

## Review

# Mesenchymal Stem Cells in Regenerative Medicine for Musculoskeletal Diseases: Bench, Bedside, and Industry

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Human bone marrow-derived mesenchymal stem cells (MSCs) can self-renew and differentiate into osteoblasts, chondrocytes, and adipocytes. MSCs have effectively emerged as a promising tool for clinical applications, specifically in musculoskeletal diseases. This article reviews the status of preclinical animal studies, clinical trials, and the efforts of the industry in using MSCs to treat musculoskeletal diseases such as bone fractures, bone defects, focal chondral lesions, osteoarthritis, spinal diseases, and tendon injuries. We also discuss the current problems encountered and potential of using MSCs in future clinical studies.

Key words: Mesenchymal stem cells (MSCs); Musculoskeletal diseases (MSDs); Preclinical animal studies; Clinical trials; Industry

## BASICS OF MSCs

Bone marrow, the home organ of hematopoietic stem cells, contains several subpopulations of stem/progenitor cells that are capable of differentiating into various non-hematopoietic cells. Among the best studied subpopulations are the cells referred to as marrow stromal cells, mesenchymal stem cells, or multipotent mesenchymal stromal cells (MSCs) (27). Such cells can be isolated by exploiting their ability to adhere to plastic tissue culture surfaces. MSCs have been identified from a variety of other tissues, including, but not restricted to, adipose tissues, Wharton's jelly of umbilical cord, and dental pulp (12). In addition, MSCs have emerged as a promising tool for clinical and commercial applications of cell transplantation and cell-based therapy, such as tissue engineering. In fact, the world's first stem cell drug uses MSCs to treat children suffering from graft-versus-host disease (GVHD) in allogeneic recipients (62). It has also been recently reported that most stem cell therapies on the market and in development utilize MSCs. Ultimately,

there are numerous reasons why MSCs have attracted so much interest in clinical applications and industry, including ease of isolation from patients, expandability in culture with maintained differentiation potentials, immune-modulating properties, and limited tendencies to form tumors.

## CRITERIA FOR DEFINING MSCs

The therapeutic potential of MSCs has created remarkably growing interest in a wide variety of biomedical disciplines but has also generated increasing difficulties in comparing outcomes. As a result, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy (ISCT) has proposed three criteria to define MSCs (17). First, MSCs must be plastic adherent when maintained in standard culture conditions. Second, MSCs must express cluster of differentiation 105 (CD105), CD73, and CD90 and lack expression of the markers CD34, CD45, CD14 or CD11b, CD79 $\alpha$  or CD19, and human leukocyte antigen (HLA)-DR in culture.

Third, MSCs must have the potential to differentiate into osteoblasts, adipocytes, and chondrocytes *in vitro*.

### **DIFFERENTIATION POTENTIALS OF MSCs**

MSCs were first demonstrated to potentially differentiate into osteogenic, adipogenic, and chondrogenic lineages *in vitro* (52) and *in vivo* (40). They also possess the ability for musculoskeletal and cardiomyocyte differentiation (40,64). In addition, MSCs have been shown to differentiate into not only mesenchymal but also nonmesenchymal lineages. For instance, MSCs have been differentiated into neuronal cells both *in vitro* (29) and *in vivo* (36). Furthermore, hepatocytic differentiation has also been observed *in vitro* (39) and *in vivo* (58). Additionally, there are many experimental animal models that utilize MSCs in cell transplantation or cell-based therapy in regenerative medicine of diseases, including musculoskeletal disorders (20), myocardial infarctions (44), hepatic failures (32), as well as neural degenerations such as spinal cord complications (76) and Parkinson's disease (14).

### **PARACRINE EFFECTS**

Although the therapeutic effects of MSCs have been demonstrated in a variety of disease models and clinical trials, the engraftment and differentiation rates of MSCs into host tissues are extremely low. For example, MSCs engrafted in mouse bone to treat osteogenesis imperfecta resulted in an engraftment rate less than 1.5% (49). Moreover, the *in vivo* tracking analysis of cell transplantation was complicated by the cellular fusion mechanism (68,75) and background tissue-specific fluorescence (33). Thus, to explain the therapeutic effects of transplanted MSCs, which had low engraftment and differentiation rates, an MSC-induced paracrine effect was proposed—a major conceptual change to the role of MSCs in therapy (53). Instead of MSCs directly differentiating into the injured cells, it is suggested that MSCs secrete paracrine factors that elicit regenerative responses, a concept supported by using only conditioned medium or secretome to enhance angiogenesis (30), promote skin wound healing (79), stimulate fracture healing (74), repair nervous degeneration (63), and treat cardiovascular diseases (65). Furthermore, cumulative evidence shows that MSCs secrete growth factors and cytokines that regulate endogenous tissue regeneration as well as modulate the immune responses and inflammation of several diseases.

### **PRECLINICAL AND CLINICAL STUDIES OF MSCs IN THE TREATMENT OF MUSCULOSKELETAL DISEASES**

Musculoskeletal diseases (MSDs) are a group of disorders that result from trauma or degeneration in either a single event or repetitive episodes. With the potential to differentiate along mesenchymal lineages, MSCs have

been widely used in cell-based therapy for the treatment of MSDs. There is a large amount of research findings on the use of MSCs for MSDs in experimental animal models and clinical settings, as reviewed previously (7,43,47,56,61). Most studies using MSCs for cell-based therapy in MSDs have shown promising results (18,25,26). We will discuss the current progress of MSC applications in scientific research and marketing availability regarding several MSDs.

