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Review

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Review

Association between Donor Age and Osteogenic Potential of Human Adipose Stem Cells in Bone Tissue Engineering

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Abstract: Adipose stem cells (ASCs) have multilineage differentiation capacity and hold great potential for regenerative medicine. Compared to bone-marrow-derived mesenchymal stem cells (bmMSCs), ASCs are easier to isolate from abundant sources with significantly higher yields. It is generally accepted that bmMSCs show age-related changes in their proliferation and differentiation potentials, whereas this aspect is still controversial in the case of ASCs. In this review, we evaluated the existing data on the effect of donor age on the osteogenic potential of human ASCs. Overall, a poor agreement has been achieved because of inconsistent findings in the previous studies. Finally, we attempted to delineate the possible reasons behind the lack of agreements reported in the literature. ASCs represent a heterogeneous cell population and the osteogenic potential of ASCs can be influenced by donor-related factors such as age, but also gender, lifestyle, and the underlying health and metabolic state of donors. Furthermore, future studies should consider experimental factors in in-vitro conditions, including passaging, cryopreservation, culture conditions, variations in differentiation protocols, and readout methods.

Keywords: ASC; osteogenic potential; osteoblast; aging; regenerative medicine; tissue engineering

1. Introduction

Human bone tissue can regenerate spontaneously after fracture or injury by a bone remodeling process consisting of three phases. The fracture callus is formed in the first phase by rapid proliferation and differentiation of stem cells. The second phase involves the endochondral ossification of the cartilage and finally the remodeling of the intramembranous and endochondral bone [1]. This spontaneous bone healing is a multilateral process that is regulated by various intrinsic and extrinsic factors and can be disrupted by different causes at various time points, resulting in a failure to heal successfully [2]. Modern surgical techniques augment natural healing by grafting autologous, allogenic, or prosthetic materials to the recipient site [3]. However, the transfer of autologous material is limited by tissue availability, and it holds the risk of donor-site morbidity, unpredictable graft absorption, infections, and structural failure. To address these issues, bone repair through osteogenic tissue engineering could combine suitable progenitor cells with appropriate scaffolds and growth factors [4]. Autologous stem cell transplantation includes cell isolation and the subsequent in-vitro expansion–eventually followed by the transplantation into the defect site. However, this strategy is challenged by the availability of suitable stem cell populations with intrinsic osteogenic potential. Admittedly, age-related changes in MSC characteristics are an important criterion that should be taken into account when considering autologous stem cell therapy for bone tissue regeneration and/or engineering. Although the clinical applicability of MSCs spans all age groups, elderly people are the primary beneficiaries of stem cell-based regenerative medicine because degenerative bone diseases and delayed or impaired fracture healing are more prevalent in the elderly population. In addition, the risk for bone deformation and fractures per se increases with

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age, while it is associated with a decreased ability for tissue regeneration and repair. Age-associated microenvironmental changes, such as metabolic alteration, hormonal disturbance, and immunological disorders, might also change stem cell niche and, thus, affect the regenerative potential of MSCs [5]. Moreover aging induces profound changes in various molecular, genetic, and epigenetic processes, resulting in alterations to the proliferation and differentiation potential of MSCs. Ultimately, this leads to disrupted tissue homeostasis and impaired repair abilities [6]. Therefore, it is not only important to find a suitable autologous cell population with the potential to regenerate bone tissue, but also crucial to choose an MSC type that is less affected by age. The most promising candidates are ASCs and bmMSCs members of the MSC family, which share unique features for osteogenic differentiation [7,8].

