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Stem Cells Trans Med 2012, 1:237-247.
doi: 10.5966/sctm.2011-0036 originally published online February 22, 2012

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Concise Review: The Clinical Application of Mesenchymal Stem Cells for Musculoskeletal Regeneration: Current Status and Perspectives

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Key Words. Tissue regeneration • Adult stem cells • Bone marrow stromal cells • Cellular therapy • Chondrogenesis • Cell transplantation • Marrow stromal stem cells • Mesenchymal stem cells

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Received October 5, 2011; accepted for publication January 12, 2012; first published online in *SCTM EXPRESS* February 22, 2012.

©AlphaMed Press
1066-5099/2012/\$20.00/0

<http://dx.doi.org/10.5966/sctm.2011-0036>

ABSTRACT

Regenerative therapies in the musculoskeletal system are based on the suitable application of cells, biomaterials, and/or factors. For an effective approach, numerous aspects have to be taken into consideration, including age, disease, target tissue, and several environmental factors. Significant research efforts have been undertaken in the last decade to develop specific cell-based therapies, and in particular adult multipotent mesenchymal stem cells hold great promise for such regenerative strategies. Clinical translation of such therapies, however, remains a work in progress. In the clinical arena, autologous cells have been harvested, processed, and readministered according to protocols distinct for the target application. As outlined in this review, such applications range from simple single-step approaches, such as direct injection of unprocessed or concentrated blood or bone marrow aspirates, to fabrication of engineered constructs by seeding of natural or synthetic scaffolds with cells, which were released from autologous tissues and propagated under good manufacturing practice conditions (for example, autologous chondrocyte implantation). However, only relatively few of these cell-based approaches have entered the clinic, and none of these treatments has become a “standard of care” treatment for an orthopaedic disease to date. The multifaceted reasons for the current status from the medical, research, and regulatory perspectives are discussed here. In summary, this review presents the scientific background, current state, and implications of clinical mesenchymal stem cell application in the musculoskeletal system and provides perspectives for future developments. *STEM CELLS TRANSLATIONAL MEDICINE* 2012;1:237–247

INTRODUCTION

Mesenchymal stem cells (MSCs) hold great promise for regenerative therapies in the musculoskeletal system. The limited resources of differentiated cells and tissues are thought to be overcome by MSC populations that can be readily harvested from bone marrow, blood, or mesenchymal tissues, including bone, cartilage, meniscus, ligaments, and tendons, among others [1]. Techniques of MSC isolation, expansion, and differentiation have been added to the therapeutic repertoire for musculoskeletal regeneration [1]. In spite of sporadic reports of adverse events that were reported in association with adult MSC delivery in experimental settings, such as heterotopic bone or tumor formation [2], controlled MSCs delivery is generally perceived as a safe procedure, and the number of clinical trials using ex vivo expanded stromal cell populations for therapeutic purposes are rapidly increasing (see <http://www.clinicaltrials.gov>).

To date, MSCs derived from adult donors are usually applied in the clinical setting, and have limited differentiation capacities. However, human embryonic stem cells (ESCs) derived from the inner cell mass of blastocysts, which exhibit unlimited in vitro self-renewal capacity and pluripotency, reflect another potential cell source for the repair of diseased human musculoskeletal tissues [3, 4]. The predisposition of ESCs to form teratomas, even after predifferentiation ex vivo because of residual undifferentiated cell populations, complicates their possible clinical application [3, 4]. Furthermore, the remaining unsolved political and ethical concerns and barriers concerning the use of ESCs currently prevent this cell type from entering the clinical and also research arena in numerous countries. To circumvent the use of ESCs or allogeneic cells in general, researchers of several groups have achieved reprogramming of adult fibroblasts to ESC-like, pluripotent stages, by retroviral transduction with variable different transcription factors, including c-MYC, Klf4, Nanog, Lin28, Oct-4,

and SOX-2 [5–8]. Although these inducible pluripotent cells elicit some promising features that might allow their application in tissue regeneration [9], more refined protocols for such applications have to be developed along with techniques to circumvent the numerous problems associated with gene transfer in the clinical setting. Therefore, MSCs from adult donors are thought to be the most promising candidate cell type to date to augment cell-based orthopaedic tissue regeneration in the clinical setting.

Despite the large amount of research findings on the use of MSCs for various regenerative approaches in the musculoskeletal system in experimental settings *in vitro* and *in vivo*, as reviewed elsewhere [1], there are only limited amounts of published clinical studies using MSC-based approaches for such purposes to date, due to medical, research, and regulatory reasons. Therefore, in this review we have chosen to highlight some unique therapeutic features of adult MSCs, summarize their current state of clinical application in the musculoskeletal system, and provide prospects for future developments.

PRINCIPLES OF ADULT MSC BIOLOGY AND DELIVERY

Adult MSCs represent usually a heterogeneous population of cells, with a positive immunophenotype for STRO-1, CD73, CD146, and CD106 and a negative one for CD11b, CD45, CD34, CD31, and CD117, based on a recommendation of the International Society for Cellular Therapy [10]. MSCs act via multifaceted pathways that are not completely understood to date to augment regeneration, including mechanisms that mediate homing of administered MSCs to sites of injury. However, two main inherent functions of MSCs can be distinguished. The first is the secretory or “trophic” function of MSCs [11], which includes the secretion of a wide spectrum of factors with immunomodulatory, anti-inflammatory, antiapoptotic, proangiogenic, proliferative, or chemoattractive capacities, among others [12–14]. Second, administered MSCs are thought to orchestrate the differentiation process together with differentiated or undifferentiated resident cells for functional tissue restoration [12, 14]. For regeneration, these MSC functions can be augmented by the coadministration of soluble growth and differentiation factors, nucleic acids, natural or synthetic biomaterials, or mechanical stimuli. Given the complexity of each of the therapeutic components involved, along with the different modes of delivery of each alone and in combination, there is currently no fully developed treatment for any orthopaedic application [1]. Given the different modes of MSC delivery as well as the time and efforts necessary for performing meaningful randomized controlled clinical trials, the search for such an optimized procedure has remained elusive. However, several studies have been conducted that use mesenchymal stromal cell-based approaches in the clinical setting (Table 1), and some of the basic principles of stromal cell delivery are now outlined.

