spins to isolate the platelets and plasma only without other blood cells.[39,47-49]

Then the PRP is activated to allow the α-granules to release the GF, in a process known as degranulation. Platelets can be activated endogenously through the collagen tissue or exogenously by thrombin or calcium chloride. Activation causes the release of stored GFs. Approximately 70–90% are released in the first 10 min and the remaining is released in the first hour. However, many human protocols activate PRP endogenously.[49] DeLong *et al*.[48] proposed a classification system to distinguish between PRP products based on three categories: The absolute number of platelets, platelets activation, and WBC counts.

Several clinical trials evaluate the benefits of using PRP as an alternative treatment for OA. Napolitano *et al*. treated 27 patients between 18 and 81 years of age and divided them into two groups: A knee arthritis group and a degenerative knee cartilage disease group. Both groups received 3 weekly interval injections of PRP. The assessments parameters Numerical Rating Scale and Western Ontario and McMaster University Osteoarthritis Index (WOMAC) showed dramatic improvement in both groups. The highest improvement was in the 6th month of the assessment.[50] Kon *et al*. reported that 91 patients diagnosed with chronic degenerative knee and different grades of OA were treated with three PRP injections. The clinical assessment was based on International Knee Documentation Committee (IKDC) and visual analog scale EQ (VAS) score at 6 and 12 months follow-up. The greatest score was at the 6-month

Table 1: Different types of molecules found in platelets

	Molecule	Biological activity
α-Granules		
Growth factor	IGF	Cell maturation, proliferation and bone matrix synthesis
	TGF-β	Promotes matrix synthesis
	PDGF	Cell proliferation, Chemoattraction
	ECGF	Endothelial cell proliferation and angiogenesis
	FGF	Fibroblast proliferation mediates angiogenesis
	VEGF	Angiogenesis
	EGF	Cell proliferation
Fibrinolytic factors	Plasminogen	Plasmin production
	α-2antiplasmin	Inactivation of plasmin
	Plasminogen activator inhibitor	Regulation of Plasmin production
Basic protein	Endostatins	Inhibit endothelial cell migration and angiogenesis
	Platelet factor 4	Inhibits angiogenesis
	β-Thromboglobulin	Platelets activation and inhibits angiogenesis
Adhesive proteins	Thrombospondin-I	Inhibits angiogenesis
	Fibronectin	Bind to the cell surface
	Vitronectin	Cell adhesion, chemotaxis
	Fibrinogen	Format fibrin during clotting cascade
Proteases and antiproteases	MMP-4	Matrix degradation
	α Antitrypsin	Inhibit proteases and enzymes
	TIMP-4	Regulation MMP and matrix degeneration
Dense Granules		
	Histamine	Attracts and activates macrophages, pro-and anti-inflammatory Effects
	ATP	Participates in platelet response to collagen
	ADP	Promotes platelet aggregation
	Catecholamines	Hormones released by the adrenal gland in response to stress
	Ca++	Platelet aggregation and fibrin formation
	Dopamine	Regulate blood pressure and heart rate, Neurotransmitter
	Serotonin	Increased capillary permeability, macrophage attraction and Vasoconstriction

IGF: Insulin-like growth factor, TGF-β: Transforming growth factor β, PDGF: Platelet-derived growth factor, VEDF: Vascular endothelial growth factor, FGF: Fibroblast growth factor, EGF: Epithelial growth factor, ECgF: Endothelial cell growth factor, MMP-4: Metalloproteinase 4, TIMP-4: Metalloproteinase inhibitor 4, ATP: Adenosine triphosphate, ADP: Adenosine diphosphate, Ca: Calcium

follow up and the score started to decrease following the 6-month assessment and at the 12-month follow-up, the score was worse but still higher than basal levels.[51] At the 24-month follow-up, the results had continuously decreased and showed a worse score compared to the 12-month follow-up but were still higher than basal levels and the greatest results were obtained from younger patients with low-grade cartilage damage. This study concluded that the PRP showed an effective result in increasing the function and quality of life and reducing pain in the short term.[44] These studies did not have a control group for comparison of results and improvement. An interesting comparison study had 150 patients between the age of 26–81 years divided into three groups. The first group received three PRPs, the second received high molecular weight HA and the third received low molecular weight HA. The clinical evaluation was based on IKDA and EQ-VAS scores at 2- and

6-month follow-ups. The results showed similar improvements in both PRP and low molecular weight HA but at the 6-month follow-up, only an improvement was found in the PRP group. This indicates that PRP has a lasting improvement in the quality of life and decreases symptoms and pain compared to HA. In addition, the best results were among active younger patients while the worst were among the older patients.[40] Patel *et al*. reported 78 patients with bilateral OA who were divided into three groups. The first group received a single WMC filtrated PRP injection, the second group received two injections and the last group received a single normal saline injection. The clinical evaluation was based on the WOMAC score and showed no improvement in the normal saline group compared to dramatic improvements in the other two groups at 6-week, 3-month, and 6-month follow-up examinations. The best results were obtained again from younger patients with low-grade cartilage degeneration.[52] Filardo *et al.* compared two types of PRP in terms of safety and efficacy using 144 patients suffering from cartilage degeneration lesions and OA and divided them into two groups. The first group received three injections of a single-spin plasma rich in growth factors (PRGF) while the other group received three injections of a double-spin PRP. The outcome evaluation was based on EQ-VAS, IKDC and Tegner scores, which showed high clinical improvements in both groups, and again. The best results were among younger patients with low-grade cartilage degenerative lesions. PRP injections also showed increased swelling and pain reactions compared to PRGF.[53]

Despite the improvements in patients treated with PRP, reducing pain and increasing the function of life, the mechanisms of PRP are still not clear. Could PRP act as an anti-inflammatory mediator or downregulate cytokines or is its effect on synovial fluid or chondrocytes? Further research needs to address the PRP mechanism.

Different methods of centrifugation, activation, the concentration of platelets, preparation, and presence or absence of other blood cells lead to an increased demand for the optimal formulation of PRP with high benefits in cartilage degeneration and OA. More research needs to identify the optimal number of injections the patient needs to the disease situation, the patient's age, activity, and sex.

