**Name of Journal:** *World Journal of Methodology*

**Manuscript NO:** 83250

**Manuscript Type:** MINIREVIEWS

**Is mandible derived mesenchymal stromal cells superior in proliferation and regeneration to long bone-derived mesenchymal stromal cells?**

Jeyaraman M *et al*. Mandible-derived MSCs *vs* long bone-derived MSCs

Madhan Jeyaraman, Tushar Verma, Naveen Jeyaraman, Bishnu Prasad Patro, Arulkumar Nallakumarasamy, Manish Khanna

**Madhan Jeyaraman,** Department of Orthopaedics, ACS Medical College and Hospital, Dr MGR Educational and Research Institute, Chennai 600056, Tamil Nadu, India

**Madhan Jeyaraman,** Department of Biotechnology, School of Engineering and Technology, Sharda University, Greater Noida 201310, Uttar Pradesh, India

**Madhan Jeyaraman, Naveen Jeyaraman, Bishnu Prasad Patro, Arulkumar Nallakumarasamy, Manish Khanna,** Department of Regenerative Medicine, Indian Stem Cell Study Group Association, Lucknow 226010, Uttar Pradesh, India

**Tushar Verma, Naveen Jeyaraman, Arulkumar Nallakumarasamy,** Department of Orthopaedic Rheumatology, Fellow in Indian Orthopaedic Rheumatology Association, Lucknow 226010, Uttar Pradesh, India

**Naveen Jeyaraman,** Department of Orthopaedics, Rathimed Speciality Hospital, Chennai 600040, Tamil Nadu, India

**Bishnu Prasad Patro, Arulkumar Nallakumarasamy,** Department of Orthopaedics, All India Institute of Medical Sciences, Bhubaneswar 751019, Odisha, India

**Author contributions:** All authors contributed equally in writing the manuscript.

**Corresponding author: Madhan Jeyaraman, MS (Orth), FEIORA, FIRM, FROSM, FASM, PhD, Assistant Professor, Research Associate,** Department of Orthopaedics, ACS Medical College and Hospital, Dr MGR Educational and Research Institute, Chennai 600056, Tamil Nadu, India. madhanjeyaraman@gmail.com

**Received:** January 14, 2023

**Revised:** February 1, 2023

**Accepted:** February 10, 2023

**Published online:**

**Abstract**

Mesenchymal stromal cells (MSCs) are cells with the characteristic ability of self-renewal along with the ability to exhibit multilineage differentiation. Bone marrow (BM) is the first tissue in which MSCs were identified and BM-MSCs are most commonly used among various MSCs in clinical settings. MSCs can stimulate and promote osseous regeneration. Due to the difference in the development of long bones and craniofacial bones, the mandibular-derived MSCs (M-MSCs) have distinct differentiation characteristics as compared to that of long bones. Both mandibular and long bone-derived MSCs are positive for MSC-associated markers such as CD-73, -105, and -106, stage-specific embryonic antigen 4 and Octamer-4, and negative for hematopoietic markers such as CD-14, -34, and -45. As the M-MSCs are derived from neural crest cells, they have embryogenic cells which promote bone repair and high osteogenic potential. *In vitro* and *in vivo* animal-based studies demonstrate a higher rate of proliferation and high osteogenic potential for M-MSCs as compared to long-bones MSCs, but *in vivo* studies in human subjects are lacking. The BM-MSCs have their advantages and limitations. M-MSCs may be utilized as an alternative source of MSCs which can be utilized for tissue engineering and promoting the regeneration of bone. M-MSCs may have potential advantages in the repair of craniofacial or orofacial defects. Considering the utility of M-MSCs in the field of orthopaedics, we have discussed various unresolved questions, which need to be explored for their better utility in clinical practice.

**Key Words:** Mandible; Long bone; Mesenchymal stromal cells; Osteogenic potential; Regeneration

Jeyaraman M, Verma T, Jeyaraman N, Patro BP, Nallakumarasamy A, Khanna M. Is mandible derived mesenchymal stromal cells superior in proliferation and regeneration to long bone-derived mesenchymal stromal cells? *World J Methodol* 2023; In press

**Core Tip:** Due to the difference in the development of long bones and craniofacial bones, the mandibular-derived MSCs (M-MSCs) have distinct differentiation characteristics as compared to that of long bones. In vitro and *in vivo* animal-based studies demonstrate a higher rate of proliferation and high osteogenic potential for M-MSCs as compared to long-bones MSCs, but *in vivo* studies in human subjects are lacking. Considering the utility of M-MSCs in the field of orthopaedics, we have discussed various unresolved questions, which need to be explored for their better utility in clinical practice.