A successful regenerative approach based on the use of autologous material usually comprises the harvest of cells either derived from blood by venous puncture or derived from bone marrow by, for example, aspiration from iliac crest or by surgical removal from a donor site, with increasing levels of invasiveness (Fig. 1, red box). Further processing of such autologous materials can be conducted via several pathways (Fig. 1, yellow box). The simplest means of cell delivery to the musculoskeletal system is direct injection of cells. Although injection of whole blood or marrow, or concentrates thereof, usually can be performed in

the operating room without major regulatory obstacles, the administration of *ex vivo* processed cell preparations requires strict compliance with good medical practice (GMP) and also nation-specific requirements. Depending on the application, the suspension can be injected directly into the diseased tissue, where the cells eventually populate the target site and stimulate repair via autocrine or paracrine pathways (Fig. 1, yellow box). Examples of such direct approaches are MSC injection therapies for the treatment of arthritis, tendinitis, or tendon ruptures [15, 16]. In cases where large tissue defects need to be restored, cells are usually delivered via a scaffold to the target tissue. Such constructs are used to regenerate structural defects in bone or cartilage [17–19]. For this purpose, large numbers of natural or synthetic biomaterials have been tested specifically for almost every target cell and tissue, as reviewed extensively elsewhere [1, 20–28]. The physicochemical characteristics of such materials should ideally facilitate appropriate cell adherence, proliferation, differentiation, and matrix synthesis, while providing biodegradability, as well as suitable mechanical strength [26, 27]. Given the heterogeneity of the different orthopaedic tissues, these requirements vary greatly, making refined strategies necessary.

An important issue for the success of any stem cell therapy is the quality of the cells administered, with regard to cellular integrity, age, senescence, proliferative and differentiative capacity, and intact paracrine function [29, 30]. In this regard, the use of cells that are harvested intraoperatively, such as whole blood, marrow, or concentrates thereof, has the advantage that cells have not been grossly manipulated and subjected to stress factors during cell culture that may impair their regenerative functions such as induced replicative senescence (Fig. 1, yellow box). However, the disadvantage is that such suspensions are heterogeneous and contain unidentified components that may be disadvantageous for therapy, such as high ratios of red blood cells or fibrin within neocartilage regenerates. *Ex vivo* cell preparations are subjected to additional external influences and stress factors but will allow for enhancements, such as predifferentiation, selection, and control of the desired subpopulation of cells (Fig. 1, yellow box).

All of the mentioned modes of cell delivery might be augmented by the use of other regenerative ingredients such as biomaterials, soluble factors, nucleic acids, or physical stimuli (Fig. 1, green box). However, the best possible combinations of these regenerative elements need to be determined. Many of these complex approaches have been reviewed extensively elsewhere for tissue engineering of cartilage, bone, muscle, tendons and ligaments, disc, and meniscus [1]. Another approach is to build on currently approved clinical practices, which are discussed below.

CLINICAL MESENCHYMAL STROMAL CELL APPLICATIONS FOR MUSCULOSKELETAL REGENERATION

Mesenchymal stromal cells have been used clinically for several regenerative approaches in the musculoskeletal system, as outlined in Table 1. Some of the specifics, including the type of cell preparation used, the nature and extent of the disease treated, and other study parameters, are detailed below.

Table 1. Present status of clinical cell delivery therapies for musculoskeletal regeneration

