**Review**

**Age effect on mesenchymal stem cell properties: A concise review**

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**Running title:** Mesenchymal stem cell aging

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**Abstract**

Mesenchymal stem cells belong to one of the multipotent stem cell types isolated from almost all tissues in human body. They function to maintain tissue homeostasis with their highly proliferative property, and they also possess the immunomodulatory property. The properties of mesenchymal stem cells can be influenced by multiple factors, among which donor ages have been indicated negatively correlated with the proliferation, migration, and differentiation of mesenchymal stem cells. Telomerase activity, telomere length and cell senescence have been studied to understand the mechanisms of the age effect on mesenchymal stem cell properties. Rejuvenation treatments are the critical research direction to attenuate the deterioration of mesenchymal stem cell properties by the age effect. This review article summarized the updated research on the impact and mechanisms of aging and age-related factors on different mesenchymal stem cell properties. In addition, the treatments to rejuvenate the aged mesenchymal stem cells will also be discussed. This review article aims to enlighten scientific researchers better preparing and nursing the autologous mesenchymal stem cells from the elderlies for future applications in tissue engineering and regenerative medicine.

**Keywords:** Mesenchymal stem cells; aging; proliferation; migration; differentiation

**Introduction**

Stem cells belong to the undifferentiated cells with the ability of self-renewal and differentiation into mature cells. They are essential for tissue growth, development, and homeostasis. Adult stem cells refer to the stem cells found in the developed tissues, and they function to maintain adult tissue specificity by homeostatic cell replacement and tissue regeneration [1]. They are presumed to be inactive within the adult tissues, but they can be stimulated to divide into a stem cell clone and a transiently amplifying cell. The latter will undergo limited divisions before terminally differentiating into mature functional tissue cells. Because of lineage restricted differentiation for adult stem cells, different types of adult stem cells are equipped with specific functions in different tissues and organs. Apart from the most-studied blood-forming hematopoietic stem cells (HSCs) [2], mesenchymal stem cells (MSCs), also known as marrow stromal cells, belong to another multipotent adult stem cell population with potentials differentiating into the mesodermal lineages, including adipocytes, chondrocytes, and osteocytes [3]. Although MSCs were first identified in bone marrow, they reside within the connective tissues of many organs, including adipose tissue, umbilical cord, and teeth [4]. MSCs isolated from the fetal tissues, including umbilical cord, umbilical cord blood, Wharton’s jelly, placenta, and amniotic membrane, are considered as the fetal MSCs, and the alterative are considered as adult MSCs. Human MSCs can be sorted with the positive selection by CD29, CD44, CD73, CD90, CD105, CD146, and STRO-1 as well as the negative selection by CD31, CD34, CD45, CD49f, and CD133 [5]. Apart from the expression of specific cell surface markers, MSCs are also defined to be growing in adherence to the plastic surface while maintained in standard culture conditions and are capable to be in vitro induced into mesenchymal lineages with appropriate medium as recommended by the International Society of Cellular Therapy [6].

MSCs have been applied as a therapeutic agent for treatment against various diseases, such as cardiovascular [7] and neurodegenerative diseases [8, 9]. Autologous MSCs are developed from the patients themselves without immune rejection, whereas allogenic MSCs are established from the selected donors allowing expansion in large scale and cryopreservation to provide a readily available source of stem cells. Current allogeneic volunteer MSC donors mostly are of young age [10]. Yet, there are substantial evidences demonstrating the aging process adversely affecting the properties of MSCs [11]. The impacts of age and age-related factors to the MSC properties are of great importance for autologous or allogeneic MSC transplantation, especially among the elderlies. In this review article, we will summarize the donor age effect on MSC properties, together with the underlying molecular mechanisms. In addition, the treatments attenuating or delaying the age effect on MSCs will also be discussed.