CELL-BASED THERAPY

The main characterization of OA is the degeneration of articular cartilage, which is a connective tissue consisting of a single type of cell named chondrocytes; their major function is to allow the skeletal structure to have load distribution and shock absorption.[12] The self-repairing ability of articular cartilage is restricted due to low cell metabolism and a vascularity which will reduce the ability of healing.[12] Different surgical repair techniques have been used that aim to increase the self-healing process, such as abrasion arthroplasty, microfracture, drilling, and Autologous Chondrocyte Implantation (ACI); however, these techniques have their limitations, which include cell to tissue availability and the formation of unwanted fibrocartilage.[54] To the best of our knowledge, there are no therapeutic options that can slow the progress of OA and cure it, except treatment to reduce pain and surgical options such as total joint replacement, which has a high percentage for failure and is not satisfying for younger patient.[55] However, in recent years, stem cells have raised hope as an alternative source of therapy for regeneration and tissue repair, due to the easy process of preparation, delivery, and large availability.[56]

CHONDROGENIC DIFFERENTIATION OF MSCS

MSCs have the potential to differentiate into chondrocytes. MSCs are fibroblast-like morphologies that change into large, round, shapes during chondrogenic differentiation. The differentiation of MSCs to chondrocytes requires a pellet culture system that culture MSCs as aggregates, first described by Johnstone *et al*. in 1998. This culture system allows the cell to cell interactions and the synthesis of ECMs which is the main characteristic of cartilage, which contains a network of highly organized collagen, mainly type 2 collagen (col2), proteoglycans, and glycosaminoglycans that can be detected by Alcian blue.[57-59] During the chondrogenesis steps, various transcription factors are involved. The early and necessary transcription factor is protein SRY-related high-mobility group box9 (Sox9), which is a member of the Sox family and controls the expression of the genes aggrecan, col2, col9, col10, col11, and the cartilage link protein. However, the mechanism by which Sox9 regulates cartilage-specific transcription is still not completely understood.[60] Another important transcription factor in chondrogenesis is BapxI, which induces chondrogenic differentiation in sclerotome by mediating Sonic Hedgehog (Shh) signaling that target Pax I and Pax 9 which, in turn, activate BapxI in sclerotome.[61,62] Furthermore, the transcription factors of the Twist subfamily act as repressors or transcriptional enhancers and include Twist, DermoI, Paraxis, HAND2, and Scleraxis. Scleraxis expresses itself during embryogenesis in developing chondrogenic cell lineages and can transactivate the expression of aggrecan. In the region of the somites, the expression of Paraxis precedes the Scleraxis to form the axial skeleton and tendons.[63] In addition to the close cell to cell contact achieved by micromass or pellet cultures, the *in vitro* chondrogenic differentiation of MSCs require the addition of chondrogenic bioactive factors such as TGF-β, bone morphogenetic protein (BMP), dexamethasone, ascorbic acid, IGF, and FGF which enhances chondrogenic differentiation.

The TGF-β family members TGF-β1, TGF-β2, and TGF-β3 are multifunctional peptides that play important roles in maintaining and including in-vitro chondrogenic differentiation of MSCs. Both TGF-β and dexamethasone represent essential factors for the differentiation of MSC into chondrocytes. TGF-β1 stimulates the chondrogenesis process in MSCs through

the transition from an initial N-cadherin-contributing state to a subsequent fibronectin-contributing stage.[64] During the adult and embryonic growth and development period, TGF-β2 participates to enhance in-vitro proliferation and redifferentiation of chondrocytes.[65] There are different opinions regarding the specific TGF-β subtypes used in chondrogenesis differentiation of MSCs; some state that any subtypes of the TGF-β can activate chondrogenic factors equally and the difference seems to be in the lot rather than the subtype.[66] Still, others conclude that TGF-β2 and TGF-β3 are more effective in promoting col2 and glycosaminoglycans.[60] BMP plays a crucial role during skeletal development, including mesenchymal cell condensation, regulation of chondrocyte maturation, and proliferation and joint formation.[67,68] The family member of BMP,[69,70] including BMP-2, BMP-4, BMP-6, and BMP-7, enhances the ECM deposition by acting synergistically to TGF-β, but cannot act with dexamethasone in classical pellet cultures to differentiate MSC to chondrocytes.[60]

Ascorbic acid is used in culture media in combination with dexamethasone to enhance the production of ECM and col2, and increase the proliferation of chondrocytes.[71]

The chondrogenesis differentiation potential of bone marrow MSC (BM-MSCs) is achieved by the use of a conditioned medium containing both dexamethasone and TGF-β, while the chondrogenesis differentiation potential of adipose tissue (AD-MSCs) requires the addition of BMP-6.[66] Garza-Veloz *et al.* concludes that the ability of AD-MSCs to differentiate into chondrocytes can be enhanced using a combination of IGF-1/FGF-2.[72] The chondrogenic differentiation of MSCs usually requires serum-free media, which reduces the cellular apoptosis induced by the serum.[60,73] Mishra *et al*. demonstrate that the use of media with PRP will significantly increase the mRNA levels of RUNX2, sox-9, aggrecan, and cellular proliferation, as well as enhance the chondrogenic differentiation of MSCs that indicate that PRP seems to be a promising supplement agent.[74] At present, different commercial chondrogenic differentiation media for MSCs are available.[60]

CLINICAL STUDIES ON MSCS TRANSPLANTATION FOR CARTILAGE REPAIR

Several kinds of literature have shown the potential of using MSCs to repair cartilage *in vivo* using animal models;[75-80] this review will only focus on the benefit of using MSCs in treating humans [Table 2]. There are two methods of implantation of MSCs, direct surgical implantation and intra-articular injection. The implantation of MSCs could be done alone or applied in combination with a scaffold.[81] The scaffold aims to build cartilage that can recapitulate the original mechanical function of native cartilage by allowing high cell suspension and cell to cell contact.[56,82] MSC, together with scaffold and bioactive molecular are the basic components of tissue engineering that will allow the regeneration and enhancement of cartilage formation.[83] Different types of scaffolds are available, such as hyaluronan gel, collagen preparation, fibrin mixed with synthesis polymers, platelet-rich fibrin glue, and PRP.[82,84]

