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Mesenchymal Stem Cell Therapy for Bone Regeneration

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Mesenchymal stem cells (MSCs) have been used in clinic for approximately 20 years. During this period, various new populations of MSCs have been found or manipulated. However, their characters and relative strength for bone regeneration have not been well known. For a comprehensive understanding of MSCs, we reviewed the literature on the multipotent cells ranging from the definition to the current research progress for bone regeneration. Based on our literature review, bone marrow MSCs have been most widely studied and utilized in clinical settings. Among other populations of MSCs, adipose-derived MSCs and perivascular MSCs might be potential candidates for bone regeneration, whose efficacy and safety still require further investigation.

Keywords: *Stem cells, Osteogenesis, Bone diseases, Review*

During the past decades, the efficacy of mesenchymal stem cells (MSCs) has been extensively investigated both in basic and clinical experiments, introducing inconsistency and controversy on this topic. According to minimal criteria for definition, MSCs are stromal cells that are plastic-adherent and able to differentiate into osteoblasts, chondroblasts, and adipocytes. They express biomarkers including CD73, CD90, and CD105, and they must not express CD14 or CD11b, CD34, CD45, CD19 or CD79α, and human leukocyte antigen-antigen D related surface molecules.[1] The cells were first found in bone marrow and have been used to promote bone healing for approximately 20 years.[2] Later, MSCs were also found in adipose tissue and vessels and could be obtained from induced pluripotent cells.[3-7] According to their sources or characteristics, MSCs could be divided into bone marrow MSCs (BMSCs), adipose-derived MSCs (ASCs), perivascular stem cells (PSCs), induced pluripotent stem cells (iPSCs), and genetically modified MSCs. However, the relative advantages of each MSCs population for bone regeneration have yet to be established.

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BONE MARROW MSC

BMSCs are MSCs extracted and cultured from bone marrow. Their effects have been broadly tested in preclinical experiments and thoroughly reviewed in many studies.[8] In treating human diseases, systematic infusion of BMSCs has been used to treat children with severe osteogenesis imperfecta. In a study by Horwitz et al.,[9] three patients, age ranging from 13 to 32 months, received 5.7 to 7.5 × 10^8 cell/kg unmanipulated nucleated cells from siblings. The treatment increased the total body bone mineral content and growth velocity of the patients. In a case control study, the treatment group had significantly higher body length increase than the control group and had similar rates of bone mineral content gain with weight-matched healthy children. A patient had an acute graft-versus-host disease and another patient had transient pulmonary insufficiency and a bifrontal hygroma, which resolved uneventfully.[10] The same authors conducted another case control study that included six patients (2 to 4 years of age) who received *ex vivo* expanded autologous BMSCs. Five of the six pa-

tients had significant improvement in growth velocity, but only one of them had substantially increased bone mineral content, and one patient had an urticarial rash that resolved after treatment.[11] In summary, BMSC infusion might be a potential intervention for treating osteogenesis imperfecta, but the evidence that supports its efficacy and safety is still insufficient.

In spinal fusion surgery, BMSCs have been used to promote bone fusion.[12] In a prospective case controlled study, a collagen/hydroxyapatite matrix soaked in BMSCs was compared with traditional iliac autograft. The BMSC-soaked matrix had a comparable effect in posterolateral fusion, whereas it had a relatively inferior effect in interbody fusion and 360° fusion. Though BMSCs might have less osteogenicity than traditional iliac transplantation in some cases, the use of BMSCs could significantly reduce the risk of donor site pain/neuroma.[13]

In treating nonunion, direct injection of the cells into the defect site is widely used. The technique was first introduced in 1991. Twenty tibial nonunion patients were treated with percutaneous injections of bone marrow aspiration and 18 of them achieved roentgenographic union in 6 to 10 months.[14] Similar outcomes appeared in later studies.[15,16] In a comparative study, patients who received *ex vivo* expanded autologous BMSCs (14 to 18 × 10^6) were compared with the patients who received autograft iliac crest transplantation. All the patients achieved successful union in 1 year. The patients treated with BMSCs had faster functional and radiographic improvements.[17] Therefore, BMSCs might be a potential candidate for treating nonunion.