**Age effect on mesenchymal stem cell yield from tissues**

A study on MSCs derived from anterior cruciate ligaments demonstrated that the mean proportion of isolated MSCs was slightly but significantly higher in older donors (67.96 ± 5.22 years) than the younger donors (29.67 ± 10.92 years) [12]. In contrast, a study on MSCs in adipose-derived stromal vascular fraction reported a negative correlation of MSC count with donor age [13]. Yet, human adipose-derived MSCs harvested from same subjects with a time window of 7 to 12 years apart (initial age of the 3 donors: 17, 21, and 72 years old) show no significant difference was found in cell yield, stromal-vascular fraction subpopulation, proliferation, and tri-lineage differentiation [14]. These studies indicated that the effect of donor ages on the cell yield of MSCs harvested from the donor tissues is still controversial.

**Age effect on mesenchymal stem cell surface marker expression**

A study reported that the subpopulations of bone marrow-derived MSCs harvested from younger donors are composed of more CD71+, CD146+, and CD274+ MSCs than that from older donors, and the fluorescence per cell of CD71, CD90, CD106, CD140b, CD146, CD166, and CD274 is negatively correlated with the donor age [15]. Similarly, human bone marrow-derived MSCs express CD13, CD44, CD90, CD105, and Stro-1 regardless of age, but those from the donors over 40 years old showed significantly lower expression of CD90, CD105, and Stro-1 [16]. Moreover, lower SSEA-4 expression was found in the elderly bone marrow-derived MSCs as compared to the young MSCs [17]. Consistently, our previous study also reported that lower expression of SSEA4 was found in human MSCs derived from periodontal ligament with donor age of > 40 years old as compared to those with donor age of ≤ 20 years old [18]. In contrast, no significant difference in cell surface marker expression was reported in bone marrow-derived MSCs from the pediatric and adult donors [19]. Human adipose-derived MSCs from all age groups also show comparable expression of CD3, CD14, CD19, CD34, CD44, CD45, CD73, CD90, and CD105 [20]. The effect of donor ages on MSC marker expression could be exhibited in the donors with older ages.

**Age effect on mesenchymal stem cell proliferation**

MSC proliferation is related to the availability and abundance of stem cells present to exert a regenerative effect. The number of colony forming unit-fibroblasts (CFU-F) colonies with alkaline phosphatase (ALP) activity in bone marrow-derived MSCs of younger donors (3 – 36 years old) is significantly higher than that of the older donors (41 – 70 years old) [21]. Consistently, there is a significant decline in the CFU-F number in bone marrow-derived MSCs from older donors (21 – 40 years old) as compared to the younger donors (0 – 20 years old) [16]. Similar results were also observed in adipose-derived stem cells that 30% decline in CFU numbers with 38% increase in population doubling time from the donors with age > 50 years old as compared to those with age < 20 years old [22]. Moreover, the cumulative population doubling of bone marrow-derived MSCs from pediatric donors is twice of that from young adult donors [19], and the doubling time is 1.7-fold longer in bone marrow-derived MSCs from the older subjects as compared to the younger subjects [23]. In addition, our previous study also found that human MSCs derived from periodontal ligament with donor age ≤ 20 years old show significantly higher proliferation than that of donor age 21 – 40 years old and > 40 years old [18]. On the contrary, no significant differences in CFU numbers of bone marrow-derived MSCs among different donor ages were also reported [24-26]. Interestingly, umbilical cord-derived MSCs from older mothers also show lower proliferative and colony forming capacity as compared to those from younger mothers [27]. Yet, other study demonstrated that human fetal membrane-derived MSCs from older mothers show higher proliferation rate than those from younger mothers [28]. Collectively, there are prominent evidences that the proliferation of MSCs would be reduced in the donors with older ages.