### **BONE REGENERATION**

In preclinical studies, MSCs have been suggested to restore large bone defects in several animal models of disease (7,9). The effectiveness of expanded MSCs from different animals behaves in a similar manner for bone regeneration *in vivo*. Our group has also successfully applied rabbit MSCs to treat calvarial defects in allogeneic recipients, with the repair ability more obvious in MSCs expanded under hypoxic conditions than that expanded under normoxic conditions (78). Clinically, when treating osteonecrosis of the femoral head with autologous bone marrow aspiration, the quantity of mineralized bone formation correlated positively with the amount of MSCs residing in the iliac crest (24). Numerous studies have also investigated the use of *ex vivo* expanded MSCs in bone regeneration. For instance, three patients with segmental bone defects were successfully treated with autologous MSCs delivered in hydroxyapatite scaffolds (54). There are several investigations that integrated MSCs into biomaterials like hydroxyapatite and calcium phosphate and showed promising features, including their ease of availabilities, osteoconductivities, and absence of immune responses (6,57,69).

### **CARTILAGE REGENERATION**

Owing to cartilage's limited quantity, extracting autologous tissue from healthy cartilage is problematic. MSCs have been demonstrated to undergo chondrogenic differentiation when encapsulated in alginate both *in vitro* (42) and *in vivo* (41), suggesting the potential for cartilage regeneration. MSCs have also been shown to enhance cartilage repair in full-thickness defects of articular cartilage (55,71,77). A clinical study also reported that full-thickness articular cartilage defects in the patellofemoral joint transplanted with autologous MSC-embedded collagen gels showed significant and lasting restoration of cartilage after follow-ups at 17 to 27 months (73). When comparing autologous MSC transplantation with autologous chondrocyte implantation (ACI) for cartilage repair, the MSC transplantation was shown to be more economical, minimized donor-site morbidity, required less surgery, and still as effective as its ACI counterpart in a 2-year follow-up (45). When treating osteochondral lesions of talus (OLT), the clinical outcomes of MSC injections with arthroscopic

marrow stimulation to OLT were superior to those with arthroscopic marrow stimulation alone, with the results measured using the visual analog scale (VAS) for pain and the American Orthopaedic Foot and Ankle Society (AOFAS) Ankle–Hindfoot Scale for ankle activity (35).

Treatment of osteoarthritis (OA) is much more difficult, since the defect is larger in size and is characterized by an inflammation environment, so there is still a lack of long-term success in using MSCs to treat OA (61). MSCs embedded in collagen gels have been transplanted to treat patients with knee OA who only underwent a high tibial osteotomy, and the effects were compared to that of patients who underwent a high tibial osteotomy. Although the clinical improvement in each of these two groups was not significantly different, the cell-transplanted group achieved better arthroscopic and histological results than the cell-free control group (72). Another clinical trial involved patients of OA who received intra-articular injections of culture-expanded, bone marrow-derived MSCs, and 63.2% of patients showed improvement at an average follow-up of 11.3 months (10). However, these data still have to be considered preliminary, as another study using intra-articular injections of MSCs to treat knee OA reported only minor improvements in the ranges of motion (13).

### SPINAL FUSION AND DISC REGENERATION

While iliac crest autografting remains the gold standard in bone fusion studies, there are many disadvantages to autograft harvest including pain, bleeding, infection, and fracture risk. The preclinical animal models that use MSCs for spinal fusion provided the framework for clinical studies in spinal fusion (81). MSCs have been applied for spinal fusion as a component of OsteoCel Plus, an alternative allograft cellular bone matrix, to treat patients who underwent a minimally invasive transforaminal lumbar interbody fusion for degenerative lumbar conditions. Twenty-one patients (91.3%) and 24 levels (92.3%) achieved radiographic evidence of solid bony arthrodesis at a 12-month follow-up (1). MSCs hybridized with  $\beta$ -tricalcium phosphate ( $\beta$ -TCP), another substitute for autografts, and utilized for lumbar spinal fusion resulted in a successful fusion rate of 93.3%, close to the fusion rate (96.2%) achieved by autologous iliac crest bone grafts (81). The regenerative ability of autologous MSCs has also been clinically investigated in degenerated intervertebral discs, where pieces of collagen sponge containing autologous MSCs were grafted. Symptoms were alleviated, and radiographic analysis showed improvements in the vacuum phenomenon and lumbar disc instability at a 2-year follow-up (80).

### TENDON HEALING

Although early clinical trials using MSCs in bone and cartilage repair are already published, information about

MSC-based therapy in tendon repair is currently limited to animal studies. However, there are many promising preclinical studies that open up possibilities for future clinical trials. MSCs have been shown to enhance Achilles tendon repair in rabbit (11) and rat models (28,48). It is interesting to note that the healing effects of rat MSCs expanded under hypoxic conditions were superior to that of MSCs cultured under normoxic conditions (28). Unfortunately, MSC application in a rotator cuff repair model did not lead to improvement of structure, composition, or strength of the healing tendon attachment site despite evidence that MSCs were present and metabolically active (22). Nevertheless, there are also large-animal models that support the transition to human clinical trials. For instance, of the 113 racehorses that received intraligamentary autologous MSC injections for superficial digital flexor tendon strains, 111 (98.2%) returned to racing with a significantly lower reinjury rate than previous studies had obtained (21).