**INTRODUCTION**

Mesenchymal stromal cells (MSCs) are cells with the ability to self-renew along with the ability to exhibit multilineage differentiation[1,2]. Initially, they were identified from the murine bone marrow (BM) as “plastic-adherent cells”, which are mainly generated from the fibroblast colony-forming units (CFU-F). Friedenstein *et al*[3] first identified CFU-F by isolating adherent cells from the BM stroma of newborn rodents which can form discrete colonies. However, these cells are regulated by various mitogenic factors such as epidermal growth factor, platelet-derived growth factor, transforming growth factor-β, basic fibroblast growth factor, and insulin growth factor-1[4-6].

Previously, MSCs were given much attention due to their precious role in creating a supportive microenvironment in the hematopoietic tissue but later their precursor role was identified for the formation of skeletal tissue/bone[7,8]. MSCs in adults have been studied extensively in animals as well as humans and have been isolated from various tissues such as BM of long bones (including ilium, femur, tibia) and mandibular bone[9-11].

International Society for Cellular Therapy has suggested the identification criteria for mesenchymal progenitors i.e. these cells can express CD-73, -90, and -105 but cannot express CD-11b or -14, -19 or -79a, -34, -45, -34 and human leukocyte antigen (HLA) -DR[12,13]. MSCs are used in the treatment of non-healing ulcers or wounds, for promoting bone regeneration in cases with non-healing or delayed healing, and MSCs can differentiate into various tissue-specific cell types, which can promote angiogenesis. Treatment with these cells has shown promising results in wound healing by various mechanisms such as promoting re-epithelialization, improving granulation tissue, promoting angiogenesis, and reducing inflammatory reactions. MSCs are utilized in the management of chronic non-healing ulcers, diabetic ulcers, bed/pressure sores, and radiation-induced burns[14].

An electronic search was conducted until Dec 2022 including articles from January 2003 to December 2022 databases such as PubMed, Web of Science, Embase, and CNKI (China Knowledge Resource Integrated Database). The terms used for the search included: “mesenchymal stromal cell”, “MSCs”, “mandible”, “long bone”, “regenerative potential”, “proliferation”, and “regeneration”. In this manuscript, we compared the proliferation and regenerative potential of mandible and long bones.

**Bone marrow-derived MSCs**

Bone marrow is the first tissue in which MSCs were identified and BM-MSCs are most commonly used in clinical settings. The Food and Drug Administration registered the first drug derived from BM-MSCs called “prochymal”, a drug against Graft *vs* Host Disease[15]. MSCs derived from the BM have a unique ability to proliferate and differentiate into various cell types in the culture i.e. fibroblasts, chondrocytes, osteocytes, adipocytes, myogenic cells, *etc.* Apart from this, MSCs also can secrete potent bioactive cytokines, which help the MSCs to regulate other cell types[16-18].MSCs can be obtained from BM of long bones which are appendicular bones derived from the mesoderm. However, maxillary and mandibular bones develop from the neural crest cells[19]. These differences in the development of the long bone and mandibular bones may reflect the difference in the properties of progenitor cells derived from different BM sites. Previous studies have reported phenotypic and functional differences in laboratory studies for cell proliferation, adipogenic potential, osteogenic potential, efficiency to form colonies, and cell surface markers[20-22]. These cells have therapeutic significance i.e. they can stimulate bone growth and promote the regeneration of the bone. MSCs have been suggested to be beneficial in the management of fractures with delayed union or non-union. These cells are documented to have certain advantages; first, these cells can migrate to the site of injury and promote regeneration; secondly, these cells suppress the local immune response; third, the quantity of the MSCs can be obtained in large amounts from patients themselves[23].

Overall, the efficacy of MSCs has been established *in vitro* studies. However, the survival of these cells *in vivo* largely depends upon depends on cell survival, osteogenic differentiation, and host cell recruitment. The major limiting factor affecting the therapeutic potential of MSCs is their low survival rates following transplantation. Literature suggests that transplanted MSCs cannot survive in the presence of temporal hypoxia or a harsh microenvironment where the MSCs of the donor are not able to survive and eventually undergo apoptosis[24]. The advantages of BM-MSCs include high stability in the culture, feasible accessibility to harvesting sites, and high osteogenic potential. The disadvantages of BM-MSCs include the painful BM harvesting process and the risk of infection by the procedure[25].

**Mandible derived MSCs**

The maxillofacial region is one of the richest sources of BM-MSCs. This region is comprised of bones particularly jaw bones, dental tissues, blood vessels, nerves, adipose tissue, and muscular tissue[11]. The MSCs from BM of the mandible (jaw) was first described in 2005 by Matsubara *et al*[20]. Neural crest cells [cranial, vagal, trunk, and cardiac] help in the development of the peripheral nervous system, orofacial and cranial bones including the mandible, melanocytes, smooth muscle cells, and endocrine cells[26,27]. The intramembranous ossification leads to the formation of craniofacial bones.