MSCs derived from bone marrow (bmMSCs) have multilineage differentiation potential, and their use in regenerative medicine is not restricted by ethical issues. However, as discussed in previous reviews [9–13], several disadvantages limit the use of bmMSCs in bone tissue engineering. For example, bmMSC isolation procedures can be associated with donor-site morbidities such as pain, infection, hematomas, seromas, nerve injuries, arterial injuries, and fractures [14]. In addition, aging negatively impacts bmMSC harvests because the cell yield declines with age [15]. Moreover, a negative age effect is observed in both the proliferation as well as the osteogenic differentiation potential of bmMSCs [15–18]. Additionally, bmMSCs isolated from elderly donors exhibit increased senescence properties and ROS-induced oxidative damage [16–18]. It has been postulated that the bmMSCs from elderly donors favor adipogenic differentiation instead of osteogenic differentiation, known as “adipogenic switch” [9,10,13].

Compared to bmMSCs, adipose stem cells (ASCs) are abundantly available, can be isolated through a minimally invasive liposuction procedure, and yield a higher number of cells [19]. Meyer et. al. obtained nearly 2-3 million cells with stem cell properties (CD34+) from a mere 10 mL sample of lipoaspirate [20]. ASCs hold the capacity to differentiate into adipocytes, chondrocytes, osteoblasts, skeletal muscle cells, and tenocytes, in vitro [21]. Regenerative potentials of ASC and ASC-derived secretomes have been substantiated in numerous studies and when combined with 3D scaffolds and microfluidic systems ASCs support tissue repair and regenerative processes [21]. Despite these great potentials for bone tissue engineering, age-related changes in ASC functions remain elusive. Understanding the age-associated changes in ASC osteogenesis is of high importance when determining the optimal therapeutical applications of ASCs

Researchers explored the effect of aging on the regenerative potential of ASCs in many studies. However, inconsistent results have been reported in the literature. Dufrane (2017) reviewed the impact of age on ASC isolation, risk of oncogenicity, and bone tissue engineering and concluded that adipose cell properties are not dependent on donor age [22]. A systematic review (2017) analyzed results from 41 in-vitro, in-vivo, and clinical studies and found a decreased proliferation and differentiation potential of ASCs with increasing age [23]. In the present review, we aimed to update the current understanding of the impact of age on the osteogenic potential of human ASCs. We excluded studies that isolated ASCs from non-human sources because studying human aging in short-lived animal models remains controversial [24]. Furthermore, ASCs derived from non-human species are not suitable for transplantation into humans due to immunological disparities. To the best of our knowledge, this is the first review that focuses on the effect of age on the osteogenic potential of ASCs derived from humans.

A literature search was performed using Google Scholar and PubMed databases and a total of 65 papers published between 2005 and 2021 were identified. Fifty papers were excluded and 15 papers based on primary research involving human (h) ASCs, met the criteria and were included in the final review (Figure 1). As summarized in Table 1 and reviewed in the following chapter, little consensus regarding the effect of donor age on the osteogenic potential of hASCs has been reached in the literature.

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Table 1. Summary of studies included in this review.

Year	Aim	Study design	Methods	Results	Conclusions	Other variables	Ref.
2009	Effect of donor age on differentiation potential of ASCs.	ASCs from females aged 20-58 years; N=27, n= 9,7,11.	ALP activity after 7 days Ca^{2+} deposition by von Kossa after 4 weeks	↓ ALP Activity with age ↓ Ca^{2+} deposition with age	Osteogenic differentiation decreases with age	**SC:** female, non-obese **AO:** u.s (liposuction) **Passage:** 1 **OI day:** up to 28 days	[25]
2009	In-vitro differentiation potential of ASCs from young and elderly females.	Young (<35) and older (>45) females N= 26.	ALP activity Assay after 14 days Ca^{2+} deposition by Alizarin Red Assay after 21 days	↓ ALP activity with age No significant difference in Ca2+ deposition	Donor age mildly affects the potential of ASCs for osteogenic differentiation in vitro	**SC:** non-obese female, BMI<30 **AO:** subcutaneous (lipo-asp.) **Passage:** 1 **OI day:** 21 days	[26]
2012	Age-associated changes in molecular characteristics of ASCs.	ASCs from healthy young (<20), middle (30-40), and adult (>50) donors. N=40; n=15,17,8.	Ca^{2+} deposition by Alizarin Red S ALP activity assay Osteogenic gene (*BMP-6, COL2A, COL10A*) expression by RT-PCR	↓ Ca^{2+} deposition ↓ ALP activity ↓ Expression of osteogenic genes	Aging processes significantly attenuate the osteogenic differentiation potential of ASCs	**SC:** healthy, BMI <29 **AO:** abdominal **Passage:** u.s**OI day:** u.s	[6]
2012	Effect of age on ASC and bmMSC from elderly patients with osteoporosis.	ASCs from young (<36) and elderly (>67) N=22, n=14,8.	Ca^{2+} deposition by Alizarin Red S Osteogenic genes (*OCA, BMP2, RUNX2, ALP*)by RT-PCR	No significant difference in Ca^{2+} deposition No significant difference in gene expression.	The osteogenic differentiation of ASCs is not impacted by age	**SC:** osteoporotic, BMI<26 **AO:** gluteal subcutaneous **Passage: 5** **OI day:** up to 14 days	[27]