**Age effect on mesenchymal stem cell migration**

The movement of stem cells and their capacity to migrate to injury sites are the determining factors of stem cell regenerative potentials. The migration ability of human adipose-derived MSCs is significantly decreased in the elderly donors as compared to the child donors with significant reduction in *CXCR4* and *CXCR7* expression in the elderly group [29]. Moreover, the migratory activity of human periodontal ligament-derived MSCs with donor age of 56 – 75 years old is significantly decreased as compared to those with donor age of 16 – 30 years old [30]. Consistently, our previous study also demonstrated that human periodontal ligament-derived MSCs with donor age > 40 years old show significantly lower migration as compared to that with donor age ≤ 20 years old and 20 – 40 years old, accompanying with lower expression of *PTK2* in the periodontal ligament-derived MSCs with donor age > 40 years old [18]. Notably, bone marrow from the aged mice can induce slower migration ability of murine MSC cell line C3H10T1/2 as compared to that from the young mice [31]. Collectively, aging, together with the aged tissue microenvironment, could reduce the migration ability of MSCs.

**Age effect on mesenchymal stem cell differentiation**

MSCs, equipped with multipotent differentiation potential, can give rise to mesenchyme tissue cells, including adipocytes, osteoblasts, chondrocytes, myocytes, and cardiomyocytes. As compared to the younger adipose-derived MSCs, the aged MSCs shows decreased chondrogenic and osteogenic potential, but in favor of shifting towards adipogenic differentiation with increasing age [32]. Yet, another study reported that the osteogenic and chondrogenic potentials of adipose-derived MSCs decline with the donor age, but the adipogenic potential of adipose-derived MSCs is independent of the donor age [20]. Advancing age has demonstrated to have a significant negative effect on the adipogenic and osteogenic differentiation potentials of human adipose-derived MSCs [29], while no differences in the differentiation efficiency in adipogenesis and osteogenesis between young (≤ 35 years old) and old (≥ 55 years old) adipose-derived MSCs have also been reported [33]. In contrast, the adipogenic and osteogenic potentials of bone marrow-derived MSCs decrease with increasing ages while the chondrogenic potential did not change [34]. Besides, the osteogenic differentiation of bone marrow-derived MSCs is more affected by age than the adipose-derived MSCs [35]. No significant differences in osteogenic differentiation capacity of bone marrow-derived MSCs between young and aged donors have also been reported [15, 26]. Under a moderate level of inflammatory stimuli, osteogenic differentiation of bone marrow-derived MSCs from elderly donors could be greatly diminished, and adipogenic differentiation remains unchanged, while the bone marrow-derived MSCs from young and intermediately aged donors show better osteogenic differentiation but reduced adipogenic differentiation [36]. For human periodontal ligament-derived MSCs, the osteogenic and adipogenic differentiation capacities of human periodontal ligament-derived MSCs have been shown to be reduced when age increases [30]. Consistently, our previous study demonstrated that the osteogenic, chondrogenic and adipogenic differentiation abilities of human periodontal ligament-derived MSCs with donor age > 40 years old are all reduced as compared to those with donor age ≤ 20 years old [18]. Collectively, the age effect on the differentiation of different mesodermal lineages of MSCs could be dependent on the originated cell sources and the microenvironments.

Apart from mesodermal lineage differentiation, we have previously demonstrated that human periodontal ligament-derived MSCs and adipose-derived MSCs can be induced to neural and retinal lineages [37-39]. It has been reported that the neuroectodermal differentiation potential of human bone marrow-derived MSCs from old donors (> 45 years old) is completely lost, with no cells showing mature neuroectodermal phenotypes and fewer cells expressing early neuroectodermal marker proteins as compared to that of the young donors (18 – 35 years old) [40]. Yet, additional studies are needed to validate the age effect on the neural differentiation of MSCs.

