A Comprehensive Review of Stem-Cell Therapy

Ameer Elbuluk, BA

Thomas A. Einhorn, MD

Richard Iorio, MD

Investigation performed at the New York University (NYU) Langone Medical Center, Hospital for Joint Diseases, New York, NY

Abstract

» Regenerative orthopaedics has been used as a biological alternative to conventional therapy and surgical intervention for treating musculoskeletal conditions associated with limited therapeutic options.

» Orthopaedic investigators have shown promising early clinical results by developing cell-based approaches to regenerate injured cartilage, tendon, ligaments, and bone.

» Despite continued research, issues regarding harvesting, delivery of treatment, cost, indications, and optimal timing of intervention must be considered.

» Multidisciplinary networks of investigators are essential to achieve the full clinical and therapeutic potential of mesenchymal stem cells in orthopaedics.

» Although mesenchymal stem cells offer great promise for the treatment of degenerative diseases and orthopaedic conditions, there is still a dearth of properly conducted controlled clinical studies.

In 1998, a team of researchers led by James Thomson reported their successful creation of the first human embryonic stem cells[1]. Their research ushered in a new era for drug discovery and transplantation medicine. In the past few decades, molecular genetics has progressed from the laboratory to the clinic in the hopes of finding new applications. The use of developmental biology focused on translational research has helped to shape the field of regenerative medicine. Rapid advances in tissue engineering and cellular therapies have caused a paradigm shift from pharmacological treatment to the construction of biological substitutes that can regenerate diseased organs or injured tissues[2]. In orthopaedics, conventional strategies for treating several musculoskeletal conditions, such as osteoarthritis, still remain ineffective[3]. Orthopaedic investigators have begun investigating cell-based techniques to develop novel therapeutic agents to address biological solutions for orthopaedic conditions.

Orthopaedic injuries that are treated with operative intervention may not heal as intended and complete function may not be regained. As a result, there has been increasing focus on understanding the pathophysiology of orthopaedic disease processes in order to develop regenerative interventions for clinical use. Regenerative orthopaedics was first recognized in 1965 with the discovery of the osteoinductive properties of bone morphogenetic proteins (BMPs)[4-6]. However, the use of BMPs has proven not to be as dramatically effective in clinical practice as was once hoped. Several studies have even highlighted the substantial clinically adverse effects of their use[7-9]. Considering the potentially harmful and

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economically expensive implications of BMP applications, the orthopaedic community turned research interests toward identifying cell-based approaches to regenerating the musculoskeletal system. One of the most promising cellular sources is mesenchymal stem cells (MSCs). MSCs hold great potential for the treatment of musculoskeletal conditions, and several recent advances in the field have shown promise. The purpose of the present comprehensive review is to provide an overview of stem cell research in orthopaedics while addressing the potential clinical applications as well as challenges of this therapy.

Concept of Stem Cells

What Are Stem Cells?

Stem cells are defined as undifferentiated cells capable of proliferation, self-renewal, and differentiation into specialized cell types[10]. Stem cells are distinguished by their ability to produce a particular lineage of cells depending on the type of stem cell and its extracellular environment. Stem cells control the replacement of several cell types that help to constitute many different organ systems, which allows scientists the opportunity to generate specific cells to replace differentiated functions lost in various disease states.

Types of Stem Cells

In mammals, there are 2 types of stem cells—embryonic and adult, which vary in origin and potential to differentiate. The main source of embryonic stem cells (ESCs) is the inner cell mass of a human blastocyst derived during embryogenesis[1]. Adult stem cells (ASCs), typically obtained from adult bone marrow, can develop into 2 types of stem cells: hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs)[11]. The ability to differentiate becomes more restricted from the embryonic to the adult stem cell population. ESCs are characterized as pluripotent and can generate all cell types of the embryo. Although ESCs have the greatest potential for self-renewal and differentiation, the legal and ethical ramifications of this research are highly controversial and have led to several reviews of legislation[12]. Moral issues surrounding the generation of human ESCs for therapeutic purposes have paved the path for the intensive study of ASCs. Despite their limitations in terms of differentiation, ACSs have the advantage of possible autologous cell therapy, which reduces the possible immune response to in vivo therapies.