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2012	Effect of aging on senescence, osteogenic factors, and osteogenesis of ASCs.	ASCs from infants (<1), adults (20-54), and elderly (>55), N=13; n=4,6,3.	Senescence (TL) by RT-PCR *RUNX2, osteocalcin* by RT-PCR ALP activity assay Ca2+ deposition by Alizarin Red S	↑ Senescence with age ↓Osteogenic gene expression compared to infant ↓ALP activity and Ca2+ deposition compared to infant.	Biological properties are conserved during the adult to the elderly period (but not compared to infants)	**AO:** abdominal (liposuction) **Passage:** 1 **OI day:** up to 21 days	[28]
2014	Impact of age on the quality of human adipose tissue-derived MSCs.	ASCs of young (<30), adult (35-50), and older (>60) donors, N=29; n=10,8,11.	Ca^{2+} deposition by von Kossa staining Senescence by β-galactosidase Staining Osteogenic genes (*osteocalcin, ALP*) by RT-PCR	↓ Ca^{2+} deposition with age ↑ Senescence with age ↓ Expression of osteocalcin and ALP with age.	Age negatively impacts stem cell osteogenic differentiation	**SC:** male/female **AO:** - (liposuction) **Passage:** 2-3 **OI day:** 21 days	[29]
2014	Effect of age on osteogenesis of female ASCs: superlot approach.	ASCs from female patients (24-81), **superlot** biobanking. N=14; n=5,4,5.	Ca^{2+} deposition by Alizarin Red S	↑ Ca^{2+} deposition with older (post-menopausal) female.	Existence of a high degree of donor-to-donor variations which is independent of age	**SC:** female **AO:** u.s **Passage:** 1 **OI day:** 14 days	[30]
2016	Effects of donor age on the biological properties of human OASC.	OASCs from young (20-38, normal) and old donors (50-67, fat pad in lower eyelid) N=20; n=10,10.	Ca^{2+} deposition by Alizarin Red S Ca^{2+} deposition by Von Kossa staining	↓ Ca^{2+} deposition with age	The benefit of autologous OASCs from elderly patients for osteogenic therapeutic purposes may be limited	**SC:** female, non-obese **AO:** lower eyelid fat pad **Passage:** 3 **OI day:** 14 days	[31]
2016	Effect of age on the osteogenic potential of ASCs.	ASCs from different age groups: >20y, >50y, >60, >70 N=32; n=8.	Ca^{2+} deposition by Alizarin Red ALP activity assay Osteogenic markers (*OPN, Col-I, OCL, and BMP-2*) by PCR	↓ Ca^{2+} deposition with age No significant difference in ALP activity	Age negatively influences the osteogenic potential of ASCs	**SC:** healthy male/female **AO:** subcutaneous **Passage:** 1 **OI day:** 21 days	[32]