SURGICAL IMPLANTATION OF MSCS

For cartilage repair, there are many clinical case reports of surgical implantation of MSCs. Gobbi *et al.* report that 15 patients between 30 and 60 years of age were diagnosed with a grade IV cartilage lesion of the knee. All patients were treated surgically with activated Bone Marrow Aspiration Concentration and covered with collagen-based membrane scaffolds. The clinical assessment was based on X-rays and magnetic resonance imaging (MRIs) at 12 and 24 months and Knee injury and osteoarthritis outcome (KOOS). IKDC, VAS, Marx, Lysholm, SF-36 (physical/mental), and Tegner scores at 6, 12, and 24 months were done to follow-up. The outcome results indicated a significant improvement of all scores and the MRI showed hyaline-like tissues in all patients.[85] Wakitani *et al.* treated 24 OA knee patients ages 49–70 at the time of High Tibial Osteotomy. The patients were divided into two groups: 12 patients received passage (P2) autologous BM-MSC cultures using Fetal Calf Serum (FCS), embedded in collagen gel sheets and applied to the cartilage defects, and covered with autologous periosteum. The other 12 patients received the same procedure without BM-MSC. 42 weeks following the operations the patients who received BM-MSCs showed white soft and hyaline-like cartilage tissue covering the defected areas. The arthroscopic and histological grading score was better in patients who received MSC compared to the control groups, although no significant clinical improvements were demonstrated.[86] A young judo player of 31 years of age suffers from full-thickness cartilage, defect grade IV. The patient was treated with BM-MSC P2 culture in autologous serum (AS), embedded in collagen gel and covered by an autologous periosteal flap. After a year, the arthroscopy and histology showed that the defect was completely covered by a smooth tissue of hyaline-like cartilage and there was a dramatic improvement in clinical symptoms.[87] Wakitani *et al.* treated nine full-thickness patellofemoral cartilage defects in three patients ages 31, 44, and 45. Collagen gel sheets were embedded with P1 BM-MSCs culture in AS implantation and covered with autologous periosteum. The clinical symptoms improved 27 months after the procedure. One patient showed fibrocartilage tissue covering the defect by histology and another patient's MRI results indicated a complete cover of the defect, both after 12 months.[88] In another study, two other patients with patella cartilage defects received BM-MSCs embedded in collagen gel and surgical implantations, then covered with autologous periosteum showed a significant improvement in clinical symptoms (movement abilities) which remained for 4 years or more.[89] In addition, Wakitani *et al*. followed up with 41 patients who received 45 transplantation BM-MSCs between 1988 to 2008. The results indicated the safety of transplantation of autologous BM-MSCs due to the

Table 2: Summary table showing the human studies included in MSCs

First author and Year of publication	Original stem cell	Patients number	Disorder	Methods
Gobbi *et al*. 2011	BMAC	15	Grade IV cartilage lesion	Surgical implantation of activated BMAC and cover with collagen-based membrane scaffold
Wakitani *et al*. 2002	BM-MSC	24	Knee OA	Surgical implantation of P2 BM-MSC culture in FCS using collagen gel sheet and cover with autologous periosteum
Kuroda *et al*. 2007	BM-MSC	1	Grade IV cartilage defect	Surgical implantation of P2 BM-MSC culture in AS embedded in collagen sheet and cover with autologous periosteum
Wakitani *et al*. 2007	BM-MSC	3	Cartilage defect in patellae	Surgical implantation of P1 BM-MSC culture in AS using collagen gel sheet and cover with autologous periosteum
Wakitani *et al*. 2004	BM-MSC	2	Cartilage defect in patellae	Surgical implantation of collagen gel sheet containing BM-MSC and cover with autologous periosteum
Haleem *et al*. 2010	BM-MSC	5	Chondral defect femoral condyle	Surgical implantation of P2 BM-MSC embedding in PR-FG scaffold and cover with autologous periosteum
Giannini *et al*. 2009/2013	BM-MSC	48	Talar osteochondral lesion	One-step arthroscopic implantation of BM-MSC with collagen powder and platelet gel or HA with platelet gel scaffold
Nejadnik *et al*. 2010	BM-MSC/ACI	72 (2 groups 35 each)	Several lesion	BM-MSC and chondrocyte harvest and culture in FBS until cell sheet P1 then implantation
Giannin *et al*. 2010	BMDC/ACI	81 10 surgical ACI 46 arthroscopic ACI 25 arthroscopic MBDC	Talar osteochondral	Surgical groups received ACI with collagen gel scaffold and both arthroscopic groups received cells with HA membrane
Teo *et al*. 2012	BM-MSC/ACI	23 3 BM-MSC 20 ACI	OLD	Both cells culture in FBS until preparation of cell sheet P1 then surgical implantation
Lee *et al*. 2012	BM-MSC	70 35 surgical 35 IA injection of BM-MSC with HA	Cartilage defect	First groups received BM-MSC cell sheet culture in FBC surgically Second groups arthroscopic microfraction then P1 BM-MSC culture in FBS injected IA followed by HA
Centeno *et al*. 2008	BM-MSC	1	Knee-OA	IA injection of P5 BM-MSC culture using PL
Centeno *et al*. 2010/2011	BM-MSC	430	Various orthopedic condition	IA injection of BM-MSC culture using PL

(*Contd..*)

Table 2: *(Continued)*

First author and Year of publication	Original stem cell	Patients number	Disorder	Methods
Davatchi *et al*. 2011	BM-MSC	4	Knee OA	IA injection of BM-MSC culture in FBS
Emadedin *et al*. 2012	BM-MSC	6	Knee OA	IA injection of P2 BM-MSC culture using HBS
Kon *et al*. 2013	Infrapatellar fat MSC	18	Knee OA	IA injection of non-expanded MSC with PRP
Pak 2011	SVF	2	Knee OA	IA injection of SVF+HA+PRP+Calcium chloride+dexamethasone
Pak *et al*. 2013	SVF	3	Chondromalacia patellae	IA injection of SVF+AH+activated PRP+calcium chloride
Hauser and Orlofsky 2013	Unfractionated WBM	7	Hip, ankle, or knee Osteoarthritis	Unfractionated whole bone marrow injection into osteoarthritic joints