**Age effect on immunomodulation of mesenchymal stem cells**

The allogenic transplantation of MSCs can be achieved because of the immunomodulatory properties of MSCs. It has been reported that adult adipose-derived MSCs (< 65 years old) inhibit the activated CD4+ T-lymphocytes more effectively than elderly adipose-derived MSCs (≥ 65 years old) with increasing mean CD4+ T-lymphocyte proliferation by 0.5 % for any 1-year increase in age [41]. However, it was also shown that gingival tissue-derived MSCs display effective immunoregulation in a mouse model of lipopolysaccharide-induced acute lung injury irrespective of donor age [42]. Similarly, human dental pulp-derived MSCs have been shown effectively regulating the CD4+ T cells; yet, their effects on Th1 and Th2 cells are not affected by the donor ages [43]. In mouse, the aged MSCs presented with a lower immunomodulatory property to induce T cell apoptosis in the co-culture system as compared to the young MSCs [44]. For our previous study, we demonstrated that human periodontal ligament-derived MSCs with donor age of 20 – 40 and > 40 years old show higher *IL6* and *CXCL8* expression [18]. Elevated expressions of IL6 and CXCL8 are also reported in adult MSCs as compared with the pediatric MSCs [45]. These could indicate that the microenvironment around the aged MSCs could be inflammatory, reflected by the accumulation of inflammatory T and B lymphocytes [44].

**Age effect on neuroprotective effect of mesenchymal stem cells**

We have previously demonstrated that human periodontal ligament-derived MSCs can protect retinal ganglion cells from optic nerve injury by secreting the brain-derived neurotrophic factor and interacting with the host cells in the retina [46]. It has been reported that bone marrow-derived MSCs from both young (16 – 18 years) and old (67 – 75 years) donors in co-culture system significantly enhance total neurite length of dorsal root ganglia neurons, and only the MSCs from young donors, but not the old donors, can further be potentiated by the treatment of growth factors [47]. Moreover, under the culture with conditioned medium of bone marrow-derived MSCs, the rescue ability of MSCs on the reduced survival of rat cortical neurons by trophic factor withdrawal decrease with increasing MSC donor age [48]. In addition, it has been suggested that the composition of the secreted bio-active materials of MSCs derived from human tooth germ is influenced by the passage number of the cells [49]. These indicate that increasing MSC age could weaken its ability of neurotrophic factor secretion and the compositions, which leads to the reduced neuroprotective effect of the aged MSCs.

**Molecular mechanisms of age effect on mesenchymal stem cells**

*Telomere length*

The length of telomere is an indicator of the mitotic capacity of a cell. Telomere shortening is considered as a hallmark of stem cell aging [50]. It has been reported that the infant adipose-derived MSCs exhibited longer telomere length than the elderly MSCs [51]. Consistently, our previous study demonstrated that human periodontal ligament-derived MSCs with donor age > 40 years old have shorter telomere length than those with donor age ≤ 20 years old [18]. However, same telomere length, regardless of the donor's age, has also been demonstrated in human adipose-derived MSCs [52]. Similarly, no difference in telomere length was found in bone marrow-derived MSCs from younger (8 months – 6 years old) and older (38 – 58 years old) donors [53]. The telomere lengths in native bone marrow-derived MSC are also not related to the ages of the donors [54]. In placenta-derived MSCs, the telomere lengths could be related to cellular division rather than aging of the mothers [55]. Collectively, the role of telomere length in the age effect on MSC properties is still controversial.

*Telomerase activity*

Telomerase (telomere terminal transferase) is a reverse transcriptase responsible for maintaining the telomere length via *de novo* telomere synthesis [56]. Telomerase activity is related to the proliferation capability of MSCs [57]. Low levels of telomerase activity were reported in bone marrow-derived MSCs in a study [54], and another study reported no telomerase activity being detected in bone marrow-derived MSCs from different ages of human donors [15]. Yet, the analysis on the microarray datasets GSE97311 and GSE68374 revealed that some of the down-regulated genes in the aged adult bone marrow-derived MSCs are involved in the telomerase activity as compared to the fetal MSCs [58]. In addition, telomerase expression was reported to be lower in bone marrow-derived MSCs from the adult rats as compared to that from the young rats [59]. The role of telomerase activity in the age effect on MSC properties requires further investigations.

