Comparative characteristic study from bone marrow-derived mesenchymal stem cells

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ABSTRACT

Tissue engineering has been extensively investigated and proffered to be a potential platform for novel tissue regeneration. The utilization of mesenchymal stem cells (MSCs) from various sources has been widely explored and compared. In this regard, MSCs derived from bone marrow have been proposed and described as a promising cell resource due to their high yield of isolated cells with colony-forming potential, self-renewal capacity, MSC surface marker expression, and multi-lineage differentiation capacities in vitro. However, there is evidence for bone marrow MSCs (BM-MSCs) both in vitro and in vivo from different species presenting identical and distinct potential stemness characteristics. In this review, the fundamental knowledge of the growth kinetics and stemness properties of BM-MSCs in different animal species and humans are compared and summarized. Finally, to provide a full perspective, this review will procure results of current information studies focusing on the use of BM-MSCs in clinical practice.

Keywords: Tissue engineering; mesenchymal stem cells; bone marrow mesenchymal stem cells; stemness characteristics

INTRODUCTION

Tissue engineering (TE) has been proposed as an advance multidisciplinary approach that incorporates principles from biological, biochemical, medical science [1], engineering, and pharmaceutical [2,3] fields. In the TE paradigm, this combination can develop bioartificial substitutes for tissues or organs, which can be used in regenerative medicine, in pharmaceutical, diagnostic, and basic research into cell functions in vivo, and to identify mechanisms involved in the aging process and disease progression [4].

The three main components used in TE are cells, scaffolds, and signaling molecules [5,6]. Among these components, promising cell resources have been investigated to assess their...
characteristics, especially stemness capability, to provide fundamental knowledge for developing a mimic tissue or organ. Due to their affordable sample collection feature and non-tumorigenicity, multipotent stem cells from various tissues derived from bone marrow, umbilical cord, amniotic fluid, adipose tissue, muscle, dental tissue [7,8], skin [9], kidney [10], liver [11], heart [12], and brain [13] have been investigated. In this regard, a common source of multipotent stem cells is bone marrow mesenchymal stem cells (BM-MSCs), which have been proposed and described as a promising cell resource from various animal sources, including avian [14], murine [15,16], rat [17], rabbit [18], feline [19], canine [20], ovine [21], bovine [22], porcine [23], equine [24], non-human primate [24], and human [25]. BM-MSCs have crucial stemness abilities, including self-renewal ability, MSCs surface marker expression, and multipotency [26,27]. However, BM-MSCs isolated from different species have been reported to exhibit both similarities and differences in the potency of their stemness characteristics, both in vitro and in vivo [28].

HISTORY OF BM-MSCs

Bone marrow-derived MSCs were the first MSCs to be described. In 1996, BM-MSCs were described as fibroblast-like cells with colony-forming ability and differentiation potential [32]. In the 1980s, researchers started to refer to these cells as osteogenic stem cells and bone marrow stromal cells, names that refer back to the source of the cell from bone marrow stroma and its osteogenic differentiation potential [33]. Later, in 1991, Caplan [34] proposed changing the terminology of osteogenic and stromal into mesenchymal. Further, in 2006, due to confusion about the terms mesenchymal stem/stromal cell, the International Society for Cell Therapy (ISCT) recommended adding the tissue origin of the cells to the name. Therefore, currently, bone marrow-derived MSCs are known as BM-MSCs.

COLLECTION SITE OF BM-MSCs

Bone marrow used for MSCs isolation can be obtained from different types of bones in different species. In humans, BM-MSCs may be isolated from sternum [35], vertebral body [36], iliac crest [36,37] and femoral shaft [36]. In animals, BM-MSCs may be sourced from different places according to the species; for example, proximal humerus, femur, and iliac crest are common areas for bone marrow aspiration in canines and felines [38,39]. Whereas, in laboratory animals such as rats [40] and mice [41], bone marrow for BM-MSCs isolation is obtained upon euthanasia from the whole femur or tibia.
STEMNESS CHARACTERISTICS OF BM-MSCs

Stemness, a natural property of MSCs, refers to the capacity of MSCs to maintain self-renewal and an undifferentiated state [42]. Stemness is important for quiescence, proliferation, and regeneration through the interaction between MSCs and their microenvironment [42,43]. In 2006, ISCT proposed several criteria to define human MSCs, including the ability to adhere to a plastic culture surface, expression of MSC surface marker antigens, and potency in multilineage differentiation [44]. However, different species may possess unique stemness characteristics, especially those associated with utilizing MSCs from specific species. Thus, in this review, we have described the MSC morphology, stemness characteristics, including their pluripotency genes, clonogenicity, cell growth kinetic, senescence, surface antigen markers, and multipotency.

Morphology of BM-MSCs

Morphological features are important in defining MSCs as they present as fibroblast-like cells. It has been reported that this feature is possessed by BM-MSCs derived from human [45], canine [46,47], feline [19], rat [48], and murine [49] sources. To the best of our knowledge, murine MSCs are smaller than human, canine, and feline cells. The morphological features of BM-MSCs from those groups are illustrated in Fig. 1.

