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. The cells were first found in bone marrow and have been used to promote bone healing for approximately 20 years. Later, MSCs were also found in adipose tissue and vessels and could be obtained from induced pluripotent stem cells. 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.

**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. 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., 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. 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.17 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 × 10⁶) 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.8,19 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 MSCs ASCs 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, CD140a, 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,35 type I collagen matrix,36 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 craniofacial 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

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SVF, which was extracted from autograft fat tissue with a Celution 800/CRS device in a study by Prins et al.\(^4\) 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.\(^2\)

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.\(^4\) 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.\(^4\)

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.\(^4\)

In summary, ASCs have advantages of easy access and abundant supply\(^8\), 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,\(^4\) and the proliferative capacity decreased with the host’s age, which is a significant drawback to senior and osteoporotic patients.\(^4\) 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.\(^3,4,48,49\) Compared with BMSCs, ASCs have demonstrated inferior osteogenicity in vitro, and the in vivo superiority remains unclear.\(^50\)

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.\(^6\) 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 compartments can be used to isolate MSCs. The most widely used compartments are Wharton’s jelly (WJ), perivascular tissue, and umbilical cord blood (UCB).\(^2,3,5,56\) 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,\(^{67-71}\) 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-kB-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.
REFERENCES

1. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells: The International Society for Cellular Therapy position statement. Cytotherapy. 2006;8(4):315-7.

2. Hernigou P, Bernaudin F, Reinert P, Kuentz M, Vernant JP. Bone-marrow transplantation in sickle-cell disease: effect on osteonecrosis: a case report with a four-year follow-up. J Bone Joint Surg Am. 1997;79(11):1726-30.

3. Lin CY, Lin KJ, Kao CY, et al. The role of adipose-derived stem cells engineered with the persistently expressing hybrid baculovirus in the healing of massive bone defects. Biomaterials. 2011;32(27):6505-14.

4. Crisan M, Corselli M, Chen WC, Peault B. Perivascular cells for regenerative medicine. J Cell Mol Med. 2012;16(12):2851-60.

5. Klontzas ME, Kenanidis EI, Heliotis M, Tsiridis E, Mantalaris A. Bone and cartilage regeneration with the use of umbilical cord mesenchymal stem cells. Expert Opin Biol Ther. 2015;15(11):1541-52.

6. Csobonyeiova M, Polak S, Zamborsky R, Danisovic L. iPS cell technologies and their prospect for bone regeneration and disease modeling: a mini review. J Adv Res. 2017;8(4):321-7.

7. Park JS, Suryaprakash S, Lao YH, Leong KW. Engineering mesenchymal stem cells for regenerative medicine and drug delivery. Methods. 2015;84:3-16.

8. Asatrian G, Pham D, Hardy WR, James AW, Peault B. Stem cell technology for bone regeneration: current status and potential applications. Stem Cells Cloning. 2015;8:39-48.

9. Horwitz EM, Prockop DJ, Fitzpatrick LA, et al. Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. Nat Med. 1999;5(3):309-13.

10. Horwitz EM, Prockop DJ, Gordon PL, et al. Clinical responses to bone marrow transplantation in children with severe osteogenesis imperfecta. Blood. 2001;97(5):1227-31.

11. Horwitz EM, Gordon PL, Koo WK, et al. 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 U S A. 2002;99(13):8932-7.

12. Ajiboye RM, Eckardt MA, Hamamoto JT, Plotkin B, Daubs MD, Wang JC. Outcomes of demineralized bone matrix enriched with concentrated bone marrow aspirate in lumbar fusion. Int J Spine Surg. 2016;10:35.

13. Neen D, Noyes D, Shaw M, Gwilym S, Fairlie N, Birch N. Healos and bone marrow aspirate used for lumbar spine fusion: a case controlled study comparing healos with autograft. Spine (Phila Pa 1976). 2006;31(18):E636-40.

14. Connolly JF, Guse R, Tiedeman J, Dehne R. Autologous marrow injection as a substitute for operative grafting of tibial nonunions. Clin Orthop Relat Res. 1991;(266):259-70.

15. Garg NK, Gaur S, Sharma S. Percutaneous autogenous bone marrow grafting in 20 cases of ununited fracture. Acta Orthop Scand. 1993;64(6):671-2.