*Features of M-MSCs*

Due to the difference in the development of long bones and craniofacial bones, M-MSCs have distinct differentiation characteristics as compared to long bones[20,21]. Yamaza *et al*[28] studied the features of M-MSCs isolated from the mouse. They reported that M-MSCs are capable of forming adherent colonies due to the presence of a colony-forming unit (CFU) and the number of colonies was 55.3 ± 9.07/1.5 × 106 cells/plate. The potential of doubling and rate of cell proliferation of M-MSCs are much higher than BM-MSCs. M-MSCs are positive for MSC-associated markers such as CD-73, -105, and -106, stage-specific embryonic antigen 4 (SSEA-4), and Octamer-4 (Oct-4) whereas it is negative for hematopoietic markers such as CD-14, -34, and -45. M-MSCs are weakly positive for c-Kit and strongly positive for Sca-1 (stem cell antigen-1).

***In vitro evidence of superiority in lineages of M-MSCs***

Lee *et al*[29] investigated the role of M-MSCs *in vitro* studies and observed the formation of mineral nodules as early as 14 d of the osteogenic differentiation, which tends to increase over time till 21 d. These cells can suppress T lymphocytes and thus have been recommended in acute graft *vs* host disease. Li *et al*[24] observed the growth of M-MSCs within 2 to 3 d of the culture and the proliferation time was also documented to be much earlier *in vitro* study. Cytometric analysis revealed strong expression of CD-29, -73, -90, and -105. M-MSCs have higher osteogenic and mineralization potential as compared to femoral BM-MSCs, but the serial passage *in vitro* reduces differentiation potentials[29]. Yamaza *et al*[28] observed M-MSCs from mice to have stronger suppressive effects on anti-CD3 antibody proliferation which activates T cells thereby suppressing T cell activation. M-MSCs produce NO in a higher amount as compared to BM-MSCs when stimulated with IFN-γ. The multilineage differentiation under osteogenic conditions revealed their differentiation into osteoblasts with increased activity of serum alkaline phosphatase (ALP) and increased mineralized nodule formation. Also, these cells exhibit higher expression of osteoblastic markers such as osteocalcin, RunX2, and ALP.

***In vivo evidence of superiority in lineages of M-MSCs***

Lee *et al*[29] reported a significantly higher rate of mineralization in the rat calvarial defects implanted with gel foam with M-MSCs as compared with gel foam only. The volume of new bone was 80.88% ± 0.68% for the gel foam with the M-MSCs group and only 49.87% ± 0.94% for only the gel foam group. Overall, M-MSCs have reported higher osteogenic potential with high site-specific bone regeneration capacity[20,21]. Various studies have documented the osteogenic potential of M-MSCs which helps in bone regeneration[30-32]. Deluiz *et al*[33] in their rat model study demonstrated that M-MSCs inoculation significantly promoted bone formation at 4 wk (22.75 ± 2.25 mm3) as well as at 8 wk (64.95 ± 5.41 mm3) as compared to acellular bone microparticles (2.34 ± 2.91 mm3 and42.73 ± 10.58 mm3 at 4 wk and 8 wk respectively). The TRAP and osteocalcin-positive cells were also higher on immunohistochemical analysis at 4 wk in the cell-seeded group as compared to the acellular group. Yamaza *et al*[28] transplanted M-MSCs into immunocompromised mice along with a carrier [hydroxyapatite/tricalcium phosphate (HA/TCP)] and demonstrated increased osteogenic potential in the form of increased bone formation.

**Long bone-derived MSCs**

MSCs were initially derived from the long appendicular bones and these bones are the principal source of MSCs in clinical settings owing to their feasible accessibility. The appendicular bones develop from mesoderm[34]. The most common location among the appendicular bone for isolation of MSCs is the iliac crest. The alternative sites include long bones (tibia, femur, humerus, radius) and sternum[34]. Literature suggests that MSCs properties as well as graft retaining properties of MSCs may vary depending upon harvesting sites[35].

***Features of long bone-derived MSCs***

As the sites of BM aspiration of appendicular bones are easily accessible, aspiration is easy[35]. These cells are positive for MSC-associated markers such as CD-29, -44, -73, -90, -105, -166, and HLA-ABC and negative for hematopoietic markers such as CD-14, -34, and -45[28,35]. The osteogenic potential of the MSCs helps in bone regeneration and bone repair. The MSCs have been utilized in the management of delayed union or non-union of fracture, osteogenesis imperfecta, osteoporosis, *etc.* Also, the MSCs can differentiate into chondrocytes, adipocytes, osteocytes, *etc*[36].