ASCs can only generate progenitor cells of a specific cell lineage (e.g., intestinal cells in villus crypts), which reduces the overall potency of the stem cell[11]. However, in the appropriate niche, ASCs can become multipotent and differentiate into multiple lineages—these cells are known as mesenchymal stem cells (MSCs)[11]. MSCs can be isolated from bone marrow, skin, synovium, adipose tissue, and many other tissues of mesenchymal origin. Recently, adipose tissue has been cited as an optimal source of MSCs because of the abundance of such cells in this tissue[12]. Other sources of MSCs, such as umbilical cord matrix, also have been considered; however, the isolation and amplification of these cells require careful laboratory manipulation and often are quite time-consuming[13,14]. Ultimately, MSCs are of particular interest in orthopaedics because of their potential to differentiate into cells that make bone, cartilage, tendon, and ligaments.

Overview of MSCs

The unique properties of MSCs in repairing skeletal defects were first described by Caplan in 1991[15]. Caplan's preliminary experiments formed the basis for using MSCs as a treatment plan to regenerate damaged articular cartilage and to help maintain bone formation. MSCs were first harvested from bone-marrow aspirates but since have been found in several other tissues in the body—a property that makes MSCs an exceptional source of regenerative capacity in orthopaedics[16]. MSCs are not only capable of dividing and differentiating into several mesenchymal phenotypes, but, of equal importance, they have been shown to produce secretory molecules and cytokines that directly influence regeneration and mediate the functional outcomes of tissues[17]. MSCs also have been shown to secrete growth factors that have both autocrine and paracrine effects. Research has identified measurable levels of bioactive factors from MSCs, such as transforming growth factor-beta (TGF-β) and epidermal growth factor (EGF), which trophically enhance host progenitors to differentiate into functional regenerative tissues[18,19]. Current evidence even suggests that these paracrine trophic effects, rather than MSC differentiation, could be largely responsible for the repair process[17,20,21]. For instance, in cases of severe tissue ischemia involving the distal third of the meniscus, the damage site can produce inflammatory signals such as stromal cell-derived factor 1 (SDF-1) that can signal and attract MSCs from the marrow to the damage site[22,23]. MSCs can then trophically enhance its regeneration by secreting factors that assist the repair process.

Therapeutically, MSCs also have the advantage of being immune-evasive and immunosuppressive[24,25]. MSCs suppress immune recognition because they do not display major histocompatibility complex (MHC) class-II cell-surface markers, which highlights their lack of an immune response during allogeneic use. MSCs secrete bioactive factors that can directly expand B and T cells to help provide a supportive microenvironment for the long-term survival of hematopoiesis. In addition, MSCs secrete immunosuppressive factors such as nitric oxide (NO) and interleukin-10 (IL-10), which help to inhibit cell proliferation and prevent host-versus-graft rejection through the suppression of T-cell responses[25-27]. Many researchers have argued that because MSCs do not express these co-stimulatory molecules, *allogeneic* MSCs could be as effective as *autologous* MSCs. The combination of trophic and immunosuppressive properties highlights

the possibility that MSCs could be delivered systemically or directly to promote angiogenesis, inhibit fibrosis, and stimulate progenitor differentiation.

Despite these several advantages, MSCs also have a number of limitations. Studies have shown that, over time, MSCs eventually become senescent and irreversibly lose their capacity to divide[28]. The current number of in vitro cell divisions before MSCs enter senescence remains unknown; however, preliminary studies have shown that prolonged culture might limit their therapeutic applications[29]. Furthermore, tissue and bone regeneration is a complex process that combines MSCs and bioactive agents within a 3-dimensional scaffold to support the development of a tissue-specific extracellular matrix[30]. The ability to establish this appropriate environment is perhaps the greatest challenge to obtaining functional MSCs. Even after identifying the correct modality to expand MSCs, substantial challenges remain for facilitating host integration and controlling tissue-specific differentiation in vivo. Currently, these issues limit the application of MSCs, and further research is required to characterize the appropriate growth factors and scaffold technology to expand their indications.