16. Goel A, Sangwan SS, Siwach RC, Ali AM. Percutaneous bone marrow grafting for the treatment of tibial non-union. Injury. 2005;36(1):203-6.

17. Ismail HD, Phedy P, Kholinne E, et al. Mesenchymal stem cell implantation in atrophic nonunion of the long bones: a translational study. Bone Joint Res. 2016;5(7):287-93.

18. Gangji V, Hauzeur JP, Matos C, De Maertelaer V, Tougouz M, Lambermont M. Treatment of osteonecrosis of the femoral head with implantation of autologous bone-marrow cells: a pilot study. J Bone Joint Surg Am. 2004;86(6):1153-60.

19. Gangji V, De Maertelaer V, Hauzeur JP. Autologous bone marrow cell implantation in the treatment of non-traumatic osteonecrosis of the femoral head: five year follow-up of a prospective controlled study. Bone. 2011;49(5):1005-9.

20. Mao Q, Jin H, Liao F, Xiao L, Chen D, Tong P. The efficacy of targeted intraarterial delivery of concentrated autologous bone marrow containing mononuclear cells in the treatment of osteonecrosis of the femoral head: a five year follow-up study. Bone. 2013;57(2):509-16.

21. Hernigou P, Trousselier M, Roubineau F, et al. Stem cell therapy for the treatment of hip osteonecrosis: a 30 year review of progress. Clin Orthop Surg. 2016;8(1):1-8.

22. Hernigou P, Flouzat-Lachaniette CH, Delambre J, et al. Osteonecrosis repair with bone marrow cell therapies: state of the clinical art. Bone. 2015;70:102-9.

23. Kawate K, Yajima H, Ohgushi H, et al. Tissue-engineered approach for the treatment of steroid-induced osteonecrosis of the femoral head: transplantation of autologous mesenchymal stem cells cultured with beta-tricalcium phosphate ceramics and free vascularized fibula. Artif Organs. 2006;30(12):960-2.

24. Sen RK, Tripathy SK, Aggarwal S, Marwaha N, Sharma RR, Khandelwal N. Early results of core decompression and autologous bone marrow mononuclear cells instillation in femoral head osteonecrosis: a randomized control study. J Arthroplasty. 2012;27(5):679-86.
25. Ma Y, Wang T, Liao J, et al. Efficacy of autologous bone marrow buffy coat grafting combined with core decompression in patients with avascular necrosis of femoral head: a prospective, double-blinded, randomized, controlled study. Stem Cell Res Ther. 2014;5(5):115.

26. Hernigou P, Poignard A, Beaufrean F, Rouard H. Percutaneous autologous bone-marrow grafting for nonunions: influence of the number and concentration of progenitor cells. J Bone Joint Surg Am. 2005;87(7):1430-7.

27. Griffin M, Iqbal SA, Bayat A. Exploring the application of mesenchymal stem cells in bone repair and regeneration. J Bone Joint Surg Br. 2011;93(4):427-34.

28. Gimble JM, Katz AJ, Bunnell BA. Adipose-derived stem cells: tissue localization, characterization, and heterogeneity. Stem Cells. 2010;28(3):518-28.

29. Baer PC, Geiger H. Adipose-derived mesenchymal stromal/stem cells heal critical size mouse calvarial defects. PLoS One. 2010;5(6):e11177.

30. Cui L, Liu B, Liu G, et al. Repair of cranial bone defects with autologous adipose-derived stem cells and coral scaffold. Biomaterials. 2010;31(12):2655-64.

31. Cai X, Lin Y, Hauschka PV, Grottkau BE. Adipose stem cells originate from perivascular cells. Biol Cell. 2011;103(9):435-47.

32. Maumus M, Peyrafitte JA, D’Angelo R, et al. Native human adipose stromal cells: localization, morphology and phenotype. Int J Obes (Lond). 2011;35(9):1141-53.

33. Cowan CM, Shi YY, Aalami OO, et al. Adipose-derived adult stromal cells heal critical-size mouse calvarial defects. Nat Biotechnol. 2004;22(5):560-7.

34. Levi B, James AW, Nelson ER, et al. Human adipose derived stromal cells heal critical size mouse calvarial defects. PLoS One. 2010;5(6):e11177.

