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Stem Cell Therapies for Treatment of Cartilage and Bone Disorders: Osteoarthritis, Avascular Necrosis, and Non-union Fractures

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Abstract

The general ease of availability and strong fundamental science of autologous mesenchymal stem cells has prompted increasing application of such biologic therapies to address inherent orthopedic challenges of limited vascularity and ability to self-repair. This article provides a concise review of emerging mesenchymal stem cell applications for bone-related pathologies including cartilage, avascular necrosis, and fractures.

Introduction

Orthobiologics is a thriving area of research and development, aimed specifically at preventing further degeneration and disease by restoring native biology, structure, and function. Cell-based therapy is a form of regenerative medicine that introduces new cells to repair damaged tissue. Because of their general ease of availability and strong fundamental science, autologous mesenchymal stem cells are the basis of increasingly applied biologic therapies to address the inherent orthopedic challenges of limited vascularity and ability to self-repair. Recent research suggests the increasing importance of subchondral bone integrity in various orthopedic conditions including osteoarthritis. Bone marrow lesions seen on T2 MRI sequences in osteoarthritic patients demonstrate histology similar to non-union fractures with necrosis and high osteoclast activity, and are becoming an important biomarker in disease progression [1]. This article provides a concise review of emerging mesenchymal stem cell applications for bone-related pathologies including cartilage, avascular necrosis (AVN), and fractures.

Cartilage Degeneration

Articular cartilage has a limited intrinsic capacity to regenerate spontaneously after injury, often leading to pain and disability. It is generally believed that cartilage lesions progress to osteoarthritis (OA), prompting intervention for symptomatic lesions to possibly prevent the evolution to OA as well as to provide symptom relief. Conventional treatment modalities may be useful for relief of symptoms in the short term; however, they do not restore the natural articular cartilage integrity or prevent the final pathway to OA [2].

The avascular, aneural, and alymphatic nature of articular cartilage hinders repair and regeneration potential once injured. Articular cartilage lesions may be focal defects resulting from direct trauma, AVN, or osteochondritis dessicans. These lesions are described as chondral (limited to the cartilage surface) or osteochondral (extending beyond the calcified cartilage layer into the subchondral bone). Chondral lesions have a poor intrinsic ability to repair themselves, as they lack blood vessels that are critical for circulation and delivery of progenitor cells as a part of the normal healing processes. Instead of progenitor cells filling chondral defects, cells from the synovial membrane migrate to the articular cartilage defect and fail to integrate completely, leading to continued degeneration. In contrast, osteochondral lesions have access to the bone marrow, which provides a supply of mesenchymal stem cells that can create the repair tissue. This tissue, however, resembles fibrocartilage, which does not integrate well with the adjacent matrix and does not

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withstand mechanical stress, resulting in eventual degeneration over time.

Articular cartilage lesions may also be more generalized, or diffuse and lacking lesion margins as in degenerative joint disease or OA. Once early OA begins, the repair capacity of articular cartilage is further compromised by a cascade of catabolic events including inflammation, recruitment of cells that release pro-inflammatory factors, and proteinase activation that leads to degeneration and cell senescence with apoptosis [3]. Disease progression is believed to result from an imbalance between pro-inflammatory cytokines (including interleukin [IL]—1a, IL-1, and tumor necrosis factor—α) and anti-inflammatory cytokines (including IL-4, IL-10, and IL-1ra) [3]. This cytokine imbalance is thought to promote proteolytic enzymes, which lead to cartilage deterioration [4,5].

In addition, the subchondral trabecular bone is thought to play an important role in OA, as subchondral bone changes are potentially both a result and a cause of cartilage loss [1].