***In vitro evidence in lineages of long bone-derived MSCs***

Li *et al*[37] observed the appearance of colonies of femur-derived MSCs (F-MSCs) was scantly on the 2nd or 3rd day. Cytometric analysis revealed strong expression of CD-29, -73, -90, and -105. The cells derived from F-MSCs have osteogenic and mineralization potential, and the serial passage *in vitro* does not reduce the ability of differentiation of these cells. Proliferation is delayed but the cloning rate is higher. The osteogenic potential as evidenced by ALP lasted beyond 21 d. Lee *et al*[29] investigated the role of F-MSCs *in vitro* study and observed mineralization within 14 days these cells express CD-44, -72, -90, and -105, but failed to express CD-34 and -45.

***In vivo evidence in lineages of long bone-derived MSCs***

The F-MSCs have increased osteogenic potential when transplanted into immunocompromised mice as evidenced by the increased bone formation in a study by Yamaza *et al*[28]. Aghaloo *et al*[22] observed a primarily cartilaginous matrix following long bone-derived MSC implantation with good osteoblastic differentiation. The periosteum of long bones contains mesenchymal progenitors which have high proportions of EdU (DNA synthesis probe)-positive cells and possess the highest clonogenic ability. Apart from this, these progenitors have a lower rate of apoptosis with high proliferative properties[38]. A comparison of mandible *vs* long bone-derived MSCs is depicted in Table 1.

**Comparison of MSCs from femur, tibia, humerus, radius, and ilium**

Recently, MSCs have been harvested from BM of long bones such as the femur (proximal and distal), tibia, humeral head, radius, ilium, *etc*[39,40]. The posterior part of the iliac crest is preferred for obtaining autologous stem cells as it contains the highest amount of nucleated cells (25.1–54.7) × 106 cells/mL, whereas the concentration of nucleated cells in the anterior iliac crest is (24.4–49) × 106 cells/mL. However, the mean number of nucleated cells in decreasing concentration has been reported from the proximal humerus (38.7 × 106 cells/mL), followed by the distal femur (25.9 × 106 cells/mL), humeral head, and proximal tibia (12.1 × 106 cells/mL)[39]. Mc Daniel *et al*[41] observed the highest BM aspirate, higher nucleated cells, and highest CFUs from the iliac crest. However, CFUs from bone marrow aspirate (BMA) of the iliac crest, femur, tibia, and humerus were 12692.3 ± 4981.4, 11235.2 ± 3451.6, 9433.9 ± 4065.1, and 9347.3 ± 3366.3 respectively whereas that from concentrated BMA aspirates, highest CFU was obtained from the iliac crest, followed by tibia, femur and least was from humerus.

**Lacunae in understanding M-MSCs**

Though M-MSCs has been utilized in animal studies and their osteogenic potential, immunomodulatory effect and clinical utility have been documented, studies in human are lacking and the mechanism depicting *in vivo* potential in therapeutic and clinical setting needs further elucidation. The factors affecting these cells when transplanted *in vivo* such as route of inoculation, time, indication for inoculation, and location of their inoculation need to be explored. Autologous M-MSCs potential is explored in previous studies, and literature elucidating the roles of allogenic M-MSCs in bone repair/regeneration with risks of rejection needs further exploration. Despite the utility of M-MSCs in the field of orthopaedics, there remain various unresolved questions, which need to be explored for their better utility in clinical practice.

**Author’s Opinions**

BM-MSCs have adherent properties that form the colonies and have osteogenic potential with the characteristic ability to differentiate into various types of cells such as osteoblasts, chondrocytes, adipocytes, *etc.* Irrespective of sites, BM-MSCs can suppress T lymphocytes and cell-mediated immunity supporting its utility in graft *vs* host disease. Concerning the accessibility and ease of obtaining the BM-MSCs, long bones are superior and the cells could be obtained as early as 2 min. However, the risk of infection is high[25] in the case where BM is derived from long bones. M-MSCs have a significantly higher number of CFUs, high proliferation rate, higher ALP activity, and high osteogenic potential as compared to MSCs derived from long bones, especially during the initial 14 d[28,41]. For the prolonged duration, the MSCs derived from BM-MSCs had higher activity and less apoptosis. The doubling time and cloning time are also superior for MSC derived from long bones as compared to M-MSCs. Therefore, we recommend the regenerative medicine researchers and experts to explore the regenerative potential of mandible derived MSCs in chondrogenesis and osteogenesis.

**CONCLUSION**

MSCs are of therapeutic significance for bone repair and regeneration. As M-MSCs are derived from neural crest cells, they have embryogenic cells which promote bone repair and have high osteogenic potential. *In vitro* and *in vivo* animal-based studies demonstrate a higher rate of proliferation and higher osteogenic potential for M-MSCs as compared to long-bones-derived MSCs, but *in vivo* studies including human subjects are still lacking. BM-MSCs have their advantages and limitations. M-MSCs may be utilized as an alternative source of MSCs which can be utilized for tissue engineering and promoting the regeneration of bone. M-MSCs may have potential advantages in the repair of craniofacial or orofacial defects.