### MSC-RELATED PRODUCTS IN INDUSTRY

It has recently been predicted that the stem cell therapy market was valued at US\$2.7 billion in 2011 and is expected to reach an estimated value of \$4.65 billion by 2016 (62). Noticeably, MSC-based cell therapy is still the major product type on the market and in development when compared to therapeutic techniques using embryonic stem cells, adult progenitor cells, and induced pluripotent stem cells. The market value of US osteobiologic products between 2009 and 2010 was about \$1.6 billion, with the stem cell products accounting for \$59 million. The sales of these products increased from 2009 to 2010 by 15.7%, making it a fast-growing subsegment in this market with an expected global value of \$600 million by 2015. Thus far, the only three products that are MSC related and available in the US in this group are OsteoCel Plus, Trinity Evolution, and LiquidGen marketed by NuVasive Inc. (San Diego, CA, USA), Orthofix Inc. (Lewisville, TX, USA), and Skye Orthobiologics LLC (Redondo Beach, CA, USA), respectively. Meanwhile, two competitors are waiting for the green light to expand into the US market: Allostem and Cellentra Viable Cell Bone Matrix (VCBM), distributed by Allosource Inc. (Centennial, CO, USA) and Biomet Inc. (Warsaw, IN, USA), respectively.

OsteoCel Plus, an allograft cellular bone matrix containing MSCs and osteoprogenitor cells combined with demineralized bone matrix (DBM) and cancellous bone, is used in spinal fusion and other orthopedic surgical procedures. Owing to its osteoinductive, osteoconductive, and osteogenic capabilities, patients can be treated with the advanced bone graft product and avoid limitations of traditional alternatives. In 2010, OsteoCel Plus was the leading product in the osteobiologics market, accounting

for over 92% of market sales. Business speculators believe that sales of OsteoCel Plus will continue to gradually increase, reaching \$74 million in revenue by 2017 (70). Although OsteoCel Plus is the dominant product on the market, it is threatened by stem cell competitors like Trinity Evolution and non-stem cell opponents such as InFuse distributed by Medtronic Inc. (Sarasota, FL, USA). OsteoCel Plus has the current advantage due to its more advanced technology and first-to-market availability; however, its growth rate will be further hindered by increased competition against other upcoming stem cell-related products.

Trinity Evolution by Orthofix Inc., also a cell-based bone matrix used in spinal fusion surgery, supplies adult MSCs, osteoprogenitor cells, and a demineralized cortical component in a minimally manipulated bone allograft. The cancellous bone used to produce Trinity Evolution is derived from freshly recovered donor tissue and processed under aseptic conditions. Preclinical studies as well as strict donor screenings have demonstrated the safety of Trinity Evolution as well as its osteoinductive and osteogenic potential contained within a natural osteoconductive matrix. After a 5-year accumulation of over 75,000 cases with Trinity Evolution, Orthofix Inc. introduced Trinity ELITE in July 2013 as an even safer and fully moldable alternative that facilitates handling procedures in surgery.

Besides the use of viable MSCs in orthopedic products, there is ongoing development of supportive materials that each contains an MSC-secreted extracellular matrix and secretome. Skye LiquidGen is an allograft tissue matrix for use as an *in vivo* wound cover to fill tissue defects or localize areas of inflammation. One of its major components is the amniotic tissue-isolated collagen that acts as a natural scaffold for cellular attachment and accelerates cell migration and proliferation *in vivo*. LiquidGen can be applied directly to the surgical site, mixed with patients' own blood, or used with other carriers to cover or fill soft tissue defects. LiquidGen is also cryopreserved to extend its shelf life and is more convenient to handle in the operation room.

There are still several MSC-related products waiting to be released in the US market. The first products that emerged in the US were AlloStem from AlloSource Inc. and Cellentra VCBM from Biomet Inc. AlloStem Stem Cell Bone Growth Substitute, an adult human stem cell bone graft, is recovered from adult human adipose tissue. This minimally processed allograft is designed to promote bone growth and healing. Adipose tissue is another rich source of a variety of stem cells, and some laboratory studies even suggest that it is the human body's primary source of stem cells. Cellentra VCBM also offers a plethora of bone-healing factors

comprised of an osteoconductive scaffold that harbors viable osteogenic cells and MSCs of verified osteoinductivities. It also provides additional inherent growth factors, including bone morphogenetic protein-2 (BMP-2), -4, -7, vascular endothelial growth factor (VEGF), transforming growth factor (TGF)- $\beta$ , platelet-derived growth factor (PDGF), insulin-like growth factor 1 (IGF-1), and fibroblast growth factor (FGF) to further improve bone growth. The cancellous bone matrix of Cellentra VCBM offers an interconnected trabecular structure for optimal osteoconductivity.

For markets outside of the US, the Korea Food and Drug Administration (KFDA) approved the manufacture and sale of Cartistem in the country for the treatment of traumatic and degenerative OA in January 2012. Cartistem contains specially selected and grown MSCs from the umbilical cord blood donated by willing mothers following KFDA regulation and guidelines. This makes Cartistem a safe stem cell-based allograft obtained from another individual rather than from the patient. However, Cartistem is currently only available in Korea through distribution by Dong-A Pharmaceuticals Co., Ltd. (Seoul, S. Korea) but has also received US Food and Drug Administration clearance to be tested in Phase I/IIa clinical trials for the possibility of US market expansion.