Reference	Number	Indication	Results
Bone defects and nonunions			
Connolly 1998 [36]	<i>n</i> = 20	Nonunions of the tibia	Case series reporting on injection of unprocessed bone marrow. Healing occurred in 18 of 20 cases after a mean of 6–8 months (± 1.25)
Warnke et al. 2004 [40]	<i>n</i> = 1	Mandibular defect	Heterotopic bone induction to form a mandible replacement inside the latissimus dorsi muscle after application of 20-ml bone marrow suspension delivery together with 7 mg BMP-7 in a human being
Hernigou et al. 2005 [53]	<i>n</i> = 60	Nonunions of the tibia	Positive correlation between the volume of mineralized callus at 4 months and the number ($p = .04$) and concentration ($p = .01$) of fibroblast colony-forming units in the graft
Dallari et al. 2007 [38]	<i>n</i> = 33	Patients undergoing high-tibial osteotomy	Significantly increased osteoid areas in bones treated with addition of a platelet gel or a platelet gel combined with bone marrow stromal cells to lyophilized bone chips
Schmid et al. 2007 [39]	<i>n</i> = 125	Bone defects of lower limb, primary, and revision THA and fractures	Bone grafting with composite transplants composed of autogenous marrow cells and industrial processed bone allografts was a good alternative to conventional bone grafting
Ochs et al. 2008 [41]	<i>n</i> = 78	Acetabular defects in revision THA	No difference in functional outcome after impaction bone grafting using a frozen nonirradiated bone bank allograft or a freeze-dried irradiated bone allograft, vitalized with autologous marrow
Hendrich et al. 2009 [42]	<i>n</i> = 101	Bone defects <ul style="list-style-type: none"> • 37 avascular necrosis hip • 32 avascular necrosis others • 12 pseudarthrosis • 20 other defects 	Safety study and surveillance, no control group, use of BMAC as suspension or via a matrix was shown to be safe at 2–24 month follow-up
Jäger et al. 2011 [43]	<i>n</i> = 39	Bone defects	Comparative efficacy study comparing BMAC delivered together with a matrix of collagen ($n = 12$) or hydroxyapatite ($n = 27$), with superior results for the HA group
Quarto et al. 2001 [44]	<i>n</i> = 3	Nonunions of long bones	After ex vivo expansion BMSCs on macroporous hydroxyapatite scaffolds showed abundant callus formation 2 months after surgery
Kitoh et al. 2007 [45]	<i>n</i> = 20	Distraction osteogenesis of long bones	Bones treated with culture-expanded BMCs and PRP showed a significantly higher healing index than a control group treated with no cell-based therapy
Kitoh et al. 2007 [46]	<i>n</i> = 46	Distraction osteogenesis of long bones	Bones treated with culture expanded BMCs and PRP showed accelerated bone healing and less complication than bones treated without a cell-based approach
Avascular necrosis of the hip			
Hernigou and Beaujean 2002 [37]	<i>n</i> = 116	Avascular necrosis of the hip ARCO I–IV	Positive correlation between the number of progenitor cells within the marrow aspirate transplanted in hips and the respective outcome
Gangji et al. 2004 [48]	<i>n</i> = 13	Avascular necrosis of the hip ARCO I–II	Significant difference in the time to collapse between core decompression and implantation of autologous bone marrow cells; better functional outcome (WOMAC, Lequesne index) within the bone marrow group
Kawate et al. 2006 [54]	<i>n</i> = 3	Avascular necrosis of the femoral head ARCO I–III	Transplantation of culture expanded and autologous bone marrow stromal cells together with β -TCP matrix and grafted fibula showed full defect healing and neo-bone formation with good results; collapsed heads still critical
Nöth et al. 2007 [19]	<i>n</i> = 3	Avascular necrosis of the femoral head ARCO II	Transplantation of culture expanded and autologous bone marrow stromal cells embedded in a β -TCP matrix showed full defect healing and no recurrence of the necrosis after 6 weeks
Cysts and benign tumors of the bone			
Wright et al. 2008 [55]	<i>n</i> = 77	Simple bone cysts	Delayed healing of bone cysts treated with intralesional injections of bone marrow aspirate compared with those treated with methylprednisolone acetate injection
Park et al. 2008 [56]	<i>n</i> = 20	Bone cysts of the calcaneus	Injection of irradiated allogeneic demineralized bone powder and autogenous bone marrow showed comparable results to treatment with open surgery and lyophilized irradiated chips of allogeneic bone
Zamzam et al. 2009 [57]	<i>n</i> = 28	Simple bone cysts	Percutaneous autogenous bone marrow injection provided healing in 82% of the cases
Köse et al. 1999 [58]	<i>n</i> = 12	Simple bone cysts	Percutaneous autogenous bone marrow injection provided healing in two of the cases, improvement in four, and recurring disease in six cases

(continued)

Table 1. (continued)

Reference	Number	Indication	Results
Lokiec et al. 1996 [59]	<i>n</i> = 10	Simple bone cysts	Percutaneous autogenous bone marrow injection to cysts in humerus, femur, and tibia provided healing in all 10 cases
Jäger et al. 2009 [60]	<i>n</i> = 10	Bone defects, nonunion, bone cysts, enchondroma	Reduction of donor site morbidity by using bone marrow aspiration together with type I collagen carrier
Spine			
Neen et al. 2006 [62]	<i>n</i> = 50	Lumbar spine fusion	No differences in clinical and radiological outcome between type I collagen/hydroxyapatite matrix combined with BMA and conventional autologous bone grafting, but no donor site morbidity
Zhang et al. 2008 [63]	<i>n</i> = 56	Posterior lumbar spinal fusion	Enriched bone marrow-derived MSCs combined with porous β -TCP showed a successful spinal fusion in 95.6% similar to iliac crest bone autograft
Cartilage lesions			
Wakitani et al. 2004 [76]	<i>n</i> = 2	Full-thickness articular cartilage defects in the patella	Two years after transplantation of culture expanded and collagen gel-embedded autologous bone marrow stromal cells, the defects were repaired with fibrocartilage
Kuroda et al. 2007 [78]	<i>n</i> = 1	Full-thickness articular cartilage defect in the femoral condyle	Seven months after surgery the defect was filled with a hyaline-like type of cartilage tissue which stained positively with Safranin-O after implantation of culture expanded and collagen gel-embedded autologous bone marrow stromal cells
Wakitani et al. 2007 [77]	<i>n</i> = 5	Full-thickness articular cartilage defect in the patellofemoral joint	Transplantation of culture expanded and collagen gel-embedded autologous bone marrow stromal cells showed functional improvement after 6 months and fibrocartilage in 1 patient investigated histologically after 12 months
Nejadnik et al. 2010 [79]	<i>n</i> = 72	Full-thickness articular cartilage defects	Transplantation of culture expanded and, collagen gel-embedded autologous BMSCs versus chondrocytes (ACI) showed functional similar results after 2 years' follow-up, but without the disadvantage of cartilage biopsy harvest in BMSC group
Centeno et al. 2010 [81]	<i>n</i> = 213	Osteoarthritis and other intraarticular pathologies	Injection of ex vivo expanded BMSCs using the platelet lysate technique, uncontrolled safety and observational trial, seven minor complications due to the injections reported, no signs of local tumor formation on magnetic resonance imaging evaluations postoperatively, 24-month follow-up
Davatchi et al. 2011 [82]	<i>n</i> = 4	OA knee	Uncontrolled pilot study, injection of ex vivo expanded MSCs derived from 30 ml of bone marrow from each patient resulted in mild clinical improvement of clinical symptoms
Tendons and ligaments			
Connell et al. 2009 [90]	<i>n</i> = 12	Lateral elbow epicondylitis	Injection of expanded autologous dermal fibroblasts improved clinical scores and response in ultrasound investigations at 1.5-, 3-, and 6-month follow-up; no control group
Clarke et al. 2011 [94]	<i>n</i> = 46	Refractory patellar tendinopathy	Injection of expanded autologous dermal fibroblasts with autologous plasma versus autologous plasma only showed faster healing response in ultrasound investigations and faster recovery clinically in the cell-based treatment group at 6 months