**REFERENCES**

1 **Dennis JE**, Carbillet JP, Caplan AI, Charbord P. The STRO-1+ marrow cell population is multipotential. *Cells Tissues Organs* 2002; **170**: 73-82 [PMID: 11731697 DOI: 10.1159/000046182]

2 **Torensma R**, ter Brugge PJ, Jansen JA, Figdor CG. Ceramic hydroxyapatite coating on titanium implants drives selective bone marrow stromal cell adhesion. *Clin Oral Implants Res* 2003; **14**: 569-577 [PMID: 12969360 DOI: 10.1034/j.1600-0501.2003.00949.x]

3 **Friedenstein AJ**, Petrakova KV, Kurolesova AI, Frolova GP. Heterotopic of bone marrow. Analysis of precursor cells for osteogenic and hematopoietic tissues. *Transplantation* 1968; **6**: 230-247 [PMID: 5654088]

4 **Hori Y**, Inoue S, Hirano Y, Tabata Y. Effect of culture substrates and fibroblast growth factor addition on the proliferation and differentiation of rat bone marrow stromal cells. *Tissue Eng* 2004; **10**: 995-1005 [PMID: 15363157 DOI: 10.1089/ten.2004.10.995]

5 **Lucarelli E**, Beccheroni A, Donati D, Sangiorgi L, Cenacchi A, Del Vento AM, Meotti C, Bertoja AZ, Giardino R, Fornasari PM, Mercuri M, Picci P. Platelet-derived growth factors enhance proliferation of human stromal stem cells. *Biomaterials* 2003; **24**: 3095-3100 [PMID: 12895582 DOI: 10.1016/s0142-9612(03)00114-5]

6 **Solchaga LA**, Penick K, Porter JD, Goldberg VM, Caplan AI, Welter JF. FGF-2 enhances the mitotic and chondrogenic potentials of human adult bone marrow-derived mesenchymal stem cells. *J Cell Physiol* 2005; **203**: 398-409 [PMID: 15521064 DOI: 10.1002/jcp.20238]

7 **Nombela-Arrieta C**, Ritz J, Silberstein LE. The elusive nature and function of mesenchymal stem cells. *Nat Rev Mol Cell Biol* 2011; **12**: 126-131 [PMID: 21253000 DOI: 10.1038/nrm3049]

8 **Bianco P**, Robey PG, Saggio I, Riminucci M. "Mesenchymal" stem cells in human bone marrow (skeletal stem cells): a critical discussion of their nature, identity, and significance in incurable skeletal disease. *Hum Gene Ther* 2010; **21**: 1057-1066 [PMID: 20649485 DOI: 10.1089/hum.2010.136]

9 **Friedenstein AJ**, Piatetzky-Shapiro II, Petrakova KV. Osteogenesis in transplants of bone marrow cells. *J Embryol Exp Morphol* 1966; **16**: 381-390 [PMID: 5336210]

10 **Jacobs FA**, van de Vyver M, Ferris WF. Isolation and Characterization of Different Mesenchymal Stem Cell Populations from Rat Femur. *Methods Mol Biol* 2019; **1916**: 133-147 [PMID: 30535691 DOI: 10.1007/978-1-4939-8994-2\_13]

11 **Botelho J**, Cavacas MA, Machado V, Mendes JJ. Dental stem cells: recent progresses in tissue engineering and regenerative medicine. *Ann Med* 2017; **49**: 644-651 [PMID: 28649865 DOI: 10.1080/07853890.2017.1347705]

12 **Horwitz EM**, Le Blanc K, Dominici M, Mueller I, Slaper-Cortenbach I, Marini FC, Deans RJ, Krause DS, Keating A; International Society for Cellular Therapy. Clarification of the nomenclature for MSC: The International Society for Cellular Therapy position statement. *Cytotherapy* 2005; **7**: 393-395 [PMID: 16236628 DOI: 10.1080/14653240500319234]

13 **Dominici M**, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop Dj, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 2006; **8**: 315-317 [PMID: 16923606 DOI: 10.1080/14653240600855905]

14 **Ahangar P**, Mills SJ, Cowin AJ. Mesenchymal Stem Cell Secretome as an Emerging Cell-Free Alternative for Improving Wound Repair. *Int J Mol Sci* 2020; **21** [PMID: 32987830 DOI: 10.3390/ijms21197038]