In treating early stage avascular and steroid-induced femoral head osteonecrosis, bone marrow concentrate injection with core decompression has demonstrated a significant effect in slowing the progression of the disease.[18,19] Besides transplantation with core decompression, BMSCs could be delivered *via* the medial circumflex femoral artery.[20] Among 78 hips (68 patients) with different etiological factors (trauma, alcohol, steroid, and idiopathic) and Ficat stage ranging from I to III, 72 hips obtained satisfactory clinical results at 5 years of follow-up. These findings indicate that both transplantation with core decompression and intra-arterial infusion of MSCs are effective interventions for osteonecrosis. However, it should be noted that BMSCs were more effective in treating early stage osteonecrosis, and there was no study that compared the effectiveness of the two administration routes.[21-25]

In summary, the efficacy of BMSCs on bone regeneration in various orthopedic diseases has been proven by cumulative evidence; however, several limitations still impede their use in clinical settings. One of the main limitations is the extremely low-yield (0.001%–0.1%). Successful bone regeneration depends on sufficient concentration and the number of MSCs transplanted in defect sites. It was suggested that the number of MSCs should be at least 30,000 to treat tibial nonunion and 35,000 to treat osteonecrosis.[21,26] However, only 0.001% to 0.01% of mononuclear cells from bone marrow are MSCs. To achieve effective concentration and quantity, a large amount of bone marrow needs to be aspirated, which might lead to additional donor site morbidity. Also, the purification process is necessary for optimal effect because poorly purified BMSCs show inconsistent morphology and finite self-renewal ability and are less likely to differentiate efficiently. Moreover, their differentiation potential is impaired by senescence, which also undermines their efficacy.[27] Therefore, standardized techniques for purification and expansion of BMSCs are needed for further clinical application.

ADIPOSE-DERIVED MSCsASCs have the phenotype of CD44+/CD73+/CD90+/CD105+/CD45–/CD31– and can be isolated *via* lipoaspiration from adipose tissue.[8] The subcutaneous fat is extracted and digested with collagenase to generate the stromal vascular fraction (SVF) that contains ASCs and endothelial and hematopoietic cells. Among them, only the multipotent cells that are plastic adherent and culturable and can be serially passaged are termed ASCs.[28-30] However, the origin of ASCs remains unclear. Cai et al.[31] reported that ASCs originated from perivascular cells, but Maumus et al.[32] stated ASCs were scattered in fat stroma, expressed CD34+, and did not express NG2, CD140α, or α-smooth muscle actin.

The osteogenic potential of ASCs was proven in various animal models,[8] rat calvarial defect,[33-35] femoral head osteonecrosis,[36] femur defect,[37] distraction osteogenesis,[38] and spine fusion,[39] and in cranial bone defects in a canine model.[40] ASCs could be used with various scaffolds, including apatite-coated poly(lactic-co-glycolic acid) scaffolds,[33,34] collagen-ceramic carriers,[37] type I collagen matrix,[39] and coral scaffolds.[35]

The potential of ASCs for bone regeneration has been investigated in several small size clinical trials. In a study by Sandor et al.,[41] 13 consecutive patients with craniomaxillofacial skeleton defect were treated with transplantation of ASCs. The abdominal fat tissue was aspirated and cultured for 10 days to 4 weeks and transplanted to the defect site with bioactive glass or β-tricalcium phosphate. Ten of the 13 patients were successfully treated.[41] The SVF without *in vitro* cell culture also has been investigated in clinical setting. Ten patients who needed maxillary sinus floor elevation was treated with freshly isolated

SVF, which was extracted from autograft fat tissue with a Celution 800/CRS device in a study by Prins et al.[42] The extracted SVF was transplanted with ceramics for elevating the vertical bone height in the posterior maxilla. No significant difference was observed between control and study sides on panoramic radiographs. But both micro-computed tomography evaluation and biopsy evaluation indicated significantly more osteogenesis in the stem cell transplanted side.[42]