Abbreviations: ACI, autologous chondrocyte implantation; ARCO, Association Research Circulation Osseous; BMA, bone marrow aspirate; BMAC, bone marrow aspirate concentrate; BMC, bone marrow cell; BMSC, bone marrow stromal cell; BMP, bone morphogenetic protein; MSC, mesenchymal stem cell; OA, osteoarthritis; PRP, platelet-rich plasma; β -TCP, β -tricalcium phosphate; THA, total hip arthroplasty; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

Bone Regeneration

Bone has a significant natural tendency towards spontaneous healing and functional restoration without significant scarring. However, there are many conditions where this feature is impaired, such as in large defects of bone due to trauma, tumor, infection, aseptic loosening, or nonunions. Therefore, substantial research efforts in the field of orthopaedics and regenerative medicine have been devoted towards the development of improved treatments for such critical bone defects [31–33]. These investigations have led to the identification of a number of factors with strong osteogenic potency, including several of the bone morphogenetic proteins (BMPs), of which the human recombinant BMP-2 and BMP-7 (OP-1) have been approved by the U.S. Food and Drug Administration (FDA) in 2004 for restricted

clinical use. Such factors might be applied together with a suitable biomaterial and/or cell preparation to devise a potentially effective approach to reconstructive surgery [34]. In fact, in the last 10–20 years, a number of clinical studies have shown that cell preparations, including bone marrow, bone marrow concentrates, and ex vivo cell preparations, can be efficiently used to facilitate bone healing in nonunions, critical-sized segmental defects, or osteonecrosis, as summarized in Table 1.

Large Bone Defects and Nonunions

Successes in several small- and large-animal models of disease have set the stage for the use of MSCs to restore large bone defects in the clinical setting [35]. In 1998, Connolly initially reported successful treatment of tibial nonunions in 18 of 20 patients examined at 6–8-month follow-up [36]. Using a similar

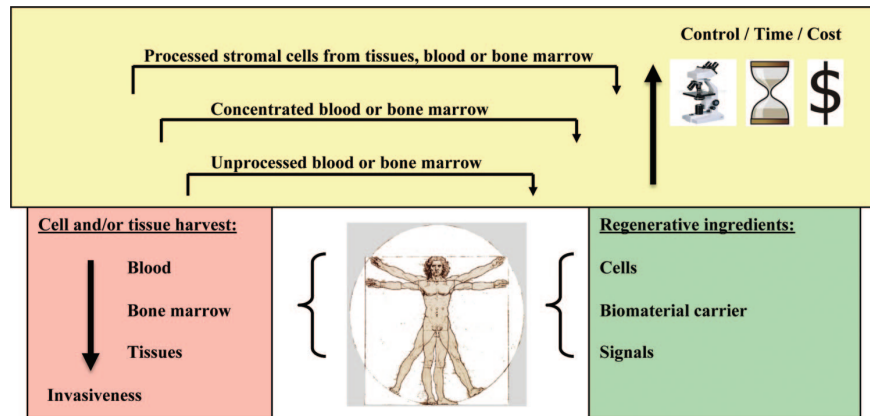


Figure 1. Schematic illustration of cell delivery for musculoskeletal regeneration. Cell delivery can be facilitated in various fashions for the purpose of musculoskeletal regeneration. First, the cells can be retrieved from the human body and readministered without further modification, such as injection of autologous blood or bone marrow. Although marrow suspensions are thought to contain a higher percentage of stromal cells than peripheral blood, both represent crude mixtures with overall low abundance of mesenchymal stem cells (MSCs). MSC density can be increased by centrifugation of whole blood or marrow or recovery from cell filters, clotting, or other concentration methods. These methods are relatively simple, making them appealing for single-step usage procedures in the operating room, and, therefore, are not associated with high regulative hurdles. However, blood and marrow concentrates or coagulates still have to be considered very crude and undefined mixtures. Alternatively, cells and in particular MSCs can be harvested from blood, bone marrow, and tissues by digestion and adherent culture. Such cell populations are usually maintained in tissue culture for amplification under controlled good medical practice conditions with usually autologous serum. This method offers the opportunity for close monitoring of the cells under safety and quality aspects, as well as further cell selection. However, this approach presents the highest regulatory levels, in the context of specific requirements of each country and continent (European Medicines Agency and U.S. Food and Drug Administration). The use of unprocessed as well as ex vivo processed cells might be enhanced and supplemented by the use of a biomaterial scaffold, soluble factor, nucleic acid, or mechanical stimulation. Additional specific regulatory requirements must be met for the use of each of these supplements, as well as for their combined usage, indicating the complexity for such approaches from a biological, medical, and regulatory perspective.