#### CURRENT PROBLEMS AND PERSPECTIVE IN CLINICAL APPLICATION OF MSCs

There are several problems that should be resolved before MSCs are clinically tested (56). Cell transplantation of MSCs for clinical use requires a great number of cells, usually ranging from  $10^8$  to  $10^9$  depending on the disease treated. Currently, there is no gold standard for an MSC expansion process, as variations in expansion conditions induce different changes in cell properties (50). Expanded MSCs have also been reported to undergo proliferative senescence with loss of stem cell properties (4,8). More importantly, the degree of homing and engraftment of these expanded MSCs in adult recipients are very low (2,37,51). The paracrine effects of MSCs are also affected by aging after long-term expansion (34). Thus, there is great interest in the identification of methods that efficiently expand enough transplantable cells.

A good protocol that expands MSCs for clinical use should meet the following guidelines. First, MSC expansion should be in large scale and not suffering from senescence, loss of proliferation capacity, or loss of differentiation potential (4,8). Second, the expanded cells should engraft or regenerate tissues after transplantation into autologous or allogeneic recipients (17). Third, the therapeutic effects of engrafted MSCs are unknowingly due to engraftment and/or paracrine-induced effects, so this detail must first be confirmed before proceeding to



clinical trials (53). There is controversy because, while MSCs have been shown to differentiate into injured cells, MSC-derived microvesicles have also been shown to protect against injuries like renal damage in mice (23). Finally, the final product must meet the ISCT minimum criteria for MSCs. In addition, the protocols and facilities used for cell manipulation and/or expansion should be regulated and approved to provide products that pass tests of identity, potency, purity, and safety (60,67).

Culturing MSCs at a low seeding density causes rapid expansion of MSCs (59); however, cells expanded at this seeding density reveal significant replicative senescence. Furthermore, ex vivo expanded MSCs failed to engraft, differentiate, or respond to host environments and also failed to secrete growth factors or cytokines (3). Recently, the beneficial effects of hypoxia on expansion of MSCs were found to be consistent even at different oxygen concentrations (19,46,66). Hypoxic cultures also enhanced the angiogenic effects of MSCs by increasing the secretion of angiogenic factors (30). Besides, in vitro migration and in vivo engraftment were increased by hypoxic exposure to MSCs for 1–2 days, detected by increases in chemokine (C-X3-C motif) receptor 1 (CX3CR1) and chemokine (C-X-C motif) receptor 4 (CXCR4) expression (31). These results suggest that hypoxic culturing or short-term hypoxic preconditioning of MSCs may provide a general method of enhancing their survival, migration, angiogenesis, and engraftment in vivo in a variety of tissues.

The immunosuppressive features of human, baboon, and murine MSCs were demonstrated in vitro (5,15,16,38) and in vivo (5). MSCs have also been applied in allogeneic transplantation in diseases such as GVHD. Nevertheless, many studies have shown that allogeneic MSCs were rejected in immunocompetent major histocompatibility complex (MHC)-mismatched recipients, and thus, the use of MSCs for allogeneic transplantation remains controversial. However, a previous study demonstrated that allogeneic hypoxic MSCs increased the effects of bone defect repair compared to the effects produced by allogeneic normoxic MSCs (78). These results suggest that hypoxic MSCs are intrinsically immunoprivileged and can serve as a “universal donor cell” in treatments.

All together, the studies currently available suggest that expanded MSCs have multiple therapeutic effects on MSDs, which can be applied for bone regeneration, cartilage defect restoration, osteoarthritis treatment, spinal fusion, disc regeneration, and tendon repair. Based on these significant benefits, accumulating MSC-related osteobiologic products are available in the market or in development. However, there is still a lack of a gold standard procedure to expand MSCs. Recently, using a hypoxic culture has been shown to enhance short-term

proliferation, long-term expansion efficiency, differentiation potential, stemness or maintenance of stem cell properties, expression of chemokine receptors, migration, and engraftment ability. Moreover, MSCs were able to survive and engraft in allogeneic recipients. The platform based on hypoxic culture will help the development of new strategies for clinical applications of MSCs.

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## REFERENCES

1. Ammerman, J. M.; Libricz, J.; Ammerman, M. D. The role of Osteocel Plus as a fusion substrate in minimally invasive instrumented transforaminal lumbar interbody fusion. *Clin. Neurol. Neurosurg.* 115(7):991–994; 2013.
2. Ankrum, J.; Karp, J. M. Mesenchymal stem cell therapy: Two steps forward, one step back. *Trends Mol. Med.* 16(5): 203–209; 2010.
3. Augello, A.; Kurth, T. B.; De Bari, C. Mesenchymal stem cells: A perspective from in vitro cultures to in vivo migration and niches. *Eur. Cell Mater.* 20:121–133; 2010.
4. Banfi, A.; Muraglia, A.; Dozin, B.; Mastrogiacomo, M.; Cancedda, R.; Quarto, R. Proliferation kinetics and differentiation potential of ex vivo expanded human bone marrow stromal cells: Implications for their use in cell therapy. *Exp. Hematol.* 28(6):707–715; 2000.
5. Bartholomew, A.; Sturgeon, C.; Siatskas, M.; Ferrer, K.; McIntosh, K.; Patil, S.; Hardy, W.; Devine, S.; Ucker, D.; Deans, R.; Moseley, A.; Hoffman, R. Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo. *Exp. Hematol.* 30(1):42–48; 2002.
6. Behnia, H.; Khojasteh, A.; Kiani, M. T.; Khoshzaban, A.; Mashhadi Abbas, F.; Bashtar, M.; Dashti, S. G. Bone regeneration with a combination of nanocrystalline hydroxyapatite silica gel, platelet-rich growth factor, and mesenchymal stem cells: A histologic study in rabbit calvaria. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol.* 115(2):e7–15; 2013.
7. Beyth, S.; Schroeder, J.; Liebergall, M. Stem cells in bone diseases: Current clinical practice. *Brit. Med. Bull.* 99:199–210; 2011.
8. Bonab, M. M.; Alimoghaddam, K.; Talebian, F.; Ghaffari, S. H.; Ghavamzadeh, A.; Nikbin, B. Aging of mesenchymal stem cell in vitro. *BMC Cell Biol.* 7:14; 2006.
9. Bruder, S. P.; Kraus, K. H.; Goldberg, V. M.; Kadiyala, S. The effect of implants loaded with autologous mesenchymal stem cells on the healing of canine segmental bone defects. *J. Bone Joint Surg. Am.* 80(7):985–996; 1998.
10. Centeno, C. J.; Schultz, J. R.; Cheever, M.; Freeman, M.; Faulkner, S.; Robinson, B.; Hanson, R. Safety and complications reporting update on the re-implantation of culture-expanded mesenchymal stem cells using autologous platelet lysate technique. *Curr. Stem Cell Res. Ther.* 6(4):368–378; 2011.
11. Chong, A. K.; Ang, A. D.; Goh, J. C.; Hui, J. H.; Lim, A. Y.; Lee, E. H.; Lim, B. H. Bone marrow-derived mesenchymal stem cells influence early tendon-healing in a rabbit Achilles tendon model. *J. Bone Joint Surg. Am.* 89(1):74–81; 2007.