Two modes of application can be used to apply MSCs: (1) cell therapy and (2) tissue engineering. Typically, the first step in developing MSCs is to aspirate bone marrow, commonly from the anterior or posterior iliac crest (which have been shown to have a higher yield for MSCs than other bone-marrow locations[31]). The aspirate is then centrifuged to concentrate the cells and the precipitate is then separated from the solution and expanded in culture media. Increasing the number and purity of cells in culture is a critical factor that can directly impact the eventual clinical effect[32]. The final step involves choosing the route of administration: percutaneous injection with a suitable scaffold or arthroscopic placement directly into a lesion. In contrast, tissue engineering combines MSCs with a 3-dimensional matrix, such as hydroxyapatite (HA), demineralized bone matrix (DBM), and/or tricalcium phosphate (TCP), to compose a construct to regenerate new tissues or organs[33]. These constructs have been successfully manufactured in the laboratory; however, clinical application is presently disallowed by the United States Food and Drug Administration (FDA) as only minimally manipulated cells (e.g., centrifuged cells) without carrier or delivery vehicle can be cleared for clinical use.

Trauma

Nonunions and Bone Defects

Despite the natural ability of most fractures to heal, some fractures still fail to unite or go on to fibrous union. Following conservative or operative treatment, a failure of bone fracture-healing occurs in 10% of all patients, or approximately 300,000 each year in the U.S[34]. In instances of delayed union or nonunion following trauma, autologous and allogeneic cancellous graft is commonly used to provide osteoconductive and osteoinductive substrates to optimize healing potential[35]. Studies have shown that treatment with DBM has led to successful consolidation at nonunion sites[36]. The use of bone graft is typically limited because of inadequate supply, transmission of infection, and donor-site morbidity. MSCs have demonstrated osteogenic potential and have been shown to be a source of bone formation to promote the regeneration of osseous defects. Although we are not aware of any Level-I randomized controlled trials regarding the use of MSCs for the treatment of nonunions, a number of successful laboratory and clinical studies have demonstrated the ability of MSCs to regenerate bone[37-40].

Several investigators have described the use of purified MSCs to treat segmental bone defects in preclinical models. Bruder et al. isolated and culture-expanded MSCs from normal human bone marrow, loaded them onto a ceramic carrier, and implanted them into critical-sized segmental defects in the femora of adult athymic rats[37]. The study revealed that femora that had been implanted with MSC-loaded ceramics were significantly ($p = 0.001$) stronger than those that received cell-free ceramics, which was one of the first demonstrations that human MSCs can regenerate bone in a clinically meaningful osseous defect. Connolly et al., in a study of 20 patients who were managed with MSCs for the treatment of nonunited tibial fractures over a 5-year period, reported sufficient callus formation to achieve union in 18 patients[38]. Garg et al., in a study on the use of percutaneous MSC grafting to stimulate healing of nonunited long-bone fractures, reported that 17 of 20 nonunions healed in 5 months[39]. Goel et al., in a prospective study on the use of percutaneous bone-marrow grafting for the treatment of established tibial nonunions associated with minimal deformity, reported clinical and radiographic union in 15 of 20 patients, with an average interval of 14 weeks between the first injection and union[40]. Hernigou et al. aspirated MSCs from the anterior iliac crest and injected them into noninfected atrophic nonunions of the tibia[32]. Bone union was obtained in 53 of 60 patients at a mean of 12 weeks. That study showed not only that percutaneous autologous bone-marrow grafting is an effective method for the treatment of atrophic tibial diaphyseal nonunions but also that optimal healing potential is actually also related to the number and concentration of progenitor cells in the graft. Despite the challenges presented by nonunions, those studies highlight evidence that MSCs may present a biological option for the treatment of nonunions and bone-healing defects.

Arthroplasty

Osteonecrosis

Osteonecrosis of the femoral head (ONFH) is a progressive disease characterized by a decreased blood supply to the bone and eventual articular cartilage collapse that typically affects younger populations[41]. Although multiple