ASCs have been used to treat osteoarthritis. A case series study enrolled 21 patients who had grade II to III osteoarthritis, and they were transplanted with SVF and platelet-rich plasma into their knee joints. The patients' visual analog scale score decreased from 7.6 ± 0.5 to 1.5 ± 0.7 after 3 months and to 1.5 ± 0.5 after 6 months, and a thicker cartilage layer was noted after 6 months of treatment.[43] However, an open label prospective clinical trial that enrolled 18 patients with grade III to IV knee osteoarthritis allocated into three groups with different doses of ASCs reported different results. In the trial, 14 days after *ex vivo* culture, the ASCs were injected into the knee under ultrasound guidance. Only patients with a low dose of cells were detected to have statistically significant improvement in clinical outcomes. In magnetic resonance imaging evaluation that was performed on six patients, possible cartilage improvement was only observed in three patients. In histological examination, the sign of stem cell grafting on cartilage surface was only observed in one patient.[44]

On the treatment of bone defect, a case series study included three bone tumor patients and three pseudarthrosis patients who failed conventional treatments including iliac crest autograft. The ASCs were extracted and cultured *ex vivo* for 80 to 143 days before being transplanted with demineralized bone matrix into the defect site. Two of the tumor patients and one pseudarthrosis patient achieved consolidation without severe complications.[45]

In summary, ASCs have advantages of easy access and abundant supply[46] although *ex vivo* expansion is still required to reduce the contamination with other cell types. However, *in vitro* cultivated ASCs have shown decreased stemness, self-renewal, or multipotency,[47] and the proliferative capacity decreased with the host's age, which is a significant drawback to senior and osteoporotic patients.[8] In addition, the safety of ASCs has not been clearly established: chromosomal abnormalities have been observed in cultured ASCs, raising concerns about the safety of ASCs.[8,48,49] Compared with BMSCs, ASCs have demonstrated inferior osteogenicity *in vitro*, and the *in vivo* superiority remains unclear.[50]

PERIVASCULAR STEM CELL

PSCs composed of two kinds of cells, pericytes (CD146+/CD31–/CD45–/CD34–) located in capillaries and microvessels and adventitial cells (CD146–/CD31–/CD45–/CD34+) located in large vessels, have a multipotent differentiation potential.[4] Similar to ASCs, PSCs can be isolated from adipose tissue due to the high vascularization and ample supply.[8] However, unlike ASCs that necessitates *in vitro* expansion for purification and adequate concentration, PSCs can be purified *via* fluorescent-activated cell sorting that requires merely a few hours.[8,51]

In preclinical experiments, PSCs have shown significantly higher osteoinductivity than control groups in a rat spine fusion model[52] and an ectopic ossification model,[53] and in a rat calvarial defect model.[54] In addition, PSCs were proven to have higher osteogenicity than SVF in *in vitro* settings and in an ectopic ossification animal model. However, there is a lack of evidence demonstrating the superiority of PSCs to other cells, and clinical trials on PSCs have not been conducted yet.

In summary, PSCs have the advantage of prospective selection immediately after extraction from the origin and possess the potential for osteoblastic differentiation. However, in terms of safety, function, and clinical potential, further investigation is required.[8]

UMBILICAL CORD BLOOD MSC

Umbilical cord compartments can be used to isolate MSCs. The most widely used compartments are Wharton's jelly (WJ), perivascular tissue, and umbilical cord blood (UCB).[5,55,56] It has been reported that the cell yield of UCB-MSCs is extremely low and the isolation of MSCs is not guaranteed as with WJ samples, but UCB-MSCs have higher osteogenic ability than WJ-MSCs.[57] *In vitro* experiments reported that UCB-MSCs had a longer culture period, a larger scale expansion, a retardation of senescence, and a higher anti-inflammation effect, but they had less osteogenic activity than BMSCs.[58-60] Therefore, the feasibility of UCB-MSCs as an alternative to BMSCs remains controversial. Moreover, this type of cell has not been tested *in vivo* for promoting bone regeneration.

INDUCED PLURIPOTENT STEM CELL

The iPSCs were created by transducing Oct4, Sox2, Klf4, and c-Myc genes to fibroblasts.[61] The possibility of replacing autologous cell with iPSCs/iPSCs-MSCs makes them one of candidates for cell-based bone defect therapies.[62-64]

Various research has demonstrated the osteogenesis ability of iPSC-derived MSCs.[62,65,66] However, their superiority to other sources of MSCs has yet to be determined. In addition, the yield of iPSCs is relatively low, the success rate of induction of iPSCs using murine adult somatic cells is less than 1%.[8] This type of cell has not been used in clinic for bone regeneration.