35. Cui L, Liu B, Liu G, et al. Repair of cranial bone defects with adipose derived stem cells and coral scaffold in a canine model. Biomaterials. 2007;28(36):5477-86.

36. Abudusaimi A, Aihemaitijiang Y, Wang YH, Cui L, Maimitiming S, Abulikemu M. Adipose-derived stem cells enhance bone regeneration in vascular necrosis of the femoral head in the rabbit. J Int Med Res. 2011;39(5):1852-60.

37. Peterson B, Zhang J, Iglesias R, et al. Healing of critically sized femoral defects, using genetically modified mesenchymal stem cells from human adipose tissue. Tissue Eng. 2005;11(1-2):120-9.

38. Sunay O, Can G, Cakir Z, et al. Autologous rabbit adipose tissue-derived mesenchymal stromal cells for the treatment of bone injuries with distraction osteogenesis. Cytotherapy. 2013;15(6):690-702.

39. Hsu WK, Wang JC, Liu NQ, et al. Stem cells from human fat as cellular delivery vehicles in an athymic rat posterolateral spine fusion model. J Bone Joint Surg Am. 2008;90(5):1043-52.

40. Liu G, Zhang Y, Liu B, Sun J, Li W, Cui L. Bone regeneration in a canine cranial model using allogenetic adipose derived stem cells and coral scaffold. Biomaterials. 2013;34(11):2655-64.

41. Sandor GK, Numminen J, Wolff J, et al. Adipose stem cells used to reconstruct 13 cases with cranio-maxillofacial hard-tissue defects. Stem Cells Transl Med. 2014;3(4):530-40.

42. Prins HJ, Schulten EA, Ten Bruggenkate CM, Klein-Nulend J, Helderman LN. Bone regeneration using the freshly isolated autologous stromal vascular fraction of adipose tissue in combination with calcium phosphate ceramics. Stem Cells Transl Med. 2016;5(10):1362-74.

43. Bui KH, Duong TD, Nguyen, NT, et al. Symptomatic knee osteoarthritis treatment using autologous adipose derived stem cells and platelet-rich plasma: a clinical study. Biomed Res Ther. 2014;1(1):2-8.

44. Pers YM, Rackwitz L, Ferreira R, et al. Adipose mesenchymal stromal cell-based therapy for severe osteoarthritis of the knee: a phase I dose-escalation trial. Stem Cells Transl Med. 2016;5(7):847-56.

45. Dufrane D, Docquier PL, Delloye C, Poirel HA, Andre W, Aouassar N. Scaffold-free three-dimensional graft from autologous adipose-derived stem cells for large bone defect reconstruction: clinical proof of concept. Medicine (Baltimore). 2015;94(50):e2220.

46. Yousefi AM, James PF, Akbarzadeh R, Subramanian A, Flavin C, Oudadesse H. Prospect of stem cells in bone tissue engineering: a review. Stem Cells Int. 2016;2016:6180487.

47. Requicha JE, Viegas CA, Albuquerque CM, Azevedo JM, Reis RL, Gomes ME. Effect of anatomical origin and cell passage number on the stemness and osteogenic differentiation potential of canine adipose-derived stem cells. Stem Cell Rev. 2012;8(4):1211-22.

48. Bellotti C, Stanco D, Ragazzini S, et al. Analysis of the karyotype of expanded human adipose-derived stem cells for bone reconstruction of the maxillo-facial region. Int J Immunopathol Pharmacol. 2013;26(1 Suppl):3-9.

49. Meza-Zepeda LA, Noer A, Dahl JA, Micci F, Myklebost O, Collas P. High-resolution analysis of genetic stability of human adipose tissue stem cells cultured to senescence. J Cell Mol Med. 2008;12(2):553-63.