etiological risk factors exist, the exact pathophysiology remains unclear. Even though various therapeutic options are available, including core decompression, osteotomy, and pharmacological agents, their efficacy has been limited, and as many as 40% of patients progress to total hip arthroplasty[42]. In turn, there has been increased focus on early interventions to preserve the native articulation. Given their osteogenic and angiogenic properties, MSCs have been introduced into areas of necrosis in the hopes of revitalizing and remodeling the necrotic bone and preventing collapse. Hernigou and Beaujean described a technique for the injection of MSCs combined with standard core decompression to repopulate the trabecular bone structure[43]. Their study included 189 hips (116 patients) that were followed for 5 to 10 years. The majority of patients with early disease (Association Research Circulation Osseous [ARCO] Stages I and II[44]) demonstrated satisfactory results (as indicted by improvement of the Harris hip score [HHS], radiographic findings, and refusal of total hip arthroplasty) at 5 years of clinical follow-up. Only 9 of the 145 hips with Stage-I or II disease at the time of intervention required total hip arthroplasty, compared with 25 of the 44 hips with Stage-III or IV disease. The authors also showed that patients who had had a greater number of progenitor cells transplanted into the hip had better outcomes. Gangji et al. conducted a controlled, double-blind, prospective study of 13 patients (18 hips) with ONFH (before collapse) who were managed with core decompression with or without concentrated bone-marrow aspirate according to the Hernigou method[45]. After 24 months, there was a significant reduction in pain and joint symptoms in the bone-marrow-graft group ($p = 0.021$). There was also a significant difference between the 2 groups in terms of the time to collapse ($p = 0.016$), and the volume of the necrotic lesions decreased by 35% in the bone-marrow-graft group.

Sen et al., in a study of 40 patients (51 hips) with ONFH who were randomized to treatment with core decompression or autologous bone-marrow mononuclear cell instillation into the core tract after core decompression, reported significantly ($p < 0.05$) better clinical outcomes (according to the HHS) and mean hip survival in patients who were managed with MSCs[46]. They also highlight that improvement was more marked in patients with poor prognostic features, including low HHS scores, the presence of radiographic changes, and edema and/or effusion on magnetic resonance imaging (MRI). Wang et al., in a study of 15 patients with Stage-II or III ONFH, evaluated a strategy involving thorough debridement that was based on the premise that a part of the technique leads to core decompression and revascularization of the femoral head[47]. The authors reported an overall success rate of 80% as determined on the basis of the HHS, radiographic progression, and the need for total hip arthroplasty. They highlight that their procedure is most effective in patients with small lesions and early-stage ONFH. Ultimately, with proper patient selection, the use of MSCs shows promising results as an effective treatment for early stages of ONFH.

Cartilage and Osteoarthritis

Articular cartilage has limited potential for self-regeneration, and injury to articular cartilage can lead to the development and progression of osteoarthritis (OA). In addition to the avascularity of articular cartilage, adult chondrocytes do not produce adequate functional matrix to compensate for damage and depletion, which contributes to arthritic changes[48]. Despite advances in pharmacological interventions for the treatment of OA, conventional strategies have not been as effective for preventing OA progression[49]. Although autologous chondrocyte implantation (ACI) has been shown to restore articular cartilage defects in otherwise normal joints, a thorough discussion of this technology is beyond the scope of the present review.

Several animal models have demonstrated the potential value of MSCs in the regeneration of articular cartilage. Shafiee et al. demonstrated that poly (vinyl alcohol)/polycaprolactone (PVA/PCL) scaffolds supported the proliferation and chondrogenic differentiation of MSCs in vitro[50]. Tay et al. showed that treatment with allogeneic undifferentiated MSCs resulted in higher Brittberg morphological scores and similar cartilage-regeneration profiles when compared with conventional autologous chondrocytes for the repair of focal articular cartilage defects[51,52]. Chiang et al., in a rabbit study, found that osteoarthritic knees that were treated with MSCs and hyaluronic acid (HA) underwent less cartilage loss, had fewer surface abrasions, and had improved cartilage when compared with contralateral knees that were treated with HA alone[53].

Nejadnik et al., in a study of 72 patients who were matched for age and lesion site, evaluated the clinical outcomes of cartilage repair with use of chondrocytes ($n = 36$) or bone marrow-derived MSCs (BMSCs) ($n = 36$)[54]. BMSCs were aspirated from the iliac crest and were cultured in media without antibiotics until being implanted beneath a periosteal patch from the proximal part of the tibia or distal part of the femur. Clinical outcomes were measured before the operation and at 3, 6, 9, 12, 18, and 24 months after the operation with use of the International Cartilage Repair Society (ICRS) Cartilage Injury Evaluation Package[55]. The authors reported that BMSCs were as effective as chondrocytes for articular cartilage repair and that their use also required 1 fewer knee operation, reduced costs, and minimized donor-site morbidity. In the study by Vega et al., 30 patients with chronic knee pain that had been unresponsive to conservative treatment and was associated with radiographic evidence of osteoarthritis were randomized to treatment with either MSCs (40×10^6 cells, administered by means of medial parapatellar injection) or intra-articular HA (60 mg,