Self-renewal and proliferation potential of BM-MSCs

To maintain pluripotency, transcriptional factors (TFs), including Oct4, Sox2, and Nanog have important roles [50]. In addition, Rex1 has been considered a pluripotency marker [51]. It has been reported that pluripotency TFs are expressed in all BM-MSCs derived from human [51-53], canine [46,54], rat [55], and murine [56] sources. Moreover, Ki67 is a proliferation marker, based on its reported expression in BM-MSCs derived from rat [55] and canine [46] but not human [57] sources. However, to date, there are no records of Rex1 and Ki67 expressions in feline and murine BM-MSCs, respectively.

The self-renewal capability of MSCs can be assessed by clonogenicity or colony-forming unit assays [58,59]. Our review noted that colonies were formed in human [59], canine [46,60,61], feline [62], rat [63,64], and murine [65-67] BM-MSCs.

The kinetics of cell growth show that proliferation is an MSC stemness characteristic. Kinetic studies allow population doubling time (PDT) to be measured and evaluated. Murine BM-MSCs from passage 2 have been reported to have a PDT of more than 80 h at week 4 and 8 [68]. On the other hand, rat BM-MSCs have a PDT of 20–30 h in passages 1 to 3, which rises markedly to 50 h and 130 h in passages 4 and 5, respectively [69]. Contrastingly, another study noted that the PDT of rat BM-MSCs decreased by up to 20 h as the passage number

![Fig. 1. Comparative morphology of BM-MSCs. Morphology (representative figures) of murine (A), canine (B), feline (C), and human (D) BM-MSCs are illustrated. BM-MSCs, bone marrow mesenchymal stem cells.](https://vetsci.org)
increased [70]. In canines, BM-MSCs derived from several large-sized dog breeds show a PDT increase of up to 100 h after 25 days of culture [60]. Meanwhile, in humans, the PDT was shorter in early-stage cultures (before passage 6) than in late-stage cultures (after passage 6), occurring at less than 48 and 96 h, respectively [57]. Unfortunately, there is no evidence of PDT in cultured feline BM-MSCs, but a different method, MTT assay, showed that feline cells were proliferative up to 120 h after low-density seeding [71]. Another study revealed that feline BM-MSCs showed exponential growth at passage 1 and followed stationary or decreasing growth patterns up to passage 3 [62].

Senescence of cells has been associated with shortened telomeres, causing irreversible cell cycle arrest [72] and leading to proliferation dysfunction in MSCs [73]. Hence, it is important to evaluate senescence by assessing senescence-associated β-galactosidase (SA-β-Gal) expression. One study observed that senescence occurred in late-stage culture (passage 6 and above) of human BM-MSCs [57]. Similarly, SA-β-Gal increased linearly as canine BM-MSCs passage numbers increased [60]. In contrast, there was no indication of SA-β-Gal presence in rat BM-MSCs at PD100 [64]. That result is supported by the previous study showing SA-β-Gal absence in passage 2 of rat BM-MSCs culture but presence in passage 6 [69]. On the other hand, the presence of senescence was relatively low in passages 3 and 4 of murine BM-MSCs [74,75]. However, no feline BM-MSCs senescence study has been reported.

**MSCs surface marker expression**

According to ISCT, cells must express CD73, CD90, and CD105 as well as negative CD11b, CD14, CD19, CD34, CD45, CD79a, and human leukocyte antigen (HLA)-DR surface markers in order to be considered MSCs. Research reported by Petrenko et al. [76] showed that human BM-MSCs, in addition to meeting the ISCT standard, expressed other MSCs surface markers such as CD10, CD29, CD44, CD133, HLA-ABC, MSCA-1, and SSEA-4. Surface markers in human MSCs differ from those of animal MSCs; for example, in canine and feline MSCs, MHC-1, CD29, and CD105 were expressed [19]. However, canine MSCs also moderately express other markers such as CD90, CD166, and CD73 [20,77,78]. Rat BM-MSCs express CD29, CD44, CD54, CD 90, and CD166 [70]. In contrast, the expressions of MSCs surface markers in mice slightly differ across strains; C57BL, DBA1, and FVBn mice express the SCA-1 surface marker, which is not expressed in BALB/C mice [79]. However, another study reported that BALB/C expressed SCA-1 [80]. Moreover, that study showed that CBA/Ca, ICR, and BALB/C mice were positive for CD106 with increasing passage. The absence of endothelial and hematopoietic cell markers CD45, CD11b, and CD34 expressions, per the ISCT standard, were reported in canine, rat, and mouse BM-MSCs [81,82].