50. Liao HT, Chen CT. Osteogenic potential: comparison between bone marrow and adipose-derived mesenchymal stem cells. World J Stem Cells. 2014;6(3):288-95.
51. Corselli M, Crisan M, Murray IR, et al. Identification of perivascular mesenchymal stromal/stem cells by flow cytometry. Cytometry A. 2013;83(8):714-20.
52. Chung CG, James AW, Asatryan G, et al. Human perivascular stem cell-based bone graft substitute induces rat spinal fusion. Stem Cells Transl Med. 2014;3(10):1231-41.
53. Askarinam A, James AW, Zara JN, et al. Human perivascular stem cells show enhanced osteogenesis and vasculogenesis with Nél-like molecule I protein. Tissue Eng Part A. 2013;19(11-12):1386-97.
54. James AW, Zara JN, Corselli M, et al. An abundant perivascular source of stem cells for bone tissue engineering. Stem Cells Transl Med. 2012;1(9):673-84.
55. Kargozar S, Lotfibakhshaiesh N, Ai J, et al. Strontium- and cobalt-substituted bioactive glasses seeded with human umbilical cord perivascular cells to promote bone regeneration via enhanced osteogenic and angiogenic activities. Acta Biomater. 2015;8(5):502-14.
56. Bosch J, Houben AP, Radke TF, et al. Distinct differentiation potential of "MSC" derived from cord blood and umbilical cord: are cord-derived cells true mesenchymal stromal cells? Stem Cells Dev. 2012;21(11):1977-88.
57. Jin HJ, Bae YK, Kim M, et al. Comparative analysis of human mesenchymal stem cells from bone marrow, adipose tissue, and umbilical cord blood as sources of cell therapy. Int J Mol Sci. 2013;14(9):17986-8001.
58. Bosch J, Houben AP, Hennicke T, et al. Comparing the gene expression profile of stromal cells from human cord blood and bone marrow: lack of the typical "bone" signature in cord blood cells. Stem Cells Int. 2013;2013:631984.
59. Maher S, Kolieb E, Sabik NA, Abd-Elhalim D, El-Serafi AT, El-Wazir Y. Comparison of the osteogenic differentiation potential of mesenchymal cells isolated from human cord blood, umbilical cord blood and placenta derived stem cells. Beni-Suef Univ J Basic Appl Sci. 2015;4(1):80-5.
60. Worringer KA, Rand TA, Hayashi Y, et al. The let-7/LIN-41 pathway regulates reprogramming to human induced pluripotent stem cells by controlling expression of prodifferentiation genes. Cell Stem Cell. 2014;14(1):40-52.
61. Xie J, Peng C, Zhao Q, et al. Osteogenic differentiation and bone regeneration of iPSC-MSCs supported by a biomimetic nanofibrous scaffold. Acta Biomater. 2016;29:365-79.
62. Ardeshiryaljami A, Soleimani M, Hosseinkhani S, Parivar K, Yaghmaei P. A comparative study of osteogenic differentiation human induced pluripotent stem cells and adipose tissue derived mesenchymal stem cells. Cell J. 2014;16(3):235-44.
63. Chen IP. The use of patient-specific induced pluripotent stem cells (iPSCs) to identify osteoclast defects in rare genetic bone disorders. J Clin Med. 2014;3(4):1490-510.
64. Sheyn D, Ben-David S, Shapiro G, et al. Human induced pluripotent stem cells differentiate into functional mesenchymal stem cells and repair bone defects. Stem Cells Transl Med. 2016;5(11):1447-60.
65. Tang M, Chen W, Liu J, Weir MD, Cheng L, Xu HH. Human induced pluripotent stem cell-derived mesenchymal stem cell seeding on calcium phosphate scaffold for bone regeneration. J Bone Miner Res. 2014;29(7-8):1295-305.
66. Barruet E, Hsiao EC. Using human induced pluripotent stem cells to model skeletal diseases. Methods Mol Biol. 2016;1353:101-18.
67. Saitta B, Passarini J, Sareen D, et al. Patient-derived skeletal dysplasia induced pluripotent stem cells display abnormal chondrogenic marker expression and regulation by BMP2 and TGFβ1. Stem Cells Dev. 2014;23(13):1464-78.
68. Matsumoto Y, Hashi Y, Schlieve CR, et al. Induced pluripotent stem cells from patients with human fibrodysplasia ossificans progressiva show increased mineralization and cartilage formation. Orphanet J Rare Dis. 2013;8:190.
69. Matsumoto Y, Ikeya M, Hino K, et al. New protocol to optimize iPS cells for genome analysis of fibrodysplasia ossificans progressiva. Stem Cells. 2015;33(6):1730-42.
70. Barruet E, Morales BM, Lwin W, et al. The ACVR1 R206H mutation found in fibrodysplasia ossificans progressiva increases human induced pluripotent stem cell-derived endothelial cell formation and collagen production through BMP-mediated SMAD1/5/8 signaling. Stem Cell Res Ther. 2016;7(1):115.
71. Quarto N, Leonard B, Li S, et al. Skeletogenic phenotype of human Marfan embryonic stem cells faithfully phenocopied by patient-specific induced-pluripotent stem cells. Proc Natl Acad Sci U S A. 2012;109(1):215-20.
72. Yu YS, Shen ZY, Ye WX, et al. AKT-modified autologous intracoronary mesenchymal stem cells prevent remodeling and repair in swine infarcted myocardium. Chin Med J (Engl). 2010;123(13):1702-8.
73. Li Y, Yu X, Lin S, Li X, Zhang S, Song YH. Insulin-like growth factor 1 enhances the migratory capacity of mesenchymal stem cells. Biochem Biophys Res Commun. 2007;356(3):780-4.
74. Tsubokawa T, Yagi K, Nakanishi C, et al. Impact of anti-apoptotic and anti-oxidative effects of bone marrow mes-
enchymal stem cells with transient overexpression of heme oxygenase-1 on myocardial ischemia. Am J Physiol Heart Circ Physiol. 2010;298(5):H1320-9.