Mesenchymal Stem Cells—Medicinal Signaling Cells

Mesenchymal stem cells are a promising therapeutic for cartilage regeneration. The exact mechanism of action of mesenchymal stem cells is not completely understood, but various means have been proposed. Through paracrine activity, mesenchymal stem cells exhibit a secretory or "trophic" function, with anti-inflammatory, immunomodulatory, pro-angiogenic, anti-apoptotic, anti-fibrotic, and wound-healing properties that have proliferative effects [2,6]. Mesenchymal stem cells have been shown to elicit differentiation of resident and non-resident cells to functional tissue, catalyzing restoration of degenerative tissue [7,8]. It has been suggested that perivascular cells, or pericytes, adhere to blood vessels and act as 1 of our body's largest reservoirs for mesenchymal stem cells. After trauma, soluble factors within the perivascular space cause the release of pericytes from microvessels. Pericytes have been described as "medicinal signaling cells" once released, where they can be activated into mesenchymal stem cells, exhibiting their homing, trophic and immunomodulatory roles [9].

Mesenchymal stem cells may be harvested from various tissues including adipose, bone marrow, synovium, and umbilical cord, as well as from peripheral blood. Thus far, the most frequently used source to treat cartilage lesions is derived from bone marrow.

Bone Marrow—Derived MSCs and Bone Marrow Concentrate

Bone marrow concentrate contains bone marrow—derived mesenchymal stem cells, hematopoetic stem cells, platelets (containing growth factors), and cytokines. Bone marrow cells consist of erythroblasts, neutrophils, eosinophils, basophils, monoid cells (monocytes containing mesenchymal stem cells and macrophages), lymphocytes, and plasma cells. These cells are present in various stages of differentiation [10]. The hematopoietic progenitor cells can morph into mesenchymal stem cells, differentiate into chondrocytes, and are more osteoinductive than adipose-derived cells [11]. Current research on undifferentiated colonization, functional bioactive components, and mechanisms of action for bone marrow—derived mesenchymal stem cells has yielded promising basic science results [6,11,12].

The anti-inflammatory and immunomodulatory properties of bone marrow—derived mesenchymal stem cells are essential in mediating tissue repair. The paracrine behavior of secreted bioactive growth factors, cytokines, and chemokines is responsible for the many functions of the mesenchymal stem cell immune response and healing potential [13]. Hematopoiesis is supported by bone marrow—derived mesenchymal stem cells through production of stem cell factor (SCF), interleukin (IL)—6, lymphocyte inhibitory factor (LIF), granulocyte macrophage-colony stimulating factor (GM-CSF), granulocyte-colony stimulating factor (G-CSF), or macrophage-colony stimulating factor (M-CSF) [14,15]. In addition, bone marrow—derived mesenchymal stem cells have been shown to exhibit homing to areas of inflammation through stromal-derived factor—1 (SDF-1) and the subsequent up-regulation of chemokine receptor type 4 (CXCR4) receptors on the cell surface [16,17].

After bone marrow aspiration, bone marrow concentrate is easily prepared using centrifugation and is available for a same-day injection procedure with minimal manipulation of cells, thus complying with U.S. Food and Drug Association (FDA) restrictions. Bone marrow concentrate is generated through density-gradient centrifugation of bone marrow aspirate. Bone marrow concentrate contains bone-marrow—derived mesenchymal stem cells, also known as bone marrow stromal cells, which have demonstrated benefit in facilitating the regeneration of cartilage.

A case study [4] demonstrated the regenerative potential of cultured bone marrow stromal cell therapy examining the use of mesenchymal cells in patients with meniscus and cartilage repair. Twenty-four weeks after percutaneous injection into the affected knee joint, the study reported significant increases in cartilage and meniscus volume, as seen on magnetic resonance imaging (MRI), with the patient attaining increased range of motion and decreased pain scores. Pilot studies have demonstrated improved patient pain scores and functional status after cartilage defect treatment with cultured bone marrow stromal cells [18]. An institutional review board—approved registry (Regenexx, IORG0002115) started in 2005 is currently collecting outcome and adverse effects data from more than 2500 patients who have received bone marrow concentrate injection treatment for various

orthopedic conditions. Preliminary and yet unpublished data collected from 539 cases of bone marrow concentrate application in the knee have demonstrated positive results. Of the patients who have returned for follow-up to date, 145 patients at 1 month, 98 patients at 1 year, 30 patients at 2 years, and 11 patients at 3 years, improvement in symptoms was demonstrated in 40%, 52%, 60%, and 68%, respectively [19].

