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Is Mandible derived MSCs superior in proliferation and regeneration to long bone-derived MSCs?

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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 (SSEA-4) and Octamer-4 (Oct-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.

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* first identified CFU-F by isolating adherent cells from the BM stroma of newborn rodents which can form discrete colonies [3]. However, these cells are regulated by various mitogenic factors such as epidermal growth factor (EGF), platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), basic fibroblast growth factor (bFGF), and insulin growth factor-1 (IGF-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 (ISCT) 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 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* 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 $55.3 \pm 9.07/1.5 \times 10^6$ 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) [28].

In vitro evidence of superiority in lineages of M-MSCs

Lee *et al* investigated the role of M-MSCs *in vitro* studies and observed the formation of mineral nodules as early as 14 days of the osteogenic differentiation, which tends to increase over time till 21 days. These cells can suppress T lymphocytes and thus have been recommended in acute graft *vs* host disease [29]. Li *et al* observed the growth of M-MSCs within 2 to 3 days 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 T *et al* 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 [28].

In vivo evidence of superiority in lineages of M-MSCs

Lee *et al* 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 [29]. 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* in their rat model study demonstrated that M-MSCs inoculation significantly promoted bone formation at 4 wk (22.75 ± 2.25 mm^3) as well as at 8 wk (64.95 ± 5.41 mm^3) as compared to acellular bone microparticles (2.34 ± 2.91 mm^3 and 42.73 ± 10.58 mm^3 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 [33]. Yamaza *et al* 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 [28].

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* 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 days [37]. Lee *et al* 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 [29].

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* observed a primarily cartilaginous matrix following long bone-derived MSC implantation with good osteoblastic differentiation [22]. 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) $\times 10^6$ cells/mL, whereas the concentration of nucleated cells in the anterior iliac crest is (24.4 – 49) $\times 10^6$ cells/mL. However, the mean number of nucleated cells in decreasing concentration has been reported from the proximal humerus (38.7×10^6 cells/mL), followed by the distal femur (25.9×10^6 cells/mL), humeral head, and proximal tibia (12.1×10^6 cells/mL) [39]. Mc Daniel *et al* 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 [41].

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 minutes. 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 days [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.

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1	T. Yamaza, G. Ren, K. Akiyama, C. Chen, Y. Shi, S. Shi. "Mouse Mandible Contains Distinctive Mesenchymal Stem Cells", Journal of Dental Research, 2010 Crossref	24 words — 1%
2	Wei Yu, John O. Nairn, Raymond J. Lanzafame. "Expression of growth factors in early wound healing in rat skin", Lasers in Surgery and Medicine, 1994 Crossref	19 words — 1%
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