**Multipotency of BM-MSCs**

The multipotency capacity of BM-MSCs derived from human, canine, feline, rat, and murine sources to differentiate into osteogenic, adipogenic, chondrogenic, and neurogenic lineages are summarized in Table 1.
Bone marrow-derived MSCs have been suggested as potential therapeutic agents in improving transplantation-related functional and pathological recovery from several diseases due to their main characteristics: self-renewal, rapid proliferation in vitro, abundant sources for isolation, and high differentiation capability. Indeed, several preclinical studies and clinical trials have been conducted in animal test models, including murine, rat, rabbit, feline, canine, ovine, bovine, and porcine models, to further translational medicine research in humans and determine the effectiveness of BM-MSCs for treatment. A scheme for moving translational research into practice is depicted in Fig. 2.

Truong et al. [83] evaluated the treatment effect of murine BM-MSCs on mouse liver cirrhosis induced by carbon tetrachloride. BM-MSCs transplantation reduced inflammation scores and an absence of cirrhosis at 21 days after BM-MSCs injection via a peripheral vein [83].

Other studies demonstrated that BM-MSCs transplantation is a promising treatment strategy for bleomycin-induced pulmonary fibrosis in rats. During the early and late BM-MSCs transplantation period, there was a reduction in pulmonary fibrosis and alveolitis [84]. In addition, BM-MSCs transplantation has been performed in a cerebral artery occlusion rat model [85]. That study noted that BM-MSCs transplantation enhanced endogenous neural progenitor cell migration in rats in a cerebral ischemic condition, which was helpful for restorative cerebral ischemic management.

Autologous and allogeneic feline BM-MSCs intrarenal administration to three cats with chronic kidney disease (CKD) has been investigated. A mild decrease in serum creatinine, a stable body weight, and mild improvement in proteinuria and urine specific gravity were statistically significant after 21 days. In the same study, a transplantation application to
treat kidney injury (AKI) in an ischemic kidney model was also investigated. Five cats were administrated for feline BM-MSCs via jugularis catheters; however, the treatment had no effect in cats with AKI, although it was effective in CKD- and AKI-induced rodent models [30].

Another study carried out by Gomes et al. [31] reported on subconjunctival transplantation of BM-MSCs in dogs with experimental corneal ulcer, and BM-MSCs were observed in the injured region. In canine dermatology, wound healing of cutaneous inflammation was accelerated by allogeneic transplantation of BM-MSCs in a Beagle dog [86].

In orthopedics, it has been possible to observe the treatment of osteosarcoma by using canine BM-MSCs and rhBMP-2 [87,88]. Another BM-MSCs application has been shown in a canine orbital wall defect model. After 24 wk, successful bone repair of an orbital wall bone defect was achieved by seeding an autologous canine BM-MSCs onto β-tricalcium phosphate scaffold for in vivo implantation [89,90]. Success was also achieved in a canine segmental bone defect model [91-93]. One of the studies revealed that new bone formation was present at 12 wk post-implant in a Beagle dog [93]. Furthermore, the use of BM-MSCs implantation in fracture repair has been successful in animal models. Autologous and/or exogenous BM-MSCs [94,95] were cultured, loaded onto ceramic cylinders, and implanted into a critically sized segmental bone defect (rat femur); after 8 wk, the implants promoted bone formation for fracture healing.

Numerous reports have shown that BM-MSCs have potency as treatments for congenital, degenerative, vascular, traumatic, and iatrogenic conditions. In veterinary medicine, BM-MSCs have assisted in the reproduction of endangered species and have been used for generating transgenic animals, producing biomedical models, and for pathological condition treatment through transplantation [31]. Some clinical trials have been carried out with BM-MSCs in companion animals, such as osteoarthritis, tendon ligament injury, and intervertebral disk degeneration trials in dogs and horses [96,97] and CKD in cats [98].

In humans, potential applications of BM-MSCs in bone TE have been studied in various bone defects. In a segmental long bone defect of the distal tibia fracture of a 58-year-old woman, bone was actively formed 6 wk after a xenogeneic transplant [99]. Another successful application was in a long-bone defect hip trauma, with bone formation present at 3–6 wk after implantation of microspheres co-immobilized with alginate [100]. Moreover, systemic infusion of allogeneic BM-MSCs produced new bone formation in children with severe osteogenesis imperfecta three months after osteoblast engraftment [101]. Overall, the efficacy of transplantation of BM-MSCs appears to be more effective in acute rather than chronic stage diseases. The advantages and disadvantages of BM-MSCs are summarized in Table 2.

**CONCLUSION**

To be referred to as stem cells, the cells must show stemness characteristics: pluripotency, gene expression, proliferation, kinetic cell growth, and senescence. The expression of pluripotency genes, such as *Oct4*, *Sox2*, and *Nanog* discriminates stem cells from other cells through the capability to differentiate into other types of cells. Moreover, the growth kinetic, proliferation, and senescence features of stem cells are important in determining their self-renewal property. Understanding the differences and uniqueness of BM-MSCs stemness characteristics across species is critical when maximizing their potential in clinical practice.
and translational research. Therefore, elucidation of stemness characteristics is crucial in the utilization of BM-MSCs in TE and clinical applications in both human and animal practice.

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