approach, Hernigou and colleagues correlated the quantity of mineralized bone formation with the number of colony-forming units (CFUs) within the grafted marrow following percutaneous injection to the tibial nonunions and reported good results in 60 patients [37]. The percutaneous injection technique was not performed in strong deformities, which could be considered to be a limitation of this procedure [37]. However, because of its biological value and low associated risks, bone marrow aspirates have been used in several other studies investigating open surgery procedures for bone healing in high-tibial osteotomies combined with lyophilized bone chips [38], healing of bone defects ($n = 125$) of variable sizes combined with devitalized bone allografts [39], and ectopic bone formation together with titanium carriers and recombinant BMP-7 [40]. No additional effects were seen when nonirradiated frozen bone-bank allografts were compared with freeze-dried irradiated bone allografts vitalized with autologous bone marrow for the treatment of acetabular defects in revision total hip arthroplasties [41].

To increase the numbers of CFU fibroblasts within the cell preparation, centrifugation methods for the generation of bone marrow concentrates have been applied in the context of bone regeneration that still avoids any ex vivo cell culture. The safety of the application of such bone marrow aspirate concentrates (BMACs) has been shown in 101 bone defects of variable etiology, including pseudarthrosis, avascular necrosis, and others [42]. A comparative follow-up study by the same group showed superior results when BMAC was applied together with a hydroxyapatite (HA) matrix, compared with a collagen matrix, in a total of 39 defects [43]. However, biomaterial-only controls were not included in this study [43].

In contrast to the single-step procedures, several studies have also investigated the use of ex vivo expanded mesenchymal stromal cells for the regeneration of bone. In an initial phase I clinical trial, segmental bone defects of three patients were successfully treated with MSCs that were used together with HA carriers [44]. When bones were treated with culture expanded bone marrow cells together with platelet-rich plasma (PRP) during distraction osteogenesis approaches that included 20 [45] or 46 [46] cases, significantly higher healing rates were observed than in the control groups without cell-based therapies [45, 46].

Osteonecrosis of the Hip

Osteonecrosis is caused by bone death in the femoral head that occurs due to poor blood supply, and there are four commonly distinguished grades to describe the severity of the disease (Association Research Circulation Osseous [ARCO]/Steinberg) [19]. Cell-based procedures have been thought to have therapeutic potential in osteonecrosis [34, 37, 47, 48], especially to augment the core decompression procedure, which was originally described by Ficat and Arlet more than 30 years ago [49], to lower the elevated levels of intraosseous pressure and enhance neovascularization and osteogenesis at the defect site [50, 51].

The first clinical trials for cell-based treatment of hip osteonecrosis used percutaneous application of different marrow suspensions via small drill holes and without any supporting matrix material [48, 52, 53]. A more recent publication reported the use of ex vivo expanded MSCs combined with β -tricalcium phosphate (β -TCP) matrix and vascularized fibula grafts in combination with core decompression for femoral head necrosis treatment in three patients [54]. In our own study, using an almost similar approach, we dispersed an ex vivo expanded autologous

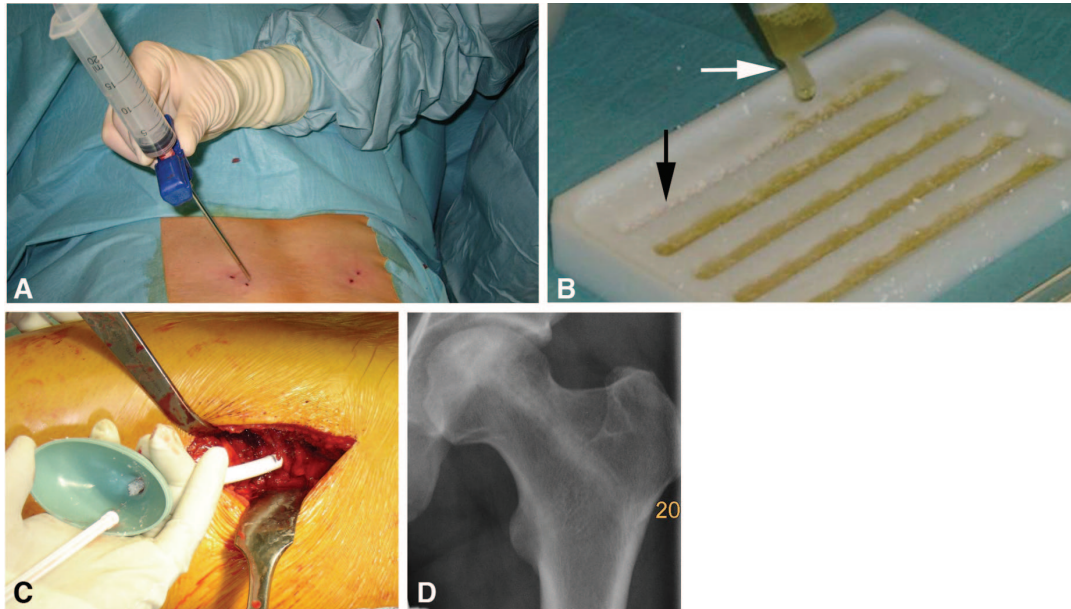


Figure 2. Example of clinical mesenchymal stromal cell delivery following ex vivo expansion for the treatment of avascular necrosis of the femoral head. (A): Autologous bone marrow aspiration from the posterior iliac crest. (B): For delivery, β -tricalcium phosphate (β -TCP) granules (black arrow) were soaked with an autologous stromal cell suspension and immersed in autologous fibrin (white arrow) to facilitate subsequent handling. (C): Adhesive stromal cell/ β -TCP constructs were delivered to the bony defects via tubings that were placed into the canals of the core decompression. (D): Postoperative radiography displays a 10-mm drill hole 6 weeks after core decompression completely filled with the stromal cell/ β -TCP construct in a 34-year-old male patient with avascular necrosis (stage ARCO II) of the femoral head.