In 2012, Emadedin et al [20], in a case series of 6 patients with severe osteoarthritis of the knee treated with cultured bone marrow stromal cells, demonstrated increased cartilage thickness and decreased edematous subchondral patches in 3 of the 6 patients, using MRI. Initial improvement in pain and function were reported; however, 6 months postinjection of cultured bone marrow-derived mesenchymal stem cells, all patients presented with recurring pain and decreased walking abilities [20]. Notably, the patients were all women volunteers (average age, 54.56 years), with 4 of 6 (67%) having a body mass index above 30 and were in need of joint arthroplasty.

The authors of this review reported our preliminary and yet unpublished clinical outcomes using bone marrow concentrate in 125 patients receiving hip, single knee, bilateral knees, shoulder, ankle, or cervical zygapophyseal joint bone marrow concentrate injections [21]. In all, 87 patients had both pre- and postinjection pain scores available for review, which demonstrated a 71% reduction in overall pain at a median follow up of 148 days, which was statistically significant. When comparing data from 87 patients with pre—post pain (complete) versus 38 patients with pre or post missing (incomplete) data, there was no evidence of selection bias, as both groups had similar characteristics (eg, age, body mass index, follow-up time, satisfaction). Comparing statistically significant results from all treated anatomic regions revealed that the single knee and bilateral knee injections had the largest improvement in pain score compared to the other joints treated. Furthermore, 92% of patients reported satisfaction with the procedure, and 95% of patients indicated that they would recommend the procedure to a friend. Contrary to prior reports in the literature of an inverse relationship, age had no correlation with outcomes in this cohort of patients up to 79 years of age reporting positive results. Bone marrow concentrate therapy has also been used as an adjunct therapy postoperatively to accelerate healing after procedures such as arthroscopic debridement, meniscal transplantation, and subchondroplasty [22]. There is some early evidence that this may improve the surgical results, but further studies are needed in this area.

Avascular Necrosis

AVN is a devastating disease characterized by the bone death caused by an interruption of the blood supply [23,24]. MRI studies have indicated that the conversion of red to fatty marrow occurs prematurely in some patients with AVN at the upper end of the femur. Consequently, intramedullary vascularity is altered; this may be a risk factor for osteonecrosis, because changes in the bone marrow and bone remodeling are linked. Another consequence is the lack of osteogenic cells and osteocyte death [25]. AVN presents often in young patients [26] and, without timely intervention, progresses to bone collapse and osteoarthritis, often resulting in unmanageable pain and disability [27]. Thus, early intervention is paramount to accomplish joint preservation [28]. Early AVN treatments remain controversial, as standard indications have not yet been established [27]. Because increased cell death and altered vascularity are common features of AVN [28], it is believed that blood vessel regeneration and collateral circulation are the most effective ways to break the pathological cycle of AVN [29].

Autologous bone marrow transplantation was proposed for the treatment of osteonecrosis in 1990 [25]. Researchers have applied intravascular infusion of mesenchymal stem cells to treat a variety of diseases, including AVN [30-38]. Intravascular infusion of mesenchymal stem cells is reported to have the advantage of minimal invasiveness and convenient operation, with fewer complications than core decompression [30-33]. Bone marrow has the hematologic component and the stromal system containing the mesenchymal stem cells [38]. The bone marrow is typically injected into the femoral head using a small trephine (Mazabraud, Collin, France). The instrument is introduced through the greater trochanter, as in conventional core decompression. Its position in the femoral head and in the necrotic segment is monitored with fluoroscopy. Hendrich et al reported retrospective data on the clinical and radiological progress of 101 patients with various bone healing deficiencies [39]. The study included 37 necroses of the head of the femur, 32 avascular necroses/bone marrow edema of other localization, 12 non-unions, and 20 other defects. The application of bone marrow concentrate was performed in the presence of osteonecrosis via a local injection as part of a core decompression ($n = 72$) or by the local adsorption of intraoperative cellular bone substitution material (scaffold) incubated with bone marrow concentrate during osteosynthesis ($n = 17$) or in further surgery ($n = 12$). Positive clinical and radiographic effects without complications were reported at 14-month follow-up [39]. Autologous bone marrow concentrate treatment has been studied for corticosteroid-induced osteonecrosis of the femoral head in systemic lupus erythematosus. At a minimum follow-up of 3 years, significant improvements in pain and Harris Hip Score were observed, suggesting therapeutic osteogenesis for corticosteroid-induced osteonecrosis of the femoral head in systemic lupus erythematosus [40].