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				↓ Expression of osteogenic markers with age			
2017	Systematical analysis of the effects of age on the quantity and quality of ASC.	ASCs were isolated from children (6-12), young individuals (22-27), adults (60-73), and the elderly, N=24; n=10,8,6.	Cellular senescence assay Ca^{2+} deposition by Alizarin Red S Osteogenic genes (*RUNX2, ALP, OCN, OPN*) by RT-PCR	ASCs from elderly donors exhibit senescent properties. ASCs from aged patients exhibit impaired osteogenic potential	While ASCs from different age populations are phenotypically similar, they present major differences at the functional level	**SC:** male/female BMI <22 **AO:** chest subcutaneous **Passage:** 3 **OI day:** up to 21 days	[33]
2017	Effect of donor age on differentiation potential of ASC.	ASCs of 260 donors (ages 5-97 years) N= 260.	Ca^{2+} deposition byAlizarin Red S	The osteogenic potential (marked by Ca^{2+} deposition) of ASCs does not correlate with donor age	The chondrogenic and osteogenic potential of ASCs were not affected by age	**SC:** male/female, median BMI =22.7 **AO:** subcutaneous **Passage:** 5 **OI day:** 21 days	[34]
2017	Cell-substrate Impedance Spectroscopy (ECIS) to track complex bioimpedance pattern of ASC osteogenesis.	ASC superlot from young (24-36), middle (48-55), elderly (60-81).	ECIS measurement throughout the osteogenic differentiation phases	ASCs from younger donors require a longer time to differentiate than ASCs from older donors.	Donor age may temporally control the onset of osteogenesis	**SC:** female **AO: u.s(**liposuction**)** **Passage: u.s** **CS: u.s** **OI day:** u.s	[35]
2018	Effect of donor age on the regenerative potential of HEASCs.	HEASCs from <20y, >20y, <45y, >55y N=13; n=4,5,4.	Ca^{2+} deposition by Alizarin Red S *RUNX2* by RT-PCR	↓ Ca^{2+} deposition with age No difference in gene expression.	Donor age has a negative influence on the osteogenic differentiation of HEASCs	**SC:** healthy donor **AO:** eyelid **Passage:** 5**OI day:** 21 days	[36]

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2020	Differentiation potential of ASCs isolated from the **lipoaspirates** of elderly and young donors.	ASCs from young (<34) & old (>54) female donors,N=18; n=9,9.	Cell mineralization assay *RUNX2* by RT-PCR	No significant difference No significant difference	Age does not significantly impact the osteogenesis of ASC	**SC:** female, BMI <30; **AO: u.s** (liposuction) **Passage:** 4-7 **OI day:** up to 28 days	[37]
2021	Association between age and ASC differentiation potential.	ASCs from young (<30) and elderly (>70), N=8.	Ca^{2+} deposition by Alizarin Red S BMP-2 by ELISA BMP-2 receptor by WB	↓ Ca^{2+} deposition with age No significant difference ↓ BMP-2 with age	Age may affect the cellular function and differentiation of ASCs	**SC:** healthy male/female, **AO: u.s** **Passage:** 3-5 **OI day:** 20 days	[38]

Abbreviations: TL = telomere length; GF= growth factor, ns = no significant, rhBMP-2 = recombinant human bone morphogenic protein -2; HEASC = Human eyelid adipose stem cell, DM= diabetic mellitus; ECM=extracellular matrix, ECIS = cell-substrate impedance spectroscopy, OASC= orbital derived adipose stem cell, GF=growth factor, OCA=osteocalcin, BMP=bone morphogenic protein, ALP= alkaline phosphatase, N= total number of participants/animal, n= sample size (number in each group), SC= sample characteristics, AO= anatomical origin, CS= centrifugal speed, OI= osteogenic induction, u.s= unspecified, Ns = non significe.