12. Da Silva Meirelles, L.; Chagastelles, P. C.; Nardi, N. B. Mesenchymal stem cells reside in virtually all post-natal organs and tissues. *J. Cell Sci.* 119(Pt 11):2204–2213; 2006.
13. Davatchi, F.; Abdollahi, B. S.; Mohyeddin, M.; Shahram, F.; Nikbin, B. Mesenchymal stem cell therapy for knee osteoarthritis. Preliminary report of four patients. *Int. J. Rheum. Dis.* 14(2):211–215; 2011.
14. Dezawa, M.; Kanno, H.; Hoshino, M.; Cho, H.; Matsumoto, N.; Itokazu, Y.; Tajima, N.; Yamada, H.; Sawada, H.; Ishikawa, H.; Mimura, T.; Kitada, M.; Suzuki, Y.; Ide, C. Specific induction of neuronal cells from bone marrow stromal cells and application for autologous transplantation. *J. Clin. Invest.* 113(12):1701–1710; 2004.
15. Di Nicola, M.; Carlo-Stella, C.; Magni, M.; Milanese, M.; Longoni, P. D.; Matteucci, P.; Grisanti, S.; Gianni, A. M. Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. *Blood* 99(10):3838–3843; 2002.
16. Djouad, F.; Plence, P.; Bony, C.; Tropel, P.; Apparailly, F.; Sany, J.; Noel, D.; Jorgensen, C. Immunosuppressive effect of mesenchymal stem cells favors tumor growth in allogeneic animals. *Blood* 102(10):3837–3844; 2003.
17. Dominici, M.; Le Blanc, K.; Mueller, I.; Slaper-Cortenbach, I.; Marini, F.; Krause, D.; Deans, R.; Keating, A.; Prockop, D.; Horwitz, E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 8(4):315–317; 2006.
18. Dong, Y.; Chen, X.; Hong, Y. Tissue-engineered bone formation in vivo for artificial laminae of the vertebral arch using beta-tricalcium phosphate bioceramics seeded with mesenchymal stem cells. *Spine* 38(21):E1300–E1306; 2013.
19. Fehrer, C.; Brunauer, R.; Laschober, G.; Unterluggauer, H.; Reitering, S.; Kloss, F.; Gully, C.; Gassner, R.; Lepperdinger, G. Reduced oxygen tension attenuates differentiation capacity of human mesenchymal stem cells and prolongs their lifespan. *Aging Cell* 6(6):745–757; 2007.
20. Gao, J.; Caplan, A. I. Mesenchymal stem cells and tissue engineering for orthopaedic surgery. *Chir. Organi. Mov.* 88(3):305–316; 2003.
21. Godwin, E. E.; Young, N. J.; Dudhia, J.; Beamish, I. C.; Smith, R. K. Implantation of bone marrow-derived mesenchymal stem cells demonstrates improved outcome in horses with overstrain injury of the superficial digital flexor tendon. *Equine Veterinary J.* 44(1):25–32; 2012.
22. Gulotta, L. V.; Kovacevic, D.; Ehteshami, J. R.; Dagher, E.; Packer, J. D.; Rodeo, S. A. Application of bone marrow-derived mesenchymal stem cells in a rotator cuff repair model. *Am. J. Sports Med.* 37(11):2126–2133; 2009.
23. He, J.; Wang, Y.; Sun, S.; Yu, M.; Wang, C.; Pei, X.; Zhu, B.; Wu, J.; Zhao, W. Bone marrow stem cells-derived microvesicles protect against renal injury in the mouse remnant kidney model. *Nephrology* 17(5):493–500; 2012.
24. Hernigou, P.; Beaujean, F. Treatment of osteonecrosis with autologous bone marrow grafting. *Clin. Orthop. Relat. Res.* 405:14–23; 2002.
25. Hernigou, P.; Poignard, A.; Beaujean, F.; Rouard, H. Percutaneous autologous bone-marrow grafting for nonunions. Influence of the number and concentration of progenitor cells. *J. Bone Joint Surg. Am.* 87(7):1430–1437; 2005.
26. Horwitz, E. M.; Gordon, P. L.; Koo, W. K.; Marx, J. C.; Neel, M. D.; McNall, R. Y.; Muul, L.; Hofmann, T. Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: Implications for cell therapy of bone. *Proc. Natl. Acad. Sci. USA* 99(13):8932–8937; 2002.
27. Horwitz, E. M.; Le Blanc, K.; Dominici, M.; Mueller, I.; Slaper-Cortenbach, I.; Marini, F. C.; Deans, R. J.; Krause, D. S.; Keating, A.; International Society for Cellular Therapy. Clarification of the nomenclature for MSC: The International Society for Cellular Therapy position statement. *Cytotherapy* 7(5):393–395; 2005.
28. Huang, T. F.; Yew, T. L.; Chiang, E. R.; Ma, H. L.; Hsu, C. Y.; Hsu, S. H.; Hsu, Y. T.; Hung, S. C. Mesenchymal stem cells from a hypoxic culture improve and engraft Achilles tendon repair. *Am. J. Sports Med.* 41(5):1117–1125; 2013.
29. Hung, S. C.; Cheng, H.; Pan, C. Y.; Tsai, M. J.; Kao, L. S.; Ma, H. L. In vitro differentiation of size-sieved stem cells into electrically active neural cells. *Stem Cells* 20(6):522–529; 2002.
30. Hung, S. C.; Pochampally, R. R.; Chen, S. C.; Hsu, S. C.; Prockop, D. J. Angiogenic effects of human multipotent stromal cell conditioned medium activate the PI3K-Akt pathway in hypoxic endothelial cells to inhibit apoptosis, increase survival, and stimulate angiogenesis. *Stem Cells* 25(9):2363–2370; 2007.
31. Hung, S. C.; Pochampally, R. R.; Hsu, S. C.; Sanchez, C.; Chen, S. C.; Spees, J.; Prockop, D. J. Short-term exposure of multipotent stromal cells to low oxygen increases their expression of CX3CR1 and CXCR4 and their engraftment in vivo. *PLoS One* 2(5):e416; 2007.
32. Ishikawa, T.; Banas, A.; Teratani, T.; Iwaguro, H.; Ochiya, T. Regenerative cells for transplantation in hepatic failure. *Cell Transplant.* 21(2–3):387–399; 2012.
33. Jackson, K. A.; Snyder, D. S.; Goodell, M. A. Skeletal muscle fiber-specific green autofluorescence: Potential for stem cell engraftment artifacts. *Stem Cells* 22(2):180–187; 2004.
34. Jiang, S.; Kh Haider, H.; Ahmed, R. P.; Idris, N. M.; Salim, A.; Ashraf, M. Transcriptional profiling of young and old mesenchymal stem cells in response to oxygen deprivation and reparability of the infarcted myocardium. *J. Mol. Cell. Cardiol.* 44(3):582–596; 2008.
35. Kim, Y. S.; Park, E. H.; Kim, Y. C.; Koh, Y. G. Clinical outcomes of mesenchymal stem cell injection with arthroscopic treatment in older patients with osteochondral lesions of the talus. *Am. J. Sports Med.* 41(5):1090–1099; 2013.
36. Kopen, G. C.; Prockop, D. J.; Phinney, D. G. Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains. *Proc. Natl. Acad. Sci. USA* 96(19):10711–10716; 1999.
37. LaBarge, M. A.; Blau, H. M. Biological progression from adult bone marrow to mononucleate muscle stem cell to multinucleate muscle fiber in response to injury. *Cell* 111(4):589–601; 2002.
38. Le Blanc, K.; Tammik, C.; Rosendahl, K.; Zetterberg, E.; Ringden, O. HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. *Exp. Hematol.* 31(10):890–896; 2003.
39. Lee, K. D.; Kuo, T. K.; Whang-Peng, J.; Chung, Y. F.; Lin, C. T.; Chou, S. H.; Chen, J. R.; Chen, Y. P.; Lee, O. K. In vitro hepatic differentiation of human mesenchymal stem cells. *Hepatology* 40(6):1275–1284; 2004.
40. Liechty, K. W.; MacKenzie, T. C.; Shaaban, A. F.; Radu, A.; Moseley, A. M.; Deans, R.; Marshak, D. R.; Flake, A. W. Human mesenchymal stem cells engraft and demonstrate site-specific differentiation after in utero transplantation in sheep. *Nat. Med.* 6(11):1282–1286; 2000.