15 **Musiał-Wysocka A**, Kot M, Majka M. The Pros and Cons of Mesenchymal Stem Cell-Based Therapies. *Cell Transplant* 2019; **28**: 801-812 [PMID: 31018669 DOI: 10.1177/0963689719837897]

16 **Mimeault M**, Hauke R, Batra SK. Stem cells: a revolution in therapeutics-recent advances in stem cell biology and their therapeutic applications in regenerative medicine and cancer therapies. *Clin Pharmacol Ther* 2007; **82**: 252-264 [PMID: 17671448 DOI: 10.1038/sj.clpt.6100301]

17 **Caplan AI**. Why are MSCs therapeutic? New data: new insight. *J Pathol* 2009; **217**: 318-324 [PMID: 19023885 DOI: 10.1002/path.2469]

18 **Caplan AI**. Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. *J Cell Physiol* 2007; **213**: 341-347 [PMID: 17620285 DOI: 10.1002/jcp.21200]

19 **Chai Y**, Jiang X, Ito Y, Bringas P Jr, Han J, Rowitch DH, Soriano P, McMahon AP, Sucov HM. Fate of the mammalian cranial neural crest during tooth and mandibular morphogenesis. *Development* 2000; **127**: 1671-1679 [PMID: 10725243 DOI: 10.1242/dev.127.8.1671]

20 **Matsubara T**, Suardita K, Ishii M, Sugiyama M, Igarashi A, Oda R, Nishimura M, Saito M, Nakagawa K, Yamanaka K, Miyazaki K, Shimizu M, Bhawal UK, Tsuji K, Nakamura K, Kato Y. Alveolar bone marrow as a cell source for regenerative medicine: differences between alveolar and iliac bone marrow stromal cells. *J Bone Miner Res* 2005; **20**: 399-409 [PMID: 15746984 DOI: 10.1359/JBMR.041117]

21 **Akintoye SO**, Lam T, Shi S, Brahim J, Collins MT, Robey PG. Skeletal site-specific characterization of orofacial and iliac crest human bone marrow stromal cells in same individuals. *Bone* 2006; **38**: 758-768 [PMID: 16403496 DOI: 10.1016/j.bone.2005.10.027]

22 **Aghaloo TL**, Chaichanasakul T, Bezouglaia O, Kang B, Franco R, Dry SM, Atti E, Tetradis S. Osteogenic potential of mandibular vs. long-bone marrow stromal cells. *J Dent Res* 2010; **89**: 1293-1298 [PMID: 20811069 DOI: 10.1177/0022034510378427]

23 **Qin Y**, Guan J, Zhang C. Mesenchymal stem cells: mechanisms and role in bone regeneration. *Postgrad Med J* 2014; **90**: 643-647 [PMID: 25335795 DOI: 10.1136/postgradmedj-2013-132387]

24 **Li X**, Liu X, Tan Y, Tran V, Zhang N, Wen X. Improve the viability of transplanted neural cells with appropriate sized neurospheres coated with mesenchymal stem cells. *Med Hypotheses* 2012; **79**: 274-277 [PMID: 22657917 DOI: 10.1016/j.mehy.2012.05.010]

25 **Oryan A**, Kamali A, Moshiri A, Baghaban Eslaminejad M. Role of Mesenchymal Stem Cells in Bone Regenerative Medicine: What Is the Evidence? *Cells Tissues Organs* 2017; **204**: 59-83 [PMID: 28647733 DOI: 10.1159/000469704]

26 **Betancur P**, Bronner-Fraser M, Sauka-Spengler T. Assembling neural crest regulatory circuits into a gene regulatory network. *Annu Rev Cell Dev Biol* 2010; **26**: 581-603 [PMID: 19575671 DOI: 10.1146/annurev.cellbio.042308.113245]

27 **Münst S**, Koch P, Kesavan J, Alexander-Mays M, Münst B, Blaess S, Brüstle O. In vitro segregation and isolation of human pluripotent stem cell-derived neural crest cells. *Methods* 2018; **133**: 65-80 [PMID: 29037816 DOI: 10.1016/j.ymeth.2017.09.012]

28 **Yamaza T**, Ren G, Akiyama K, Chen C, Shi Y, Shi S. Mouse mandible contains distinctive mesenchymal stem cells. *J Dent Res* 2011; **90**: 317-324 [PMID: 21076121 DOI: 10.1177/0022034510387796]

29 **Lee DJ**, Kwon J, Current L, Yoon K, Zalal R, Hu X, Xue P, Ko CC. Osteogenic potential of mesenchymal stem cells from rat mandible to regenerate critical sized calvarial defect. *J Tissue Eng* 2019; **10**: 2041731419830427 [PMID: 30886687 DOI: 10.1177/2041731419830427]