Kumar Sen et al reported on 51 osteonecrotic hips in 40 patients who were randomly divided into 2 treatment groups [41]. Patients in group A (25 hips) were treated with core decompression, and those in group B (26 hips) received autologous bone marrow mononuclear cell instillation into the core tract after core decompression. The clinical score and mean hip survival were significantly better in group B than in group A. Adverse prognostic features at initial presentation, including poor Harris Hip Score, radiographic changes, edema, and/or effusion on MRI had significantly better clinical outcome and hip survival in group B than in group A [41].

Targeted intra-arterial delivery has been used as a strategy for intravascular implantation of stem cells [42]. In 2013, Mao et al reported their 5-year results using the medial circumflex femoral artery to deliver concentrated autologous bone marrow mononuclear cells from bone marrow harvested from the anterior iliac crest to treat AVN of the femoral head. In all, 72 of 78 hips (92%) achieved a satisfactory clinical result, whereas only 6 hips (8%) progressed to clinical failure and required total hip arthroplasty. The mean Harris Hip Score increased from 59 points at baseline to 74 points at 60 months. Five years after the treatment, 3 of 10 hips (30%) in Ficat stage III had deteriorated to clinical failure, whereas only 3 of 68 hips (4%) in a combination of Ficat stages I and II had progressed to clinical failure ($P < .05$). Kaplan-Meier survival analysis showed a significant difference in the time to failure between the precollapse hips (Ficat stages I and II) and the postcollapse hips (Ficat stage III) at 5-year follow-up (log-rank test; $P < .01$). No complication was found in any patients. They concluded that targeted intra-arterial delivery of autologous bone marrow mononuclear cells via medial circumflex femoral artery is a safe, effective, and minimally invasive treatment strategy for early-stage osteonecrosis of the femoral head. It is capable of relieving symptoms, improving hip function, and delaying progression of the disease. The clinical outcome appears to be better when it is applied before the collapse, with early intervention.

Fractures

Electron microscopy has shown that bone marrow cells are responsible for the manufacturing of the bony callus. Consolidation of a fracture is often delayed in heavy smokers and drinkers. There is a significant adipose involution of the bone marrow in these patients and therefore a potential decrease in progenitor cell number. Challenges in consolidation of a fracture may be connected to an overall deterioration in the numbers of progenitor cells in the bone marrow from underlying physiologic obstacles [24].

Bone marrow aspirate contains osteoprogenitor cells that have osteogenic and osteoinductive properties [22]. Bone marrow aspirate from the iliac crest has been applied to nonunion sites with limited morbidity; however, the number of stem cells available from bone marrow aspirate is limited. A trocar identical to that used to aspirate the marrow is generally placed intraosseously, either at the site of the pseudarthrosis or in the ends of the fracture adjacent to it. Typically, weight bearing is not allowed during the first month after bone marrow transplantation, to avoid mechanical instability interfering with the progression of tissue regeneration and healing. After 1 month, after callus is observed on radiographs, partial weight bearing is allowed with plaster or external fixation. A period of 1 month is observed between partial weight bearing and full weight bearing, with radiographic monitoring for cortical bridging or removal of fracture lines, after which the casting or fixation is removed [24].