bone marrow cell preparation (tissue repair cells, Aastrom, Ann Arbor, MI, <http://www.aastrom.com>) on β -TCP carrier granules to fill stage II (ARCO) osteonecrosis areas after debridement in three patients [19]. Follow-up examinations showed intact femoral heads and entirely filled bone canals at 6 weeks upon radiographic and magnetic resonance imaging (MRI) examination (Fig. 2). It remains to be proven, in large-scale randomized controlled clinical trials compared with no-cell controls, whether such procedures can improve long-term clinical outcome after core decompression.

Bone Cysts and Tumors

For the treatment of bone cysts, marrow injections have proven useful in several clinical studies, with some variability in the healing outcome after simple marrow injections ranging from 18%–100% [55–60]. However, intralesional marrow injections have shown to be not as efficient as intralesional injection with methylprednisolone in a comparative study [55]. When marrow aspirates were injected together with a bone powder, similarly satisfactory results were obtained compared with open surgery and grafting of allogenic lyophilized bone chips [56]. In addition, collagen type I has been shown to be an appropriate carrier for the use of marrow aspirates in this setting [60].

Spinal Fusion

For spinal fusion, MSCs have been successfully tested in several small and large animal disease models as reviewed in [61]. These preclinical data set the stage for two initial phase I clinical trials for this indication [62, 63]. The first involved the use of bone marrow aspirated together with hydroxyapatite biomaterial car-

riers to augment lumbar spine fusion, compared with conventional bone grafting from the iliac crest in 55 patients, and yielded similar results in both groups, whereas the donor site morbidity in the latter one was acknowledged [62]. Similar successful spinal fusion rates (95%) were seen when ex vivo bone marrow stromal cells were combined with porous β -TCP for posterior lumbar spine fusion in 56 patients compared with iliac crest bone autograft [63].

From numerous in vitro and in vivo experimental studies of bone healing, it is conceivable that other osteogenic or angiogenic agents, such as growth factors (e.g., bone morphogenetic proteins: BMP-2, BMP-7), may also be incorporated in the procedure to elicit a greater healing response at the osseous defect site [64–68]. Overall, the experimental outcomes have been remarkable, suggesting the feasibility of future clinical application of this technology. However, it remains to be seen whether such approaches will be sufficient to induce an efficient osteogenic response in settings where patients are older, diabetic, traumatized, or smokers, and more rigorous evaluations in preclinical studies are needed.

Cartilage

Localized Articular Cartilage Defects

Substantial experimental research in the field of cell-based treatment of cartilage pathologies, recently reviewed in [15], has set the stage for the use of cell-based treatments in the clinical setting. For focal articular cartilage defects, the autologous chondrocyte implantation (ACI) procedure, first described by Brittberg and colleagues [69], became an established treatment

modality for defects larger than 4 cm² or as a second-line treatment, where initial treatments such as microfracture failed to induce satisfactory healing [70]. For this procedure, a cartilage biopsy is harvested arthroscopically from a non-load-bearing area, followed by enzymatic digestion to release chondrocytes, which are subsequently expanded before reimplantation. The first generation of ACI used a periosteal flap sutured onto the defect, followed by injection of the cell suspension underneath the periosteum within the defect cavity, and then sealing with fibrin glue. This technique elicited predominantly good to very good long-term clinical results in the majority of the patients [71, 72]. Because of several problems that were noted using this technology, including transplant hypertrophy, calcification, delamination, and cell leakage [73, 74], the ACI was subsequently developed further using biocompatible scaffolds in which the chondrocytes were seeded in three-dimensional (3D) constructs that are implanted in the second and third generation of ACI procedures [19, 75].

To circumvent autologous tissue harvest from healthy cartilage, which is very limited in quantity, initial clinical attempts were directed towards the use of MSCs seeded in 3D collagen carriers for the repair of focal full-thickness cartilage defects [76]. In this study, full-size patella defects in two patients were treated with collagen gel constructs that were seeded with ex vivo expanded MSCs and covered with a periosteal flap, and good defect filling, as well as markedly improved patient outcome, was observed at 1-year follow-up [76]. The same group also reported on the treatment of nine defects, in five knees of three patients, with similar good results at 12 months [77]. A biopsy of the regenerate tissue from one patient after 12 months revealed formation of a fibrocartilage repair tissue that was rich in proteoglycans [77]. Using a similar approach, this technology was also used to treat a full-thickness cartilage defect in the medial femoral condyle of one individual, with similar good clinical and histological outcomes [78]. A recent randomized controlled cohort study compared the use of autologous bone-marrow-derived MSCs (BMSCs) with the transplantation of autologous chondrocytes by means of the ACI procedure, and similar functional outcomes were observed in both groups in up to 2 years' follow-up, with less cost and donor site morbidity in the BMSC group [79].