However, other than the bone repairing ability, iPSC-derived disease models from patients with genetic mutations help us to understand the origins and pathologies of certain diseases. The iPSCs have been used to model infrequent genetically influenced disorders, including fibrodysplasia ossificans progressiva, metatropic dysplasia,[67-71] craniometaphyseal dysplasia[64] and Marfan syndrome.[72]

GENETICALLY MODIFIED MSC: COMPARISONS WITH OTHER CELLS

The MSCs can be modified at the genomic level to improve survival, enhance migration, produce growth factors, and deliver medication.[7] To increase the survival of MSCs *in vivo*, protein kinase B (Akt1),[73] adrenomedullin, B-cell lymphoma-2,[74] and heme oxygenase-1 can be transfected.[75] Bone formation can be elevated by transfecting bone morphogenetic protein (BMP)-2, transforming growth factor-β, latent membrane protein-1, insulin-like growth factor-1, and growth differentiation factor-5.[76-78] Homing of BMSCs to the defect site could be enhanced *via* injection of BMSCs cotransduced with an adenovirus expressing C-X-C chemokine receptor type 4 (CXCR-4) and runt-related transcription factor 2 (RunX2),[79] intravenous injection of retrovirus-engineered BMSCs overexpressing receptor activator of nuclear factor-κB-Fc and CXCR-4,[80] or intravenous injection of peptidomimetic ligand-bisphosphonate (alendronate, Ale), all of which proved to improve the bone formation and bone strength.[81,82]

Besides BMSCs, ASCs can also be genetically modified for bone repair. In a study by Lin et al.,[3] the ASCs were transfected with FLP/FRT recombination that prolonged BMP-2/vascular endothelial growth factor (VEGF) expression in New Zealand white rabbit ASCs for more than 28 days. The modified ASCs were transplanted into a 10 mm femur defect. The ASCs expressed the BMP-2/VEGF for 28 days and the treatment group achieved complete osseous reunion in the defect.[3] A great number of similar experiments have been conducted, which consistently indicated the enormous potential of genetically modified MSCs.[83-87]

Additionally, transduction of iPSCs with an adenovirus expressing RunX2 enhanced osteogenesis *in vitro*.[88] Transplantation of the special AT-rich sequence-binding protein 2-overexpressing iPSCs enhanced new bone formation in a mouse calvarial defect model.[89] Though the efficacy of genetically modified MSCs has been proven in preclinical experiments, it has not been investigated in clinical experiments.

CURRENT LIMITATIONS AND PROSPECTS

Major barriers that limit the clinical application of MCSs include requirement of *in vitro* expansion, donor-related heterogeneity in the quality of MSCs, and lack of standardized procedures in manipulation of the cells.

Among different sources of MSCs, only BMSCs have been extensively researched and proven to treat various orthopedic diseases. However, the usage of the cells was limited by the complications in the donor site and the *ex vivo* culture procedures. The BMSCs might be replaced by ASCs that could be obtained *via* lipoaspiration, but the long *ex vivo* culture period remains a limitation. Therefore, PSCs seem to be a better candidate for replacement of BMSCs than ASCs: PSCs can be isolated with lipoaspiration and purified *via* fluorescent-activated cell sorting without *ex vivo* culture. However, the effect and safety of PSCs have not been studied in human subject research. Genetically modified MSCs might be a potent tool for bone regeneration, but their safety should be confirmed before clinical use.

CONCLUSIONS

BMSCs have been most extensively studied both in preclinical and clinical experiments: the effects of BMSCs in fracture healing, spinal fusion, and osteonecrosis have been sufficiently demonstrated. ASCs possess osteogenesis capacity, but their efficacy and safety still need to be proven in further research. PSCs and genetically modified MSCs might be potential candidates to replace BMSCs for bone regeneration, but their efficacy and safety have yet to be determined in further research.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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