BMAC: Activated bone marrow aspiration concentration, BM-MSC: Bone marrow mesenchymal stromal cell, OA: Osteoarthritis, AS: Autologous serum, P: Passage, PR-FG: Platelet-rich fibrin glue, ACI: Autologous chondrocyte implantation, BMDC: Bone marrow-derived cells, OCD: Osteochondral defect, FBS: fetal bovine serum, FCS: Fetal calf serum, PL: Platelet lysate, IA: Intra-articular injection, HBS: Hyclone bovine serum, HA: Hyaluronic acid, SVF: Stromal vascular fraction, WBM: Whole bone marrow

absence of infection and tumors in the patients during long-term follow-up.[90] Haleem *et al.* reported that five young patients diagnosed with full-thickness cartilage defects of femoral condyles were treated with autologous BM-MSCs which culture using fetal bovine serum (FBS) for two passages and then was placed on platelet-rich fibrin glue (PR-FG) scaffolds implanted surgically and then covered with autologous periosteum. The clinical evaluation based on Lysholm and revised Hospital for Special Surgery Knee scores, X-Ray, and MRI at the 6 and 12 months follow-ups. The results showed improvement in all patient symptoms; three patients showed complete coverage of the defected surface with native cartilage, while two patients showed incomplete coverage by MRI.[91] Giannin *et al.* treated 48 patients diagnosed with talar osteochondral lesions ages 14–50-years-old. The patients received BM-MSCs with either scaffold of collagen powder with platelet gel, or HA with platelet gel, which is done by one step arthroscopic transplantation technique. The clinical evaluation was based on the American Orthopaedic Foot and Ankle Society (AOFAS), MRI, and histology for 4 years. The histology results showed regenerative tissue progression. The clinical scores lowered between 24 and 36 months significantly and were negatively affected by the time between trauma and surgery, but the AOFAS score improved at the 24 month follow-up. MRI T2- mapping analysis showed regenerated tissue similar to hyaline cartilage and its quality correlated with clinical results directly.[92,93] An interesting comparison study by Nejadnik *et al*. comparing ACI and BM-MSCs outcomes in 72 matched patients divided them into two groups, with 36 patients in each group. The clinical assessments were based on IKDC, ICRS, Tegner activity level scale, and Lysholm Knee Scale at 3, 6, 9, 12, 18, and 24 months after implantation. Both chondrocytes and BM-MSCs were harvested and cultured in FBS until the preparation of cell sheets P1 and then implanted. Both groups showed great improvements in quality of life, with better physical role function in BM-MSCs groups compared to the ACI groups; in terms of clinical outcomes, there were no differences. Nejadnik *et al*. concluded that both groups had similar effects; however, BM-MSC required only one-step surgery, lower cost, and donor-site morbidity.[94] Giannin *et al.* evaluated the treatment of cartilage defects by presenting the results and comparing three types of techniques for treating talar osteochondral, including open ACI surgery, arthroscopic ACI, and arthroscopic bone marrow-derived cells (BMDC) in 3 patients, were 10, 25, and 46, respectively. In open ACI surgery, the collagen gel was used, while the HA membrane was used in arthroscopic ACI. The clinical evaluation was based on X-ray, MRI, and AOFAS scores. All three groups showed an improvement in AOFAS scores; however, the BMDC reduced the morbidity and cost.[95] Teo *et al.* reported the treatment of 23 young patients diagnosed with patellar Osteochondritis dissecans (OLD). The patients' ages were between 12 and 21-years-old; twenty of them received ACI and three received BM-MSCs. Both chondrocyte and stem cell culture were used FBS until the preparation of the cell sheet P1. The clinical assessment was based on IKDC subjective, Tenger-Lysholm scales and Lysholm-Gillquist scores at 6, 12, and 24 months follow-up. All three groups showed improvement in assessments except for two patients who showed hypertrophy.[96] Lee *et al.* investigated the clinical outcome and safety of injected BM-MSCs and HA after arthroscopic microfracture and compared it with (control) surgical implantation of BM-MSCs cell sheets. Seventy matched patients with symptoms of cartilage defect were divided into two groups, each with 35 patients. The first group received intra-articular injections of P1 BM-MSC culture

in FBS, followed by HA injections after the arthroscopic microfracture. The other group received surgically implanted BM-MSC after culturing them in FBS until cell sheet. The evaluation is based on using ICRS Cartilage Injury Evaluation Package, IKDC subjective, the Tegner activity scale and the Lysholm scale. Both groups showed similar improvements in the short term and intra-articular injections are safe and minimally invasive.[97]

INTRAARTICULAR INJECTION OF MSCS

The intra-articular injection technique is considered the easiest method due to its potential advantage as a less invasive method, its minimal recovery time and its cheap price.[98] Centeno *et al.* treated a 36-year-old man diagnosed with knee OA by culture BM-MSCs in platelet lysate (PL) until P5 and then giving an intra-articular injection. The post-injection 3 months later showed improvement and a decrease in the VAS score; the MRI analysis before and after the procedure demonstrated an increase in meniscus.[99] The same author reported that in 2010, 227 patients were diagnosed with various orthopedic conditions between 2005 and 2009 and treated with P5 BM-MSC cultures in PL. Then in 2011 updated paper with the addition of 113 patients were treated. The follow-up indicated no tumor formations and less morbidity detected based on HHS criteria, compared with surgical procedure.[100,101] Davatchi *et al.* treated four patients with knee OA by P1 BM-MSCs cultures in FBS. After intra-articular injections of BM-MSC, the patients improved in walking time and stair climbing at the in follow-up evaluation.[102] Emadedin *et al.* noted the improvement in six patients of quality of life 6 months post intra-articular injections of P2 BM-MSCs cultures in Hyclone Bovine Serum (HBS). The patients diagnosed with knee OA showed evidence of increased thickness of cartilage during an MRI, but after the first 6 months, the pain started to appear.[103] Koh *et al.* evaluated the results of intra-articular injections of non-expanded MSCs isolated from intrapatellar fat pads with PRP; the clinical results were based on Lysholm scores, VAS, Western Ontario and McMaster University OA Index and MRI pre and post operations. The results indicated an improvement in MRI scores, reduced pain, and improved knee function.[104] Pak treated two old female patients diagnosed with knee OA using adipose tissue MSC. The patients received intra-articular injections of Stromal Vascular Fraction (SVF), along with HA, PRP calcium chloride, and dexamethasone. There were significant improvements in patient quality of life with positive MRI results.[105] Furthermore, another study done by the same author where he treated three patients with intra-articular injections of SVF for chondromalacia patellae, found similar results. SVF is mixed with calcium chloride, activated PRP, and HA. The patients' outcome evaluations were based on MRI, VAS, Function rating index, and physical therapy assessments pre and post-treatments. Significant improvement in terms of pain at the 1 year follow-up was concluded, no serious side effects and MRI results showed improvements in the damaged tissue.[106]

However, more clinical trials are required to address the ability of MSC to regenerate cartilage and its anti-inflammatory effect, especially in OA patients since the synovial fluid in OA patients prevent the ability of MSC to differentiate into chondrocytes.[107,108]

CONCLUSIONS

Cell-based therapies represent promising and effective methods for cartilage regeneration. The development in cell-based therapy has been massive, starting from two-step surgery in ACI, to one-step surgery in BM-MSC, and now the intra-articular injection of SVF. MSCs are alternative sources of cells that could differentiate into chondrocytes, are easy to isolate and culture, and have an anti-inflammatory ability compared with chondrocytes. Furthermore, some animal and human experiments using MSCs in treating cartilage defects gave encouraging results, making MSCs the future hope for the regeneration of cartilage. In addition, more research is needed to analyze the MSC profile and the mechanism, which will address the specific need of other GFs, autologous products (serums, PRPs), and scaffolds to allow the regeneration of cartilage.