Although promising, the clinical data on MSC delivery to cartilage defects still have to be considered as very preliminary. Several randomized controlled clinical studies that investigate the use of bone marrow concentrates and MSCs via delivery as suspension or three-dimensional constructs to cartilage defects are under way and officially registered (<http://www.clinicaltrials.gov>). However, to our knowledge these data are not published yet, and the data emerging from these controlled *in vivo* studies in the next few years need to be analyzed to determine whether MSC-based treatments can compete with current treatment modalities.

Arthritides

As opposed to focal articular cartilage defects, approaches to treat arthritides such as rheumatoid arthritis (RA) or osteoarthritis (OA) with stem cell-based approaches have to take into consideration the larger sizes of the defects, as well as the underlying disease process, as vulnerable neocartilage constructs may face rapid degradation when delivered to inflamed joints. Therefore, the underlying pathology has to be effectively under con-

trol, or any cell-based treatment of OA or RA is unlikely to be successful long-term.

For the treatment of OA, the intraarticular injection of MSC suspensions represents conceptually the simplest approach. Using such an approach, cells are distributed throughout the joint space to provide their therapeutic effects, as seen in an *in vivo* study in goats, where delivery of autologous stromal cell suspensions were able to mediate regeneration of inflamed menisci after resection, along with reduction of arthritic changes to the cartilage, including osteophytic remodeling, and subchondral sclerosis [80]. Initial casuistic pilot studies are in the literature that evaluated injection of ex vivo expanded marrow stromal cell populations in patients with OA of the knee [81, 82], and they reported safety and feasibility of the procedure; MRI evaluations of one patient revealed increased cartilage and meniscus thickness after 24 months. However, these data still have to be considered as very preliminary, and long-term evaluations in controlled randomized clinical trials are necessary to determine efficacy.

Because of their immunosuppressive effects, MSCs from autologous and allogeneic origin have been considered for several autoimmune diseases, including RA [83, 84]. Allogeneic MSC transplantation in four patients with refractory RA was considered safe but revealed only short-term clinical improvement in two patients, and none of the patients reached the stage of remission [85]. However, in RA this treatment option has not been pursued further, because of disease relapses and the good success with standard therapies that were complemented with the use of the antagonist in recent years [84].

As opposed to systemic transplantation, fibroblastic cells were used to deliver anti-inflammatory cytokines to RA diseased joints intraarticularly following genetic modification experimentally and clinically [86]. In the first clinical trial, the IL-1 receptor antagonist (IL-1Ra) was chosen as the transgene and delivered to the metacarpophalangeal joints of nine individuals with severe RA via synovial fibroblasts, which were genetically modified using a retrovirus [87]. This phase I study revealed the safety of the procedure and also transgene expression in all treated joints [87]. Comparable studies were performed at other centers with different vector- and cell preparations and showed comparable outcomes, which are reviewed elsewhere [86]. However, it remains to be seen whether such costly and laborious genetic therapies will find the way to broad clinical application, and the safety issue remains of concern in diseases that are not life-threatening.

Tendon

Because of the beneficial experiences of cell-based treatments of tendinopathy in experimental studies [88], autologous blood injections were used clinically with some success for the treatment of medial [89] or lateral epicondylitis with [90, 91] or without dry needling, as well as refractory patellar tendinopathy [92]. A recent systematic review of four injection therapies for lateral epicondylitis, namely, prolotherapy, polidocanol, whole blood, and PRP, revealed that all were comparatively effective without any major differences that could be extracted from the literature [93]. In the quest for the development of improved treatments for the tendinopathies, the injection of ex vivo expanded autologous dermal fibroblasts has been evaluated as an effective and safe treatment for refractory lateral elbow epicondylitis in an initial pilot study [90]. The same group of investigators compared

the injection of dermal fibroblasts together with autologous serum versus autologous serum only for the treatment of refractory patellar tendinopathy, and they reported a faster healing response as observed by ultrasound and faster clinical recovery in the cell-based treatment group at 6 months [94].

Other Orthopaedic Applications

In the field of regenerative orthopaedics, there are solid research data that support the beneficial use of MSCs for treatment of meniscus [95], intervertebral disc [96], ligaments and tendon [97, 98], or muscle injuries [99], which are extensively reviewed elsewhere [1]. For all of these applications, clinical trials are currently under way that use MSCs from autologous or allogeneic origin (<http://www.clinicaltrials.gov>). Taken together, these promising features of cell-based therapy approaches support the feasibility of the development of these treatments to clinical use, and the initial clinical trials will clarify whether such approaches will find the way to broad clinical application.

Apart from applications in musculoskeletal regeneration, there is a rapidly growing body of data from clinical trials that have used MSCs for the treatment of myocardial infarction, nerve regeneration after stroke or spinal cord injuries, graft versus host disease, certain Mendelian hereditary disorders, and others, which have been recently extensively reviewed elsewhere [100–103]. Of particular interest to the orthopaedic field are the studies that used allogeneic bone marrow-derived MSC transplantation to replace defective collagen type I synthesis by osteoblasts of patients suffering from osteogenesis imperfecta (OI), a genetic disorder that leads to a variety of skeletal pathologies, including deformities and incidental fractures [104, 105]. In this context, transplantation of allogeneic MSCs from foreign donors [105], or human leukocyte antigen (HLA)-compatible sibling donors [104], led to successful cell engraftment, significant increase of bone mineral content, and reduced bone fracture frequencies in children with OI. Therefore it remains to be seen whether such therapies can be further developed to broad successful clinical application.