administered as a single dose)[56]. The patients were followed for 1 year with regard to disability, pain, and quality of life. The patients in the MSC group exhibited significant ($p < 0.005$) improvement of algofunctional indices for OA and improved cartilage quality as measured with T2 relaxation measurements. Buda et al. reported on 30 patients with osteochondral lesions of the knee who underwent a 1-step procedure in which bone marrow was harvested from the posterior iliac crest and arthroscopically implanted with a collagen membrane scaffold into the lesion site[57]. After 3 years of follow-up, control MRI and biopsy samples showed osteochondral regeneration at the lesion site. Each of the studies described above highlights the potential of MSCs to inhibit the progression of OA.

Autologous Chondrocyte Implantation

Another cell-based treatment that has been implemented is autologous chondrocyte implantation (ACI). To fully appreciate the benefits of MSCs, it is useful to compare MSC-based treatments with ACI. ACI is a complex procedure that involves surgical removal of articular cartilage, the use of collagenases to digest the extracellular matrix and isolate the chondrocytes, and expansion of the cell count in vitro. After the cultivation of sufficient cells, the cultured chondrocytes are injected into periosteal graft covering the affected lesion[58]. Although ACI has shown success in promoting cartilage growth, studies have raised several concerns regarding the procedure and the eventual de-differentiation of the chondrocytes[59]. ACI requires harvesting healthy cartilage tissue for chondrocyte cultivation, which is a critical step that is bypassed when MSCs are used. The self-renewal capacity and multilineage differentiation potential of MSCs allow researchers to avoid the surgical step of cartilage biopsy that is required in ACI, which, depending on the depth and size of the harvested tissue, has been reported to be a potential cause of donor-site morbidity[60]. Other complications associated with the use of ACI for cartilage repair include hypertrophy of the cartilage or periosteal graft material, graft failure, and extended culture times[61]. In short, ACI has shown encouraging results to improve joint function without violating intact hyaline cartilage.

Sports

ACL Reconstruction

Although operative intervention remains the current standard of treatment for anterior cruciate ligament (ACL) tears, long-term clinical success rates still have not exceeded 85% to 90%[62,63]. The success of ACL reconstruction has largely been limited by delayed graft incorporation into the bone and failure to recreate the complex anatomy of the native ACL, leading to variability in long-term function and graft fixation[64]. Silva et al. prospectively randomized 20 patients to ACL reconstruction with adult non-cultivated BMSCs and 23 patients to ACL reconstruction without adult non-cultivated MSCs[65]. There was no difference between the groups in terms of the signal-to-noise ratio of the interzone on MRI at 3 months, and the authors concluded that BMSCs did not accelerate graft-to-bone healing in ACL reconstruction. Soon et al. performed bilateral ACL reconstruction with use of Achilles tendon allografts in 36 rabbits[66]. One limb received a graft coated with autogenous MSCs in a fibrin glue carrier, whereas the contralateral limb served as a control and received no MSCs. The study showed that although the MSC-enhanced grafts had significantly higher load-to-failure rates than the controls ($p < 0.05$), the stiffness and Young's modulus were lower in the treatment group. That study highlights that MSCs could have a role in tendon-bone healing during ACL reconstruction, but these grafts need to be further studied to investigate their usefulness.

Rotator Cuff and Tendons

Another research focus has been on using MSCs to improve the biological environment around healing tendons. Tendons are primarily composed of collagen and do not heal as quickly as other soft tissues because of their lack of vascularity[67]. MSCs have been used in animal models to increase the healing of the graft to bone and eventual long-term strength and overall function. Gulotta et al., in a study of 98 rats that underwent unilateral detachment and repair of the supraspinatus tendon, found that the addition of MSCs to the healing rotator cuff insertion site did not improve the structure, composition, or strength of the healing tendon attachment site[68]. However, Lim et al. reported that tendon grafts coated with MSCs showed accelerated tunnel healing from the tibial plateau to the tibial tuberosity and early remodeling of the tendon-bone junction in a rabbit model[69]. Ellera Gomes et al. investigated 14 patients with complete rotator cuff tears who were treated with transosseous stitches through mini-open incisions, with subsequent injection of bone marrow mononuclear cells (BMMCs), obtained from the iliac crest prior to surgery, into the tendon borders[70]. After a minimum duration of follow-up of 1 year, the mean UCLA shoulder score[71] increased from 12 ± 3.0 to 31 ± 3.2 and MRI analysis demonstrated tendon integrity in all 14 cases. The authors concluded that the implantation of BMMCs in rotator cuff sutures appears to be a safe and promising alternative to other biological approaches currently used to enhance tissue quality in affected tendons.