Significance statement

OA is one of the most commonly occurring degenerative joint problems, affecting more than one-quarter of the population over the age of 18 years. It impairs the quality of life to a great extent. Unfortunately, the precise molecular pathways behind OA onset and progression are still unknown, and the therapeutic regimen for its management is not well established. Hence, based on recently released scientific findings, it is worthwhile to present the existing knowledge on the progression and management of OA. This review examines the many therapeutic options available and discusses their benefits and drawbacks, with a particular focus on cell-based therapies. These therapies represent potential and successful cartilage regeneration strategies. This review will serve as a comprehensive source of knowledge for scientists and researchers working on the topic of OA.

REFERENCES

1. Coleman CM, Curtin C, Barry FP, O'Flatharta C, Murphy JM. Mesenchymal stem cells and osteoarthritis: Remedy or accomplice? Hum Gene Ther 2010;21:1239-50.
2. Peat G, McCarney R, Croft P. Knee pain and osteoarthritis in older adults: A review of community burden and current use of primary health care. Ann Rheum Dis 2001;60:91-7.
3. Woolf AD, Pfleger B. Burden of major musculoskeletal conditions. Bull World Health Organ 2003;81:646-56.
4. Felson DT. Osteoarthritis of the knee. N Engl J Med 2006;354:841-8.

5. De Lange-Brokaar BJ, Ioan-Facsinay A, Van Osch GJ, Zuurmond AM, Schoones J, Toes RE, *et al*. Synovial inflammation, immune cells and their cytokines in osteoarthritis: a review. Osteoarthritis Cartilage 2012;20:1484-99.
6. Benito MJ, Veale DJ, FitzGerald O, van den Berg WB, Bresnihan B. Synovial tissue inflammation in early and late osteoarthritis. Ann Rheum Dis 2005;64:1263-7.
7. Pelletier JP, Martel-Pelletier J, Abramson SB. Osteoarthritis, an inflammatory disease: Potential implication for the selection of new therapeutic targets. Arthritis Rheum 2001;44:1237-47.
8. Goldring MB, Goldring SR. Articular cartilage and subchondral bone in the pathogenesis of osteoarthritis. Ann N Y Acad Sci 2010;1192:230-7.
9. Gerwin N, Hops C, Lucke A. Intraarticular drug delivery in osteoarthritis. Adv Drug Deliv Rev 2006;58:226-42.
10. Muir H. The chondrocyte, architect of cartilage. Biomechanics, structure, function and molecular biology of cartilage matrix macromolecules. Bioessays 1995;17:1039-48.
11. Bruckner P, van der Rest M. Structure and function of cartilage collagens. Microscopy Res Tech 1994;28:378-84.
12. Goldring MB. The role of the chondrocyte in osteoarthritis. Arthritis Rheum 2000;43:1916-26.
13. Aigner T, McKenna L. Molecular pathology and pathobiology of osteoarthritic cartilage. Cell Mol Life Sci 2002;59:5-18.
14. Buckwalter JA, Mankin HJ. Articular cartilage: Tissue design and chondrocyte-matrix interactions. Instr Course Lect 1998;47:477-86.
15. Zhang W, Moskowitz RW, Nuki G, Abramson S, Altman RD, Arden N, *et al*. OARSI recommendations for the management of hip and knee osteoarthritis, Part II: OARSI evidence-based, expert consensus guidelines. Osteoarthritis Cartilage 2008;16:137-62.
16. Kon E, Filardo G, Drobnic M, Madry H, Jelic M, van Dijk N, *et al*. Non-surgical management of early knee osteoarthritis. Knee Surg Sports Traumatol Arthrosc 2012;20:436-49.
17. Lin J, Zhang W, Jones A, Doherty M. Efficacy of topical non-steroidal anti-inflammatory drugs in the treatment of osteoarthritis: Meta-analysis of randomised controlled trials. BMJ 2004;329:324.
18. McAlindon TE, LaValley MP, Gulin JP, Felson DT. Glucosamine and chondroitin for treatment of osteoarthritis: A systematic quality assessment and meta-analysis. JAMA 2000;283:1469-75.
19. Towheed T, Maxwell L, Anastassiades TP, Shea B, Houpt JB, Welch V, *et al*. Glucosamine therapy for treating osteoarthritis. Cochrane Database Syst Rev 2005;2005:CD002946.
20. National Collaborating Centre for Chronic Conditions (Great Britain), and National Institute for Clinical Excellence (Great Britain). (2008). Osteoarthritis: National Clinical Guidelines for Care and Management in Adults. Royal College of Physicians. Available from: https://www.nice.org.uk/guidance/cg59 [Last accessed on 2022 Apr 15].
21. Uthman I, Raynauld JP, Haraoui B. Intra-articular therapy in osteoarthritis. Postgrad Med J 2003;79:449-53.
22. Strauss EJ, Hart JA, Miller MD, Altman RD, Rosen JE. Hyaluronic acid viscosupplementation and osteoarthritis: Current uses and future directions. Am J Sports Med 2009;37:1636-44.
23. Liddle AD, Pegg EC, Pandit H. Knee replacement for osteoarthritis. Maturitas 2013;75:131-6.
24. Zhu S, Lu P, Liu H, Chen P, Wu Y, Wang Y, *et al*. Inhibition of Rac1 activity by controlled release of NSC23766 from chitosan microspheres effectively ameliorates osteoarthritis development *in vivo*. Ann Rheum Dis 2015;74:285-93.
25. Goldring MB. Osteoarthritis and cartilage: the role of cytokines. Curr Rheumatol Rep 2000;2:459-65.
26. Frizziero A, Giannotti E, Oliva F, Masiero S, Maffulli N. Autologous conditioned serum for the treatment of osteoarthritis and other possible applications in musculoskeletal disorders. Br Med Bull 2013;105:169-84.
27. Arend WP, Joslin FG, Massoni RJ. Effects of immune complexes on production by human monocytes of interleukin 1 or an interleukin 1 inhibitor. J Immunol (Baltimore, Md. 1950) 1985;134:3868-75.
28. Arend WP, Smith MF Jr., Janson RW, Joslin FG. IL-1 receptor antagonist and IL-1 beta production in human monocytes are regulated differently. J Immunol 1991;147:1530-6.
29. Arend WP, Leung DY. IgG induction of IL-1 receptor antagonist production by human monocytes. Immunol Rev 1994;139:71-8.
30. Pelletier JP, Caron JP, Evans C, Robbins PD, Georgescu HI, Jovanovic D, *et al*. *In vivo* suppression of early experimental osteoarthritis by interleukin-1 receptor antagonist using gene therapy. Arthritis Rheum 1997;40:1012-9.
31. Frisbie DD, Ghivizzani SC, Robbins PD, Evans CH, McIlwraith CW. Treatment of experimental equine osteoarthritis by *in vivo* delivery of the equine interleukin-1 receptor antagonist gene. Gene Ther 2002;9:12-20.
32. Frisbie DD, McIlwraith CW. Evaluation of gene therapy as a treatment for equine traumatic arthritis and osteoarthritis. Clin Orthop Relat Res 2000;379 Suppl:S273-87.
33. Zhang X, Mao Z, Yu C. Suppression of early experimental osteoarthritis by gene transfer of interleukin-1 receptor antagonist and interleukin-10. J Orthop Res 2004;22:742-50.
34. Fernandes J, Tardif G, Martel-Pelletier J, Lascau-Coman V, Dupuis M, Moldovan F, *et al*. *In vivo* transfer of interleukin-1 receptor antagonist gene in osteoarthritic rabbit knee joints: Prevention of osteoarthritis progression. Am J Pathol 1999;154:1159-69.
35. Chevalier X, Giraudeau B, Conrozier T, Marliere J, Kiefer P, Goupille P. Safety study of intraarticular injection of interleukin 1 receptor antagonist in patients with painful knee osteoarthritis: A multicenter study.