41. Ma, H. L.; Chen, T. H.; Low-Tone Ho, L.; Hung, S. C. Neocartilage from human mesenchymal stem cells in alginate: Implied timing of transplantation. *J. Biomed. Mater. Res. A* 74(3):439–446; 2005.
42. Ma, H. L.; Hung, S. C.; Lin, S. Y.; Chen, Y. L.; Lo, W. H. Chondrogenesis of human mesenchymal stem cells encapsulated in alginate beads. *J. Biomed. Mater. Res. A* 64(2): 273–281; 2003.
43. Mafi, R.; Hindocha, S.; Mafi, P.; Griffin, M.; Khan, W. S. Sources of adult mesenchymal stem cells applicable for musculoskeletal applications – A systematic review of the literature. *Open Orthop. J.* 5(Suppl 2):242–248; 2011.
44. Mayorga, M.; Finan, A.; Penn, M. Pre-transplantation specification of stem cells to cardiac lineage for regeneration of cardiac tissue. *Stem Cell Rev.* 5(1):51–60; 2009.
45. Nejadnik, H.; Hui, J. H.; Feng Choong, E. P.; Tai, B. C.; Lee, E. H. Autologous bone marrow-derived mesenchymal stem cells versus autologous chondrocyte implantation: An observational cohort study. *Am. J. Sports Med.* 38(6):1110–1116; 2010.
46. Nekanti, U.; Dastidar, S.; Venugopal, P.; Totev, S.; Ta, M. Increased proliferation and analysis of differential gene expression in human Wharton's jelly-derived mesenchymal stromal cells under hypoxia. *Int. J. Biol. Sci.* 6(5):499–512; 2010.
47. Noth, U.; Rackwitz, L.; Steinert, A. F.; Tuan, R. S. Cell delivery therapeutics for musculoskeletal regeneration. *Adv. Drug Deliv. Rev.* 62(7–8):765–783; 2010.
48. Okamoto, N.; Kushida, T.; Oe, K.; Umeda, M.; Ikehara, S.; Iida, H. Treating Achilles tendon rupture in rats with bone-marrow-cell transplantation therapy. *J. Bone Joint Surg. Am.* 92(17):2776–2784; 2010.
49. Otsuru, S.; Gordon, P. L.; Shimono, K.; Jethva, R.; Marino, R.; Phillips, C. L.; Hofmann, T. J.; Veronesi, E.; Dominici, M.; Iwamoto, M.; Horwitz, E. M. Transplanted bone marrow mononuclear cells and MSCs impart clinical benefit to children with osteogenesis imperfecta through different mechanisms. *Blood* 120(9):1933–1941; 2012.
50. Pérez-Ilzarbe, M.; Díez-Campelo, M.; Aranda, P.; Tabera, S.; Lopez, T.; del Cañizo, C.; Merino, J.; Moreno, C.; Andreu, E. J.; Prósper, F.; Pérez-Simón, J. A. Comparison of ex vivo expansion culture conditions of mesenchymal stem cells for human cell therapy. *Transfusion* 49(9):1901–1910; 2009.
51. Phinney, D. G.; Prockop, D. J. Concise review: Mesenchymal stem/multipotent stromal cells: The state of transdifferentiation and modes of tissue repair—Current views. *Stem Cells* 25(11):2896–2902; 2007.
52. Pittenger, M. F.; Mackay, A. M.; Beck, S. C.; Jaiswal, R. K.; Douglas, R.; Mosca, J. D.; Moorman, M. A.; Simonetti, D. W.; Craig, S.; Marshak, D. R. Multilineage potential of adult human mesenchymal stem cells. *Science* 284(5411):143–147; 1999.
53. Prockop, D. J. Repair of tissues by adult stem/progenitor cells (MSCs): Controversies, myths, and changing paradigms. *Mol. Ther.* 17(6):939–946; 2009.
54. Quarto, R.; Mastrogiacomo, M.; Cancedda, R.; Kutepov, S. M.; Mukhachev, V.; Lavroukov, A.; Kon, E.; Marcacci, M. Repair of large bone defects with the use of autologous bone marrow stromal cells. *N. Engl. J. Med.* 344(5):385–386; 2001.
55. Raghunath, J.; Salacinski, H. J.; Sales, K. M.; Butler, P. E.; Seifalian, A. M. Advancing cartilage tissue engineering: The application of stem cell technology. *Curr. Opin. Biotechnol.* 16(5):503–509; 2005.