Young et al., in a rabbit Achilles tendon gap model, reported significantly greater load-related structural and material properties at all time intervals in tendons that were treated with MSCs than in contralateral (control) tendons that were treated with suture alone with natural cell recruitment[72]. Chong et al., in a rabbit study, tested the hypothesis that MSCs can accelerate tendon-healing after primary repair of a tendon injury[73]. The authors found no differences between the treatment and control groups with regard to the gross

morphology of the tendons but noted that biomechanical testing showed an improved modulus in the treatment group at 3 weeks. In a recent clinical case-controlled study, Hernigou et al. showed that BMSC injection as an adjunctive therapy during rotator cuff repair significantly ($p < 0.05$) enhanced the healing rate and improved the quality of the repair surface as determined with ultrasound and MRI[74]. At the time of the 10-year follow-up, intact rotator cuffs were found in 39 (87%) of 45 patients in the MSC-treated group, compared with only 20 (44%) of 45 patients in the control group. However, despite these successful findings, there is still no consensus on whether the use of MSCs is effective for enhancing rotator cuff healing.

Meniscus

Following conservative treatment of meniscal tears, arthroscopic resection is typically necessary. However, this procedure has been associated with early onset of OA and other complications[75,76]. In turn, the lack of noninvasive treatment for meniscal damage presents a major therapeutic challenge. Moriguchi et al., in a porcine model, showed that, after 6 months, scaffold-free, tissue-engineered construct (TEC)-treated defects were consistently repaired with a fibrocartilaginous tissue, with considerable tissue integration to the adjacent host meniscal tissue, whereas untreated defects were either partially repaired or not repaired[77]. Moreover, TEC treatment significantly ($p = 0.008$) reduced the size and severity of posttraumatic chondral lesions on the tibial plateau. Dutton et al. also studied the use of MSCs to enhance the healing of avascular meniscal tears[78]. Histological and macroscopic findings showed that the repair of meniscal tears in the avascular zone was significantly ($p < 0.001$) improved with MSCs, but the improvement only constituted about 25% of the normal biomechanical properties as expressed by Young's modulus. Vangsness et al. performed a randomized, double-blind, controlled study of 55 patients who underwent a partial medial meniscectomy[79]. Patients were randomized to receive an injection of 50×10^6 allogeneic MSCs (Group A), 150×10^6 allogeneic MSCs (Group B), or HA (control). The authors reported that there was a significant ($p = 0.022$) increase in meniscal volume (defined a priori as a 15% threshold) as determined by means of quantitative MRI and a significant ($p = 0.04$) reduction in visual analog pain scores when the patients in the MSC groups were compared with those in the control group.

Spine

Spinal Fusion

In certain patients, lumbar spinal fusion remains the only option to relieve back symptoms and restore spinal function. Autograft from the iliac crest has been the gold standard for fusion material, but the morbidity associated with the harvest and the desire for an off-the-shelf material or more efficient process have influenced investigators to find better methods[80-83]. In the study by Neen et al., 50 patients who were managed with Healos (a Type-I collagen/hydroxyapatite matrix; DePuy) soaked in bone-marrow aspirate were compared with 50 patients who were managed with autograft from the iliac crest[84]. The rates of radiographic fusion were equivalent for the 2 groups, with no significant difference in subjective and objective clinical outcomes. More importantly, there were no lasting complications in the Healos group, compared with a 14% rate of persistent donor-site complications in the autograft group. Gan et al. used a new method based on enriched bone marrow-derived MSCs combined with porous β-TCP in a study of 41 patients undergoing posterior spinal fusion[85]. After 34.5 months, 95.1% of the patients in the MSC group showed consolidation and only 2 had nonunion.