J Rheumatol 2005;32:1317-23.
36. Meijer H, Reinecke J, Becker C, Tholen G, Wehling P. The production of anti-inflammatory cytokines in whole blood by physico-chemical induction. Inflamm Res 2003;52:404-7.
37. Fox BA, Stephens MM. Treatment of knee osteoarthritis with Orthokine®-derived autologous conditioned serum. Expert Rev Clin Immunol 2010;6:335-45.
38. Baltzer AW, Moser C, Jansen SA, Krauspe R. Autologous conditioned serum (Orthokine) is an effective treatment for knee osteoarthritis. Osteoarthritis Cartilage 2009;17:152-60.
39. Foster TE, Puskas BL, Mandelbaum BR, Gerhardt MB, Rodeo SA. Platelet-rich plasma: From basic science to clinical applications. Am J Sports Med 2009;37:2259-72.
40. Kon E, Mandelbaum B, Buda R, Filardo G, Delcogliano M, Timoncini A, *et al*. Platelet-rich plasma intra-articular injection versus hyaluronic acid viscosupplementation as treatments for cartilage pathology: From early degeneration to osteoarthritis. Arthroscopy 2011;27:1490-501.
41. Smyth NA, Murawski CD, Fortier LA, Cole BJ, Kennedy JG. Platelet-rich plasma in the pathologic processes of cartilage: Review of basic science evidence. Arthroscopy 2013;29:1399-409.
42. Bendinelli P, Matteucci E, Dogliotti G, Corsi MM, Banfi G, Maroni P, *et al*. Molecular basis of anti-inflammatory action of platelet-rich plasma on human chondrocytes: Mechanisms of NF-κB inhibition via HGF. J Cell Physiol 2010;225:757-66.
43. Steinert AF, Middleton KK, Araujo PH, Fu FH. Platelet-rich plasma in orthopaedic surgery and sports medicine: Pearls, pitfalls, and new trends in research. Oper Tech Orthop 2012;22:91-103.
44. Filardo G, Kon E, Buda R, Timoncini A, Di Martino A, Cenacchi A, *et al*. Platelet-rich plasma intra-articular knee injections for the treatment of degenerative cartilage lesions and osteoarthritis. Knee Surg Sports Traumatol Arthrosc 2011;19:528-35.
45. Kon E, Filardo G, Di Martino A, Marcacci M. Platelet-rich plasma (PRP) to treat sports injuries: Evidence to support its use. Knee Surg Sports Traumatol Arthrosc 2011;19:516-27.
46. Engebretsen L, Steffen K, Alsousou J, Anitua E, Bachl N, Devilee R, *et al*. IOC consensus paper on the use of platelet-rich plasma in sports medicine. Br J Sports Med 2010;44:1072-81.
47. Metcalf KB, Mandelbaum BR, McIlwraith CW. Application of platelet-rich plasma to disorders of the knee joint. Cartilage 2013;4:295-312.
48. DeLong JM, Russell RP, Mazzocca AD. Platelet-rich plasma: The PAW classification system. Arthroscopy 2012;28:998-1009.
49. Mishra A, Harmon K, Woodall J, Vieira A. Sports medicine applications of platelet rich plasma. Curr Pharm Biotechnol 2012;13:1185-95.
50. Napolitano M, Matera S, Bossio M, Crescibene A, Costabile E, Almolla J, *et al*. Autologous platelet gel for tissue regeneration in degenerative disorders of the knee. Blood Transfus 2012;10:72.
51. Kon E, Buda R, Filardo G, Di Martino A, Timoncini A, Cenacchi A, *et al*. Platelet-rich plasma: Intra-articular knee injections produced favorable results on degenerative cartilage lesions. Knee Surg Sports Traumatol Arthrosc 2010;18:472-9.
52. Patel S, Dhillon MS, Aggarwal S, Marwaha N, Jain A. Treatment with platelet-rich plasma is more effective than placebo for knee osteoarthritis: A prospective, double-blind, randomized trial. Am J Sports Med 2013;41:356-64.
53. Filardo G, Kon E, Pereira Ruiz MT, Vaccaro F, Guitaldi R, Di Martino A, *et al*. Platelet-rich plasma intra-articular injections for cartilage degeneration and osteoarthritis: Single-versus double-spinning approach. Knee Surg Sports Traumatol Arthrosc 2012;20:2082-91.
54. Santo VE, Gomes ME, Mano JF, Reis RL. Controlled release strategies for bone, cartilage, and osteochondral engineering-Part I: Recapitulation of native tissue healing and variables for the design of delivery systems. Tissue Eng Part B Rev 2013;19:308-26.
55. Gomoll AH, Filardo G, De Girolamo L, Esprequeira-Mendes J, Marcacci M, Rodkey WG, *et al*. Surgical treatment for early osteoarthritis. Part I: Cartilage repair procedures. Knee Surg Sports Traumatol Arthrosc 2012;20:450-66.
56. Diekman BO, Guilak F. Stem cell-based therapies for osteoarthritis: Challenges and opportunities. Curr Opin Rheumatol 2013;25:119-26.
57. Johnstone B, Hering TM, Caplan AI, Goldberg VM, Yoo JU. *In vitro* chondrogenesis of bone marrow-derived mesenchymal progenitor cells. Exp Cell Res 1998;238:265-72.
58. Fernandes H, Mentink A, Bank R, Stoop R, van Blitterswijk C, de Boer J. Endogenous collagen influences differentiation of human multipotent mesenchymal stromal cells. Tissue Eng Part A 2010;16:1693-702.
59. Choi KM, Seo YK, Yoon HH, Song KY, Kwon SY, Lee HS, *et al*. Effect of ascorbic acid on bone marrow-derived mesenchymal stem cell proliferation and differentiation. J Biosci Bioeng 2008;105:586-94.
60. Vater C, Kasten P, Stiehler M. Culture media for the differentiation of mesenchymal stromal cells. Acta Biomater 2011;7:463-77.
61. Murtaugh LC, Zeng L, Chyung JH, Lassar AB. The chick transcriptional repressor Nkx3.2 acts downstream of Shh to promote BMP-dependent axial chondrogenesis. Dev Cell 2001;1:411-22.
62. Rodrigo I, Hill RE, Balling R, Münsterberg A, Imai K. Pax1 and Pax9 activate Bapx1 to induce chondrogenic differentiation in the sclerotome. Development 2003;130:473-82.
63. Jorgensen C, Djouad F, Bouffi C, Mrugala D, Noël D. Multipotent mesenchymal stromal cells in articular diseases. Best Pract Res Clin Rheumatol 2008;22:269-84.
64. Li WG, Xu XX. The expression of N-cadherin, fibronectin