*Cell senescence*

Cellular senescence is a special form of durable cell cycle arrest, leading to the gradual decline in the ability of cell proliferation, differentiation, and physiological function over time. Senescent cells are characterized by durable growth arrest, expression of anti-proliferative molecules, such as p16INK4a, and activation of damage sensing signaling pathways, including p38 and NF-κB [60]. A significant increase in quiescence of G2 and S phase was reported in adipose-derived MSCs from the aged donors with increased expression of *CHEK1* and p16(ink4a) genes with age [22]. The donor age of adipose-derived MSCs is associated with an increase in the expression of senescence-associated β-galactosidase staining with p16 and p21 gene expression higher in adipose-derived MSCs from the aged donors (> 50 years) than the young donor (< 40 years) [20]. The increase in senescence-associated β-galactosidase-positive cells in the elderly human adipose-derived mesenchymal stem cells is accompanied with the increased mitochondrial-specific reactive oxygen species production and the p21 expression [29]. Similarly, the percentage of senescence-associated β-galactosidase-positive cells is tremendously increased in bone marrow-derived MSCs from the aged donors (> 60 years old) as compared to the young donors (< 30 years old) [61]. Moreover, the numbers of p21-positive and p53-positive cells were also found to be significantly higher in bone marrow-derived MSCs from the aged donors (> 40 years old) as compared to the young donors (7 – 18 years old) [16]. Critically, NAP1L2 is a regulator for cell senescence of bone marrow-derived MSCs through the activation of NF-κB pathway [62], whereas follistatin is a marker for human bone marrow-derived MSC aging [63]. For the gingival tissue-derived MSCs, increase in p53 and sirtuin-1 expression was shown in MSCs from the elderly donors (59 – 80 years old) as compared to the young donors (13 – 31 years old) [42]. Yet, no evidence of cellular senescence was reported in bone marrow-derived MSCs from the pediatric and adult donors [19]. Collectively, the evidences of the involvement of cell senescence in the age effect of MSC properties are substantial (**Figure 1**).

**Rejuvenation of the aged mesenchymal stem cells**

Rejuvenation refers to the restoration of youthful vigor. Multiple strategies have been studied to rejuvenate the aged mesenchymal stem cells (**Figure 2**) so as to improve their properties for treatments.

*Sorting of juvenile subpopulations*

MSCs are heterogeneous in population [1]. We have previously isolated the pluripotent neural crest subpopulation from human periodontal ligament-derived MSCs [64], suggesting that there could be “juvenile” cells residing in the aged MSCs as rare subpopulation. Consistent with our study, 8% of SSEA-4-positive subpopulation was identified in human bone marrow-derived MSCs from the elderly donors and exhibits a “youthful” phenotype that is similar to that of young MSCs with the number of cells increased by 17,000 folds [17]. Moreover, it has been shown that the sorted CD264+ human bone marrow-derived MSCs have elevated β-galactosidase activity, decreased differentiation potential and inefficient in colony formation relative to the CD264- MSCs [65], indicating that CD264- is a selection method for the “juvenile” MSCs. Yet, CD271 might not be the marker for the isolation of the “juvenile” cells from the aged MSCs [66].