Intervertebral Disc Degeneration

Therapeutic approaches to discogenic back pain typically rely on conservative treatment and surgical options such as fusion with or without discectomy. Although these options can provide symptomatic relief, they do not address the underlying issue. The difficulty in understanding how MSCs can best be used therapeutically for this condition is that animal models do not mimic the slow, progressive degenerative changes seen in humans. Sakai et al. transplanted autologous MSCs into the discs of rabbits that had undergone a procedure proven to induce degeneration[86]. The study showed that MSC-transplanted model subjects had preserved disc structure with minimal degeneration at all time periods compared with degeneration-induced models. Furthermore, primary morphological features of disc degeneration, cell depletion in the nucleus pulposus, and disorientation of oval anular structure were prevented by injection of atelocollagen gel embedded with MSCs. In the study by Henriksson et al., 3 lumbar discs in each of 9 minipigs were injured by means of aspiration of the nucleus pulposus and then were injected with human MSCs[87]. The authors found that human MSCs survived in the porcine disc for at least 6 months and expressed typical chondrocyte markers suggesting differentiation toward disc-like cells.

Potential Challenges

Despite progress in the field of regenerative orthopaedics, a number of issues still need to be addressed prior to the general adoption of these clinical therapies. Stem-cell research also has come under close scrutiny by the FDA[88]. Currently, the only stem cell-based treatment approved by the FDA is bone-marrow transplantation[89]. There has been an ongoing debate about whether autologous MSCs are biological drugs subject to FDA approval or simply human cellular products. In early 2014, the U.S. Court of Appeals for the District of Columbia Circuit upheld a 2012 ruling that a patient's stem cells for therapeutic use fall under the aegis of the FDA[90]. The FDA strongly contends that any process that includes culturing, expansion, and the addition of growth factors or antibiotics requires regulation as the process constitutes substantial

manipulation. The FDA also has raised concern regarding the risk of tumorigenesis and the formation of ectopic tissue. However, Hernigou et al., in a study investigating the hypothesis that regenerative cell-based therapies could result in increased risk of local tumor recurrence, found no increase in this risk at an average of 15.4 years after cell-based therapy involving autologous MSCs[91]. Moreover, in a retrospective analysis of nearly 1,900 patients who were managed with bone marrow-derived concentrated cells, Hernigou et al. found no increase in the risk of cancer at the treatment site or elsewhere after an average duration of follow-up of 12.5 years[92]. Both of those investigations showed no justification for the current FDA position as the cancer risk remains theoretical and has not been shown to be increased in orthopaedic patients managed with MSCs.

Identifying the optimal time to incorporate regenerative therapies into the treatment of orthopaedic disease states is another key issue related to stem-cell therapy. Many questions remain with regard to the ideal patient population and the exact indications for when bioactive agents should be introduced to patient care. Another important prerequisite to MSC therapy is the ability to generate adequate numbers of the correct cells that specifically target the involved tissue. Cell expansion requires having the appropriate 3-dimensional extracellular matrix that is structurally and biomechanically compliant with the demands of host tissue. In order to be effective for therapy, cells also must be immunologically compatible and able to fully integrate with the native environment. Scientists must continue to build on existing animal models to answer these questions. Cost-effectiveness and insurance approval are other issues that will need to be addressed as stem-cell therapy becomes more commonly integrated into clinical orthopaedic practice.

Conclusion

Although MSCs have shown promise in orthopaedics, more high-quality, evidence-based research is required to better understand how to utilize these cells innovatively and effectively. Several orthopaedic conditions still have inadequate and costly treatment strategies that warrant further investigations of cell-based therapies. Delivery mechanisms, the timing of intervention, dosage size, and immunogenicity are issues that require a network of multidisciplinary investigators (including orthopaedic surgeons, molecular biologists, immunologists, bioengineers, and economists) for future study. Current evidence suggests that MSCs may provide an option for various musculoskeletal diseases, but several challenges remain in the translation of these regenerative therapies to clinical practice. Further research and long-term clinical trials are necessary to better understand MSCs and determine their exact role in the orthopaedic armamentarium.

Ameer Elbuluk, BA[1],
Thomas A. Einhorn, MD[1],
Richard Iorio, MD[1]

[1]Department of Orthopaedic Surgery, NYU Langone Medical Center, Hospital for Joint Diseases, New York, NY

E-mail address for A. Elbuluk: ameer.elbuluk@nyumc.org
E-mail address for T.A. Einhorn: thomas.einhorn@nyumc.org
E-mail address for R. Iorio: richard.iorio@nyumc.org

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