during chondrogenic differentiation of MSC induced by TGF-beta(1). Chin J Traumatol 2005;8:349-51.
65. Goessler UR, Bugert P, Bieback K, Deml M, Sadick H, Hormann K, *et al*. *In-vitro* analysis of the expression of TGFbeta-superfamily-members during chondrogenic differentiation of mesenchymal stem cells and chondrocytes during dedifferentiation in cell culture. Cell Mol Biol Lett 2005;10:345-62.
66. Richter W. Mesenchymal stem cells and cartilage *in situ* regeneration. J Intern Med 2009;266:390-405.
67. Pogue R, Lyons K. BMP signaling in the cartilage growth plate. Curr Top Dev Biol 2006;76:1-48.
68. Scimeca M, Bonanno E, Piccirilli E, Baldi J, Mauriello A, Orlandi A, *et al*. Satellite cells CD44 positive drive muscle regeneration in osteoarthritis patients. Stem Cells Int 2015;2015:469459.
69. Abula K, Muneta T, Miyatake K, Yamada J, Matsukura Y, Inoue M, *et al*. Elimination of BMP7 from the developing limb mesenchyme leads to articular cartilage degeneration and synovial inflammation with increased age. FEBS Lett 2015;589:1240-8.
70. Caron MM, Emans PJ, Cremers A, Surtel DA, Coolsen MM, van Rhijn LW, *et al*. Hypertrophic differentiation during chondrogenic differentiation of progenitor cells is stimulated by BMP-2 but suppressed by BMP-7. Osteoarthritis Cartilage 2013;21:604-13.
71. Na K, Choi SJ, Kim S, Sun BK, Woo DG, Chung HM, *et al*. Enhancement of cell proliferation and differentiation by combination of ascorbate and dexamethasone in thermo-reversible hydrogel constructs embedded with rabbit chondrocytes. Biotechnol Lett 2007;29:1453-7.
72. Garza-Veloz I, Romero-Diaz VJ, Martinez-Fierro ML, Marino-Martinez IA, Gonzalez-Rodriguez M, *et al*. Analyses of chondrogenic induction of adipose mesenchymal stem cells by combined co-stimulation mediated by adenoviral gene transfer. Arthritis Res Ther 2013;15:1-13.
73. Wang CY, Chen LL, Kuo PY, Chang JL, Wang YJ, Hung SC. Apoptosis in chondrogenesis of human mesenchymal stem cells: Effect of serum and medium supplements. Apoptosis 2010;15:439-49.
74. Mishra A, Tummala P, King A, Lee B, Kraus M, Tse V, *et al*. Buffered platelet-rich plasma enhances mesenchymal stem cell proliferation and chondrogenic differentiation. Tissue Eng Part C Methods 2009;15:431-5.
75. Veronesi F, Maglio M, Tschon M, Aldini NN, Fini M. Adipose-derived mesenchymal stem cells for cartilage tissue engineering: State-of-the-art in in vivo studies. J Biomed Mater Res A 2014;102:2448-66.
76. 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;25:2964-71.
77. Murphy JM, Fink DJ, Hunziker EB, Barry FP. Stem cell therapy in a caprine model of osteoarthritis. Arthritis Rheum 2003;48:3464-74.
78. Desando G, Cavallo C, Sartoni F, Martini L, Parrilli A, Veronesi F, *et al*. Intra-articular delivery of adipose derived stromal cells attenuates osteoarthritis progression in an experimental rabbit model. Arthritis Res Ther 2013;15:1-16.
79. Lu CH, Yeh TS, Yeh CL, Fang YH, Sung LY, Lin SY, *et al*. Regenerating cartilages by engineered ASCs: Prolonged TGF-β3/BMP-6 expression improved articular cartilage formation and restored zonal structure. Mol Ther 2014;22:186-95.
80. Matsumoto T, Okabe T, Ikawa T, Iida T, Yasuda H, Nakamura H, *et al*. Articular cartilage repair with autologous bone marrow mesenchymal cells. J Cell Physiol 2010;225:291-5.
81. Wang J, Liao L, Tan J. Mesenchymal-stem-cell-based experimental and clinical trials: Current status and open questions. Expert Opin Biol Ther 2011;11:893-909.
82. Roberts S, Genever P, McCaskie A, De Bari C. Prospects of stem cell therapy in osteoarthritis. Regen Med 2011;6:351-66.
83. Kai D, Jin G, Prabhakaran MP, Ramakrishna S. Electrospun synthetic and natural nanofibers for regenerative medicine and stem cells. Biotechnol J 2013;8:59-72.
84. Wu W, Zhang J, Dong Q, Liu Y, Mao T, Chen F. Platelet-rich plasma-a promising cell carrier for micro-invasive articular cartilage repair. Med Hypotheses 2009;72:455-7.
85. Gobbi A, Karnatzikos G, Scotti C, Mahajan V, Mazzucco L, Grigolo B. One-step cartilage repair with bone marrow aspirate concentrated cells and collagen matrix in full-thickness knee cartilage lesions: Results at 2-year follow-up. Cartilage 2011;2:286-99.
86. Wakitani S, Imoto K, Yamamoto T, Saito M, Murata N, Yoneda M. Human autologous culture expanded bone marrow mesenchymal cell transplantation for repair of cartilage defects in osteoarthritic knees. Osteoarthritis Cartilage 2002;10:199-206.
87. Kuroda R, Ishida K, Matsumoto T, Akisue T, Fujioka H, Mizuno K, *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;15:226-31.
88. 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;1:74-9.
89. Wakitani S, Mitsuoka T, Nakamura N, Toritsuka Y, Nakamura Y, Horibe S. Autologous bone marrow stromal cell transplantation for repair of full-thickness articular cartilage defects in human patellae: Two case reports. Cell Transplant 2004;13:595-600.
90. Wakitani S, Okabe T, Horibe S, Mitsuoka T, Saito M, Koyama T, *et al*. Safety of autologous bone marrow-derived mesenchymal stem cell transplantation for cartilage repair in 41 patients with 45 joints followed for up to 11 years and 5 months. J Tissue Eng Regen Med 2011;5:146-50.