30 **Moshaverinia A**, Chen C, Xu X, Akiyama K, Ansari S, Zadeh HH, Shi S. Bone regeneration potential of stem cells derived from periodontal ligament or gingival tissue sources encapsulated in RGD-modified alginate scaffold. *Tissue Eng Part A* 2014; **20**: 611-621 [PMID: 24070211 DOI: 10.1089/ten.TEA.2013.0229]

31 **Fujii Y**, Kawase-Koga Y, Hojo H, Yano F, Sato M, Chung UI, Ohba S, Chikazu D. Bone regeneration by human dental pulp stem cells using a helioxanthin derivative and cell-sheet technology. *Stem Cell Res Ther* 2018; **9**: 24 [PMID: 29391049 DOI: 10.1186/s13287-018-0783-7]

32 **Gao RT**, Zhan LP, Meng C, Zhang N, Chang SM, Yao R, Li C. Homeobox B7 promotes the osteogenic differentiation potential of mesenchymal stem cells by activating RUNX2 and transcript of BSP. *Int J Clin Exp Med* 2015; **8**: 10459-10470 [PMID: 26379836]

33 **Deluiz D**, Delcroix GJ, D'Ippolito G, Grau-Monge C, Bonnin-Marquez A, Reiner T, Tinoco EMB, Amadeu T, Pires FR, Schiller PC. Human Bone Marrow-Derived Mesenchymal Stromal Cell-Seeded Bone Biomaterial Directs Fast and Superior Mandibular Bone Augmentation in Rats. *Sci Rep* 2019; **9**: 11806 [PMID: 31413279 DOI: 10.1038/s41598-019-48236-8]

34 **Hernigou P**, Poignard A, Manicom O, Mathieu G, Rouard H. The use of percutaneous autologous bone marrow transplantation in nonunion and avascular necrosis of bone. *J Bone Joint Surg Br* 2005; **87**: 896-902 [PMID: 15972899 DOI: 10.1302/0301-620X.87B7.16289]

35 **Lee BK**, Choi SJ, Mack D, Oh SH. Isolation of mesenchymal stem cells from the mandibular marrow aspirates. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2011; **112**: e86-e93 [PMID: 21872505 DOI: 10.1016/j.tripleo.2011.05.032]

36 **Saeed H**, Ahsan M, Saleem Z, Iqtedar M, Islam M, Danish Z, Khan AM. Mesenchymal stem cells (MSCs) as skeletal therapeutics - an update. *J Biomed Sci* 2016; **23**: 41 [PMID: 27084089 DOI: 10.1186/s12929-016-0254-3]

37 **Li C**, Wang F, Zhang R, Qiao P, Liu H. Comparison of Proliferation and Osteogenic Differentiation Potential of Rat Mandibular and Femoral Bone Marrow Mesenchymal Stem Cells In Vitro. *Stem Cells Dev* 2020; **29**: 728-736 [PMID: 32122257 DOI: 10.1089/scd.2019.0256]

38 **Lu W**, Gao B, Fan J, Cheng P, Hu Y, Jie Q, Luo Z, Yang L. Mesenchymal Progenitors Derived from Different Locations in Long Bones Display Diverse Characteristics. *Stem Cells Int* 2019; **2019**: 5037578 [PMID: 31089329 DOI: 10.1155/2019/5037578]

39 **Vasiliadis AV**, Galanis N. Human bone marrow-derived mesenchymal stem cells from different bone sources: a panorama. *Stem Cell Investig* 2020; **7**: 15 [PMID: 32964008 DOI: 10.21037/sci-2020-013]

40 **Muthu S**, Jeyaraman M, Jain R, Gulati A, Jeyaraman N, Prajwal GS, Mishra PC. Accentuating the sources of mesenchymal stem cells as cellular therapy for osteoarthritis knees-a panoramic review. *Stem Cell Investig* 2021; **8**: 13 [PMID: 34386542 DOI: 10.21037/sci-2020-055]

41 **McDaniel JS**, Antebi B, Pilia M, Hurtgen BJ, Belenkiy S, Necsoiu C, Cancio LC, Rathbone CR, Batchinsky AI. Quantitative Assessment of Optimal Bone Marrow Site for the Isolation of Porcine Mesenchymal Stem Cells. *Stem Cells Int* 2017; **2017**: 1836960 [PMID: 28539939 DOI: 10.1155/2017/1836960]