Samir Kassem et al published results on 20 patients with internally fixed fractures with delayed union or nonunion with a mean of 9.65 months between initial surgery and marrow injection [43]. Of the 20 fractures, 19 achieved clinical and radiological union, on average after 2.95 months. Padha et al studied 50 cases of post-traumatic delayed and non-unions and noted that 46 of 50 cases (92%) had successful union, whereas 4 had failures, with percutaneous bone marrow injection [44].

Braly et al reported the outcomes of percutaneous autologous bone marrow injection for nonunion or delayed union of the distal tibial metaphysis in patients with prior plating [45]. Of the 11 patients, 9 attained bony union within 6 months of bone marrow injection. The authors concluded that percutaneous autologous bone marrow injection is a minimally invasive, safe, and inexpensive treatment option for distal metaphyseal tibial nonunions or delayed unions after internal fixation and should be considered when the retained hardware is intact and stable [45].

Hernigou et al reported their results in 60 noninfected atrophic nonunions of the tibia in which a volume of 20 cm^3 of bone marrow concentrate was injected [46]. They concluded that percutaneous autologous bone-marrow grafting is an effective and safe method for the treatment of an atrophic tibial diaphyseal nonunion. Importantly, they also noted that the efficacy is related to the number of progenitors in the graft, and the number of progenitors available in the iliac crest bone marrow aspirate was less than optimal without concentration [46]. Various aspirations and cell concentration techniques have been used to increase the number of progenitor cells, with reported union rates ranging from 62.5% to 90% [46-50]. Notably, these reports are of case series, lacking control groups to differentiate between the effect of the bone marrow aspirate or bone marrow

concentrate and the other concomitant interventions. Importantly, how a bone marrow aspirate injection into a long-standing nonunion results in bone union without debridement of the intervening fibrous tissue remains unknown.

Discussion

Mesenchymal stem cells are being used for their therapeutic potential to enhance the regeneration of cartilage and bone to prevent or modify disease progression and bone marrow concentrate, with its mesenchymal stem cell and hematopoetic stem cell populations, along with abundant growth factors, exhibits anti-inflammatory, immunosuppressive, osteoinductive, and chondrogenic qualities. The exact mechanisms of action of bone marrow concentrate remain unknown, however, it is postulated that the cells either induce proliferation and differentiation of resident stem cells, and/or possess innate differentiation potential. Along with mesenchymal stem cells, hematopoetic stem cells, and progenitor cells, exosomes are also considered to have a possible role in facilitating regeneration, particularly in secreting proteins that may direct repair [51]. An exosome is a nanoparticle or secreted granule found in blood, saliva, and stem cell cultures, which has a function in cell-to-cell communication, paracrine signaling, autocrine signaling [51]. Future research may focus on the transfer of genetic information, which is now thought to occur with exosomes. Watson reported that the key to understanding how cell or platelet-based therapy works may not be the cell itself but understanding how the secreted exosomes work, suggesting an influence on exosome production, content, secretion, and selection of specific target cells [51]. Further studies are needed to better understand the role of bone marrow concentrate therapy in reducing pain and increasing function in patients with musculoskeletal disease. Future exploration through dosage-based trials, bioactive adjuncts, and protein scaffolding may reveal the optimal method for application of regenerative medicine. Clinically, treatment of patients with bone marrow concentrate is readily performed by visualized guided needle injection for musculoskeletal disease therapy. Further studies are needed to determine the exact mechanisms of action among the cells, growth factors, and exosomes, as well as the long-term durability of the tissue treated.

Although sufficient human safety and efficacy data are increasing, there are sparse data for the therapeutic effectiveness of same-day bone marrow concentrate therapy in patients with musculoskeletal disorders. There is no standardized technique for bone marrow concentrate or injection protocol at this time. Many significant questions remain unanswered, such as the ideal cell harvest technique, cell preparation, as well as optimal windows for various indications and injection protocols. These early regenerative medicine therapies will likely serve as precursors for more customized, refined cellular therapies using serum and genetic biomarkers to better understand the mechanism of action and to identify optimal methods of treatment. The integration of mesenchymal stem cell therapy into routine clinical use is intriguing but must be preceded by the effective demonstration of long-term safety and efficacy through publication of rigorous research studies.

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