91. Haleem AM, Singergy AA, Sabry D, Atta HM, Rashed LA, Chu CR, *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;1:253-61.
92. Giannini S, Buda R, Vannini F, Cavallo M, Grigolo B. One-step bone marrow-derived cell transplantation in talar osteochondral lesions. Clin Orthop Relat Res 2009;467:3307-20.
93. Giannini S, Buda R, Battaglia M, Cavallo M, Ruffilli A, Ramponi L, *et al*. One-step repair in talar osteochondral lesions: 4-year clinical results and t2-mapping capability in outcome prediction. Am J Sports Med 2013;41:511-8.
94. 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;38:1110-6.
95. Giannini S, Buda R, Cavallo M, Ruffilli A, Cenacchi A, Cavallo C, *et al*. Cartilage repair evolution in post-traumatic osteochondral lesions of the talus: From open field autologous chondrocyte to bone-marrow-derived cells transplantation. Injury 2010;41:1196-203.
96. Teo BJ, Buhary K, Tai BC, Hui JH. Cell-based therapy improves function in adolescents and young adults with patellar osteochondritis dissecans. Clin Orthop Relat Res 2013;471:1152-8.
97. Lee KB, Wang VT, Chan YH, Hui JH. A novel, minimally-invasive technique of cartilage repair in the human knee using arthroscopic microfracture and injections of mesenchymal stem cells and hyaluronic acid-a prospective comparative study on safety and short-term efficacy. Ann Acad Med Singap 2012;41:511-7.
98. 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;17:1289-97.
99. Centeno CJ, Busse D, Kisiday J, Keohan C, Freeman M, Karli D. Regeneration of meniscus cartilage in a knee treated with percutaneously implanted autologous mesenchymal stem cells. Med Hypotheses 2008;71:900-8.
100. Centeno CJ, Schultz JR, Cheever M, Robinson B, Freeman M, Marasco W. Safety and complications reporting on the re-implantation of culture-expanded mesenchymal stem cells using autologous platelet lysate technique. Curr Stem Cell Res Ther 2010;5:81-93.
101. Centeno CJ, Schultz JR, Cheever M, Freeman M, Faulkner S, Robinson B, *et al*. Safety and complications reporting update on the re-implantation of culture-expanded mesenchymal stem cells using autologous platelet lysate technique. Curr Stem Cell Res Ther 2011;6:368-78.
102. 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;14:211-5.
103. Emadedin M, Aghdami N, Taghiyar L, Fazeli R, Moghadasali R, Jahangir S, *et al*. Intra-articular injection of autologous mesenchymal stem cells in six patients with knee osteoarthritis. Arch Iran Med 2012;15:422-8.
104. Koh YG, Jo SB, Kwon OR, Suh DS, Lee SW, Park SH, *et al*. Mesenchymal stem cell injections improve symptoms of knee osteoarthritis. Arthroscopy 2013;29:748-55.
105. Pak J. Regeneration of human bones in hip osteonecrosis and human cartilage in knee osteoarthritis with autologous adipose-tissue-derived stem cells: A case series. J Med Case Rep 2011;5:296.
106. Pak J, Lee JH, Lee SH. A novel biological approach to treat chondromalacia patellae. PLoS One 2013;8:e64569.
107. Hauser RA, Orlofsky A. Regenerative injection therapy with whole bone marrow aspirate for degenerative joint disease: A case series. Clin Med Insights Arthritis Musculoskelet Disord 2013;6:65-72.
108. Heldens GT, Blaney Davidson EN, Vitters EL, Schreurs BW, Piek E, van den Berg WB, *et al*. Catabolic factors and osteoarthritis-conditioned medium inhibit chondrogenesis of human mesenchymal stem cells. Tissue Eng Part A 2012;18:45-54.

Source of Support: Nil. **Conflicts of Interest:** None declared.