56. Razzouk, S.; Schoor, R. Mesenchymal stem cells and their challenges for bone regeneration and osseointegration. *J. Periodontol.* 83(5):547–550; 2012.
57. Reddy, S.; Wasnik, S.; Guha, A.; Kumar, J. M.; Sinha, A.; Singh, S. Evaluation of nano-biphasic calcium phosphate ceramics for bone tissue engineering applications: In vitro and preliminary in vivo studies. *J. Biomater. Appl.* 27(5):565–575; 2013.
58. Sato, Y.; Araki, H.; Kato, J.; Nakamura, K.; Kawano, Y.; Kobune, M.; Sato, T.; Miyanishi, K.; Takayama, T.; Takahashi, M.; Takimoto, R.; Iyama, S.; Matsunaga, T.; Ohtani, S.; Matsuura, A.; Hamada, H.; Niitsu, Y. Human mesenchymal stem cells xenografted directly to rat liver are differentiated into human hepatocytes without fusion. *Blood* 106(2):756–763; 2005.
59. Sekiya, I.; Larson, B. L.; Smith, J. R.; Pochampally, R.; Cui, J. G.; Prockop, D. J. Expansion of human adult stem cells from bone marrow stroma: Conditions that maximize the yields of early progenitors and evaluate their quality. *Stem Cells* 20(6):530–541; 2002.
60. Sensebe, L.; Gadelorge, M.; Fleury-Cappellesso, S. Production of mesenchymal stromal/stem cells according to good manufacturing practices: A review. *Stem Cell Res. Ther.* 4(3):66; 2013.
61. Steinert, A. F.; Rackwitz, L.; Gilbert, F.; Noth, U.; Tuan, R. S. Concise review: The clinical application of mesenchymal stem cells for musculoskeletal regeneration: Current status and perspectives. *Stem Cells Transl. Med.* 1(3):237–247; 2012.
62. Syed, B. A.; Evans, J. B. Stem cell therapy market. *Nat. Rev. Drug Discov.* 12(3):185–186; 2013.
63. Teixeira, F. G.; Carvalho, M. M.; Sousa, N.; Salgado, A. J. Mesenchymal stem cells secretome: A new paradigm for central nervous system regeneration? *Cell. Mol. Life Sci.* 70(20):3871–3882; 2013.
64. Toma, C.; Pittenger, M. F.; Cahill, K. S.; Byrne, B. J.; Kessler, P. D. Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. *Circulation* 105(1):93–98; 2002.
65. Toussaint, L. L.; Whipple, M. O.; Abboud, L. L.; Vincent, A.; Wahner-Roedler, D. L. A mind-body technique for symptoms related to fibromyalgia and chronic fatigue. *Explore* 8(2):92–98; 2012.
66. Tsai, C. C.; Chen, Y. J.; Yew, T. L.; Chen, L. L.; Wang, J. Y.; Chiu, C. H.; Hung, S. C. Hypoxia inhibits senescence and maintains mesenchymal stem cell properties through down-regulation of E2A-p21 by HIF-TWIST. *Blood* 117(2):459–469; 2011.
67. United States Pharmacopeia. Thirty-Two Revision Chapter 1046 (USP 32:1046). Rockville, MD: United States Pharmacopeial Convention Inc.; 2009.
68. Vassilopoulos, G.; Wang, P. R.; Russell, D. W. Transplanted bone marrow regenerates liver by cell fusion. *Nature* 422(6934):901–904; 2003.
69. Viateau, V.; Manassero, M.; Sensebe, L.; Langonne, A.; Marchat, D.; Logeart-Avramoglou, D.; Petite, H.; Bensidhoum, M. Comparative study of the osteogenic ability of four different ceramic constructs in an ectopic large animal model. *J. Tissue Eng. Regen. Med.*; 2013.
70. Visiongan Ltd. Translational regenerative medicine: Market prospects 2012–2021. London, United Kingdom; Visiongan Ltd.; 2012.
71. Wakitani, S.; Goto, T.; Pineda, S. J.; Young, R. G.; Mansour, J. M.; Caplan, A. I.; Goldberg, V. M. Mesenchymal cell-based