**Footnotes**

**Conflict-of-interest statement:** All authors declare no conflict of interests.

**Open-Access:** This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

**Provenance and peer review:** Invited article; Externally peer reviewed.

**Peer-review model:** Single blind

**Peer-review started:** January 14, 2023

**First decision:** January 31, 2023

**Article in press:**

**Specialty type:** Orthopedics

**Country/Territory of origin:** India

**Peer-review report’s scientific quality classification**

Grade A (Excellent): 0

Grade B (Very good): B

Grade C (Good): C

Grade D (Fair): 0

Grade E (Poor): 0

**P-Reviewer:** Chen G, China; Saei M, Iran **S-Editor:** Liu JH **L-Editor:** A **P-Editor:** Liu JH

**Table 1 Comparison of mandible *vs* long bone-derived MSCs**

|  |  |  |  |
| --- | --- | --- | --- |
| **Features**  | **Ref.** | **Mandible derived MSCs** | **Long bone-derived MSCs** |
| Aspiration time | Lee *et al*[35], 2011 | 10 min | 2 min |
| No. of colonies | Yamaza *et al*[28], 2011 | 55.3 ± 9.07/ 1.5 × 106 cells/plate (Higher) | 5.33 ± 0.58/ 1.5 × 106 cells/plate |
| Li *et al*[37], 2019) | The appearance of colonies was early within 2-3 d of inoculation into the culture | The appearance of colonies of Femur- MSCs was scantly on the 2nd or 3rd day as compared to M-MSCs |
| Osteogenic potential | Yamaza *et al*[28], 2011 | High | Low |
| Matsubara *et al*[20], 2005) | High | Low |
| Aghaloo *et al*[22], 2010) | higher activity of ALP and OCN expression suggesting higher osteogenic potential  | Comparatively lower osteogenic potential |
| Li *et al*[37], 2019 | After 21 d, M-MSCs showed loss of morphology, and dry staining was observed; *Runx2* gene expression was higher  | After 21 d, F-MSCs showed obvious cell morphology  |
| Doubling rate and cell proliferation | Yamaza *et al*[28], 2011 | High | Low |
| Lee *et al*[29], 2019 | Proliferation time (OD-0.82  ±  0.26) was also documented to be much earlier as compared to F-MSCs but doubling time was lower (22.6  ±  2.22  h)  | Proliferation time was much delayed (OD-1.13  ±  0.41) as compared to M-MSCs but doubling time was earlier (35  ±  3.19  h) |
| Li *et al*[37], 2019 | Proliferation time was also documented to be much earlier as compared to F-MSCs | Proliferation time was much delayed as compared to M-MSCs |
| Arrangement of cells | Li *et al*[37], 2019 | On day 2, triangular, while after cell (tightly) fusion- these cells are arranged as paving stones | On day 2, elongated fibroblast-like morphology, while after cell (tightly) fusion- F-MSCs show vortex-like cloning center |
| Cell expression | Yamaza *et al*[28], 2011 | Positive for MSC-associated markers such as CD-73, -105, and -106, SSEA-4, and Oct-4; Negative for hematopoietic markers such as CD-14, -34, and -45; Expresses SSEA-4 (6.4%) and Oct-4 (6%) in much higher proportion as compared to long bones | Positive for MSC-associated markers such as CD-73, -105, and -106, SSEA-4, and Oct-4; Negative for hematopoietic markers such as CD-14, -34, and -45. Expresses SSEA-4 (4.2%) and Oct-4 (2.6%) in lower proportion |
| Lee *et al*[35] , 2011 | Negative for hematopoietic stem cells such as for CD-14, -34, -45, and HLA-DR whereas positive for MSC markers such as CD-29, -44, -73, -90, -105, -166, and HLA-ABC | Negative for hematopoietic stem cells such as for CD-14, -34, -45, and HLA-DR whereas positive for MSC markers such as CD-29, -44, -73, -90, -105, -166, and HLA-ABC |
| Li *et al*[37], 2019 |  Strongly expressed CD-29, -73, -90, and -105 but negative for CD-31 and -34 | Strongly expressed CD-29, -73, -90, and -105 but negative for CD-31 and -34 |
| Mineralization | Aghaloo *et al*[22], 2010 | Mandible BMSC were significantly larger and calcification was also more as compared to long bones; Tissue volume and bone volume was also larger | Less calcified as compared to M-MSCs |
| Lee *et al*[29], 2019 | Mineralization appears within 14 d of osteogenic differentiation (mean-1.57 ± 0.05) | The mineral formation is higher (1.98  ±  0.05) as compared to M-MSCs at 14 d |
| Histology | Aghaloo *et al*[22], 2010 | Characterized by increased and mature lamellar bone with marked osteoblastic rimming of bony trabeculae  | The bone formed was primarily of the cartilaginous matrix with only peripheral bone formation |

ALP: Alkaline phosphatase; BMSC: Bone mesenchymal stem cell; HLA: Human leukocyte antigen; MSCs: Mesenchymal stromal cells; M-MSCs: Mandibular-derived MSCs; F-MSCs: Femur-derived MSCs; OCN: Osteocalcin; SSEA-4: Stage-specific embryonic antigen 4; Oct-4: Octamer-4.