76. Hodgkinson CP, Gomez JA, Mirotou M, Dzau VJ. Genetic engineering of mesenchymal stem cells and its application in human disease therapy. Hum Gene Ther. 2010;21(11):1513-26.

77. Lu CH, Chang YH, Lin SY, Li KC, Hu YC. Recent progresses in gene delivery-based bone tissue engineering. Biotechnol Adv. 2013;31(8):1695-706.

78. Kimelman Bleich N, Kallai I, Lieberman JR, Schwarz EM, Pelled G, Gazit D. Gene therapy approaches to regenerating bone. Adv Drug Deliv Rev. 2012;64(12):1320-30.

79. Lien CY, Chih-Yuan Ho K, Lee OK, Blunn GW, Su Y. Restoration of bone mass and strength in glucocorticoid-treated mice by systemic transplantation of CXCR4 and cbfa-1 co-expressing mesenchymal stem cells. J Bone Miner Res. 2009;24(5):837-48.

80. Cho SW, Sun HJ, Yang JY, et al. Transplantation of mesenchymal stem cells overexpressing RANK-Fc or CXCR4 prevents bone loss in ovariectomized mice. Mol Ther. 2009;17(11):1979-87.

81. Guan M, Yao W, Liu R, et al. Directing mesenchymal stem cells to bone to augment bone formation and increase bone mass. Nat Med. 2012;18(3):456-62.

82. Yao W, Lane NE. Targeted delivery of mesenchymal stem cells to the bone. Bone. 2015;70:62-5.

83. He X, Dziak R, Yuan X, et al. BMP2 genetically engineered MSCs and EPCs promote vascularized bone regeneration in rat critical-sized calvarial bone defects. PLoS One. 2013;8(4):e60473.

84. Zou D, Zhang Z, He J, et al. Repairing critical-sized calvarial defects with BMSCs modified by a constitutively active form of hypoxia-inducible factor-1α and a phosphate cement scaffold. Biomaterials. 2011;32(36):9707-18.

85. Zou D, Zhang Z, Ye D, et al. Repair of critical-sized rat calvarial defects using genetically engineered bone marrow-derived mesenchymal stem cells overexpressing hypoxia-inducible factor-1α. Stem Cells. 2011;29(9):1380-90.

86. Zou D, Zhang Z, Ho J, et al. Blood vessel formation in the tissue-engineered bone with the constitutively active form of HIF-1α mediated BMSCs. Biomaterials. 2012;33(7):2097-108.

87. Yu L, Lu J, Zhang B, et al. miR-26a inhibits invasion and metastasis of nasopharyngeal cancer by targeting EZH2. Oncol Lett. 2013;5(4):1223-8.

88. Tashiro K, Inamura M, Kawabata K, et al. Efficient adipocyte and osteoblast differentiation from mouse induced pluripotent stem cells by adenoviral transduction. Stem Cells. 2009;27(8):1802-11.

89. Ye JH, Xu YJ, Gao J, et al. Critical-size calvarial bone defects healing in a mouse model with silk scaffolds and SATB2-modified iPSCs. Biomaterials. 2011;32(22):5065-76.