- repair of large, full-thickness defects of articular cartilage. *J. Bone Joint Surg. Am.* 76(4):579–592; 1994.
72. Wakitani, S.; Imoto, K.; Yamamoto, T.; Saito, M.; Murata, N.; Yoneda, M. Human autologous culture expanded bone marrow mesenchymal cell transplantation for repair of cartilage defects in osteoarthritic knees. *Osteoarthritis Cartilage* 10(3):199–206; 2002.
  73. Wakitani, S.; Nawata, M.; Tensho, K.; Okabe, T.; Machida, H.; Ohgushi, H. Repair of articular cartilage defects in the patello-femoral joint with autologous bone marrow mesenchymal cell transplantation: Three case reports involving nine defects in five knees. *J. Tissue Eng. Regen. Med.* 1(1):74–79; 2007.
  74. Wang, C. Y.; Yang, H. B.; Hsu, H. S.; Chen, L. L.; Tsai, C. C.; Tsai, K. S.; Yew, T. L.; Kao, Y. H.; Hung, S. C. Mesenchymal stem cell-conditioned medium facilitates angiogenesis and fracture healing in diabetic rats. *J. Tissue Eng. Regen. Med.* 6(7):559–569; 2012.
  75. Wang, X.; Willenbring, H.; Akkari, Y.; Torimaru, Y.; Foster, M.; Al-Dhalimy, M.; Lagasse, E.; Finegold, M.; Olson, S.; Grompe, M. Cell fusion is the principal source of bone-marrow-derived hepatocytes. *Nature* 422(6934):897–901; 2003.
  76. Wright, K. T.; El Masri, W.; Osman, A.; Chowdhury, J.; Johnson, W. E. Concise review: Bone marrow for the treatment of spinal cord injury: Mechanisms and clinical applications. *Stem Cells* 29(2):169–178; 2011.
  77. Yan, H.; Yu, C. Repair of full-thickness cartilage defects with cells of different origin in a rabbit model. *Arthroscopy* 23(2):178–187; 2007.
  78. Yew, T. L.; Huang, T. F.; Ma, H. L.; Hsu, Y. T.; Tsai, C. C.; Chiang, C. C.; Chen, W. M.; Hung, S. C. Scale-up of MSC under hypoxic conditions for allogeneic transplantation and enhancing bony regeneration in a rabbit calvarial defect model. *J. Orthop. Res.* 30(8):1213–1220; 2012.
  79. Yew, T. L.; Hung, Y. T.; Li, H. Y.; Chen, H. W.; Chen, L. L.; Tsai, K. S.; Chiou, S. H.; Chao, K. C.; Huang, T. F.; Chen, H. L.; Hung, S. C. Enhancement of wound healing by human multipotent stromal cell conditioned medium: The paracrine factors and p38 MAPK activation. *Cell Transplant.* 20(5):693–706; 2011.
  80. Yoshikawa, T.; Ueda, Y.; Miyazaki, K.; Koizumi, M.; Takakura, Y. Disc regeneration therapy using marrow mesenchymal cell transplantation: A report of two case studies. *Spine* 35(11):E475–480; 2010.
  81. Zhang, P.; Gan, Y. K.; Tang, J.; Hao, Y. Q.; Wang, Y.; Sun, Y. H.; Zhu, Z. A.; Dai, K. R. [Clinical study of lumbar fusion by hybrid construct of stem cells technique and biodegradable material]. *Zhonghua Wai Ke Za Zhi* 46(7):493–496; 2008.