PERSPECTIVES FOR STEM CELL THERAPIES FOR MUSCULOSKELETAL REGENERATION

In the orthopaedic and sports medicine community, regenerative biological therapies are rapidly emerging and have received increasing attention over the last years. For example, the clinical application of PRP has been added to the repertoire of treatment options to enhance healing for almost all musculoskeletal tissues, including tendon, bone, cartilage, muscle, and others, and almost outpaced the research in the field [106]. However, as more and more clinical data surface, the limitations of PRP therapies have become evident [106]. It is perceivable that stem cells might prove ideal to complement or enhance such readily available treatment modalities, where desired results are often not achieved. It remains to be seen for every specific application whether the addition of cellular entities derived from whole blood or marrow, concentrates thereof, or ex vivo processed cell preparations (Fig. 1) is required and effective to augment optimized healing results.

Another great potential for healing enhancement is the facilitation of endogenous tissue stem cells. In view of the mounting evidence demonstrating the paracrine and trophic functions of

MSCs, it is not known to date whether the stem cell therapies outlined above in this review act by stimulating regenerative cell populations in situ, and to what extent this mechanism contributed to the therapeutic effects seen. Also, does PRP attract MSCs, and what are the relevant mechanisms of action? As information on tissue stem cells becomes more evident, local resident cell populations might be a more effective cell therapy target in the future [1]. Once the relevant regenerative mechanisms of stem cells are more precisely understood, it is conceivable that these functions might be augmented with the use of appropriate growth factors and biomaterials. For bone regeneration, human recombinant BMP-2 and BMP-7 (OP-1) have been brought to broad clinical application and found their place in the therapeutic regimen of bone diseases, where standard therapies failed [107]. For such cases, the addition of MSCs might further improve the approach, as the preliminary clinical data suggest [40]. These may represent the steps of the “therapeutic escalator” that lead to the regeneration of other musculoskeletal tissues, such as cartilage, where no satisfying treatment options are available in the clinic. These approaches will require that the most appropriate material(s), factor(s), and cell preparation(s) for the target tissue are identified.

Furthermore, what researchers and clinicians have to clarify before they pursue therapeutic efforts is what they can and cannot realistically expect from their stem cell or regenerative therapy. In other words, what are the aspects of healing they would like to enhance, and which tools are therefore needed? An MSC-based therapy per se might not be the solution for every problematic defect in the musculoskeletal system, and chances and risks have to be well considered to avoid failures that might be disadvantageous for the patient and the field. Such information needs to be communicated to patients and also to the sponsors and regulators of the health system, in order to gain public support for the development of novel therapies for more effective treatments in the future that will prove to be cost-effective in the long term.

Lastly, several ethical and regulatory aspects have to be considered before such cell-based treatments can be conducted. Any application of cells that involves ex vivo expanded or processed cells is considered advanced therapy medicinal products (ATMPs) by the competent international and national regulatory agencies, for example, the FDA or the European Medicines Agency. Approval of such ATMPs usually requires several specific quality-control and safety criteria that are based on the use of standardized GMP conditions for cell processing, the conduct of appropriate preclinical animal models, and meaningful controlled phase I/II clinical trials [108, 109]. Meeting these stringent criteria will help guarantee that the efficacy and safety of innovative stem cell interventions will be rigorously established, while also protecting study participants. However, the laborious and time-consuming nature of these regulatory processes must be taken into consideration in developing clinical applications of such innovative stem cell-based approaches.

CONCLUSION

There is an increasing demand for MSC-based regenerative approaches in the musculoskeletal system, the current clinical status of which is listed in Table 1. MSCs have been delivered as whole blood or marrow, concentrates, or ex vivo expanded cell

populations, either in suspension or seeded in carrier matrices with or without stimulating factors, to injured tissues. However, there is no orthopaedic application where MSC-based therapies have been advanced to broad clinical application. Delivery of MSCs for the repair of bone, cartilage, and tendon cells has shown safety and initial efficacy in several phase I clinical trials, but large comparative prospective randomized clinical trials are required for adequate comparison of the MSC-based therapies to standard treatment modalities. In other arenas, such as meniscus, intervertebral disc, ligament, or muscle regeneration, MSCs have only been used experimentally with some success. Such MSC-based therapies are thought to be improved by the use of certain bioactive materials and stimuli that aid distinct aspects of repair. However, the ideal combinations of cell preparation, bioactive factor(s), and material(s) for each application have to be identified that also meet the desired safety and cost requirements in a satisfactory manner. Only when such therapies for non-life-threatening diseases can show improved outcomes compared with standard treatments will they be able to prove long-term cost-effectiveness and find their place in the therapeutic regimen of the discipline.

ACKNOWLEDGMENTS

This work is supported in part by the Deutsche Forschungsgemeinschaft (DFG STE1051/2-1, A.F.S. and U.N.), Bayerische Forschungsförderung (FORZEBRA TP2-WP5, A.F.S. and U.N.), the Interdisziplinäres Zentrum für Klinische Forschung Würzburg (IZKF D-101 and D-219, A.F.S.), the European Union (seventh Framework Program-HEALTH VasuBone and ADIPOA, U.N. and L.R.), and the Commonwealth of Pennsylvania Department of Health (R.S.T.).

AUTHOR CONTRIBUTIONS

A.F.S., L.R., U.N., R.S.T.: conception and design, manuscript writing, final approval of manuscript, financial support; F.G.: conception and design, manuscript writing, final approval of manuscript.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicate no potential conflicts of interests.

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