*Treatments*

Hypoxic preconditioning induced by 2, 4-dinitrophenol has been shown to be able to improve the regeneration potential of aging bone marrow-derived MSCs into pancreatic β-cells [67]. Similarly, hypoxic preconditioning can improve the *in vivo* angiogenic capacities of human adipose-derived MSCs from the older donors [68]. Moreover, preconditioning the bone marrow-derived MSCs with repetitive electromagnetic stimulation can enhance CFU-F and cell proliferation in bone marrow-derived MSCs, more effectively from the older donors than the young donors, via transient nitric oxide production and extracellular signal regulated kinase 1/2 activation [69]. For gene modulation, *SIRT3* overexpression can protect human bone marrow-derived MSCs from older donors against oxidative damage by activating catalase and manganese-dependent SOD through FOXO3a, and improved their cell myocardial repair effect [70]. The improvement of myocardial repair by the aged MSCs can also be achieved by modulating the macrophage migration inhibitory factor that overexpressing macrophage migration inhibitory factor in human bone marrow-derived MSCs from older donors can reduce cellular senescence, activate autophagy, induce angiogenesis, prevent cardiomyocyte apoptosis, and improve the heart function and cell survival after myocardial infarction [71]. In addition, treatment with L-carnitine has been demonstrated to increase the gene expression of human telomerase reverse transcriptase and telomere length in human adipose tissue-derived MSCs isolated from the healthy aged volunteers [72]. For the osteogenic differentiation, we have previously demonstrated that treatment of 5 μmol/L curcumin can enhance the osteogenic differentiation of human bone marrow-derived MSCs via matrix metalloproteinase-13 expression and activity [73]. Treatments with 17β-estradiol and glycinol have also been demonstrated to rescue the age-related reduction in osteogenic differentiation of bone marrow-derived MSCs isolated from older donors through estrogen receptor signaling [74], whereas treatment of 5-azacytidine induces the proliferation and improves the osteogenic differentiation potential of adipose-derived MSCs from older donors with DNA demethylation and increased *TET2* and *TET3* gene expression [75]. Furthermore, it has been reported that culture the bone marrow-derived MSCs from aged human donors on a poly(ethylene glycol)-poly(ε-caprolactone) copolymer substrate can decrease levels of detected intracellular ROS levels in the aged MSCs and promoting the osteogenic differentiation [76].

**Challenges and future prospects**

Aging is a life-long process of living toward old age, which is characterized by the progressive loss of physiological functions that could lead to diseases and death. The effect of aging on MSCs is complex and complicated, involving genetic martial deterioration, non-coding RNAs, exosomes, protein imbalance, mitochondrial dysfunction, reactive oxygen species as well as the mTOR, and insulin/IGF-1-like signaling pathways [77]. However, as a life-long process, MSCs are not just influenced by aging. In the real world, other environmental exposures and behaviors can also influence the properties of MSCs [78, 79]. The influences of these personalized factors also need to be considered in the analysis of the donor effect. Refine phenotyping and grouping with larger sample sizes could help to resolve the effects of specific factors on MSC properties. Single cell and spatial transcriptomics could also help to delineate the specific aging cells among the heterogeneous subpopulations of MSCs [80].

The induced pluripotent (iPS) stem cells [81] demonstrated as an example of rejuvenation. There is still lack of consensus on the standard/clinically recognized rejuvenation strategies for the aged MSCs although numerous anti-aging strategies have been proposed [82]. Yet, MSCs possess diversified properties for different treatment approaches [9], different rejuvenation approaches might be needed for different MSC properties. Further studies are needed to optimize the condition and quality of MSCs in the treatment regime for each MSC property. Despite the uncertainties on the application of the aged MSCs, MSC therapy would still be a promising and important strategy for the treatment of different diseases.

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**Competing Interests**

The authors declare no potential conflicts of interest.

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**Figure Legends**

**Figure 1: Cell senescence in aged mesenchymal stem cells**

Increase in senescence-associated β-galactosidase staining, quiescence of G2 and S phases, expressions of p16, p21, p38, p53, and sirtuin-1, activation of nuclear factor-κB (NF-κB), nucleosome assembly protein 1 like 2 (NAP1L2), and follistatin have been shown contributing to cell senescence in the aged mesenchymal stem cells (MSCs).

**Figure 2: Rejuvenation of the aged mesenchymal stem cells**

Sorting of juvenile subpopulations among the aged mesenchymal stem cells (MSCs), hypoxic preconditioning, repetitive electromagnetic stimulation, sirtuin-3 (*SIRT3*) and macrophage migration inhibitory factor overexpression, treatment with L-carnitine, 17β-estradiol, glycinol, and 5-azacytidine, and culturing on the poly(ethylene glycol)-poly(ε-caprolactone) (PEG-PCL) copolymer substrate have been studied as the rejuvenation strategies on the aged MSCs.

**Figure 1**

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**Figure 2**

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