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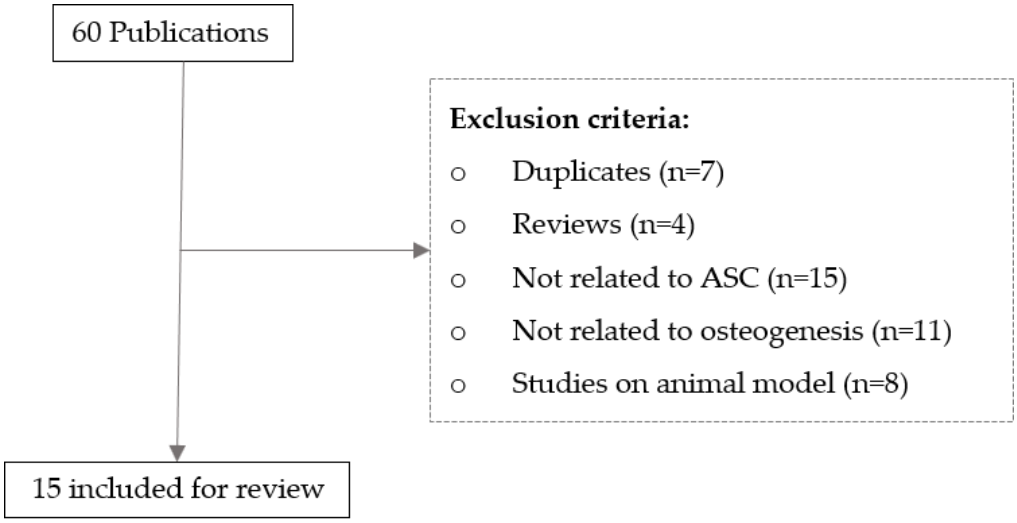

Figure 1. Flow chart demonstrating the selection process in this review.

2. Review of previous studies

Among 15 original studies included in this review, seven studies have reported no significant influence of age on the osteogenic differentiation capacity of hASCs. For example, Chen et al. evaluated the effect of age on the osteogenic potential of hASCs from 14 young patients with hip fracture (36.4 ± 11.8 years) and 8 elderly patients (71.4 ± 3.6 years) with osteoporosis. The results from their study indicate that age is not an influential factor in terms of matrix mineralization and calcification of hASCs. However, the mRNA expression of *osteocalcin* and *ALP* genes decreased in hASCs of elderly donors. [27]. Wu et al. investigated the effect of age on the osteogenic potential of hASCs isolated from infant (<1 year), adult (20-54 years), and elderly (>55 years) donors. Although *RUNX2 and osteocalcin* mRNA expression and matrix mineralization were higher in infant ASCs, these parameters were overall comparable to those from adult and elderly donors [28]. A complex relationship between donor age and the osteogenic potential of hASCs from female donors was revealed in a study by Zhu et al.; Age-associated decline in hASC osteogenesis was not significant in the study population (20-58 years). However, a significant decline in osteogenesis, in terms of decreased matrix calcification in the von Kossa staining, was seen when female donors entered their 40s which suggests that these changes may be associated with estrogen loss during the transition of women from the pre-to-perimenopause [25]. To overcome the high inter-individual variability between various donors, Bodle et al. generated ASC "superlots", i.e. pooled donor cell populations derived from four to five age-clustered pre-(24-36 years), peri-(48-55 years), and post-menopausal (60-81 years) female donors. With this superlot approach, the authors could show that despite high donor-to-donor variability, young hASCs are primed to the adipogenic lineage whereas old hASCs (60-81 years) preferentially differentiate osteogenically [30]. A report from Kawagishi-Hotta and colleagues included hASCs from a large number of donors (n=260) aged 5-97 years old. The authors concluded that age negatively impacts adipogenic differentiation but not chondrogenic and osteogenic differentiation, measured by Oil Red O staining, sulfated glycosaminoglycans content, and Alizarin Red S staining, respectively. The principle component analysis (PCA) for ASC-characteristics revealed that the proliferation and multi-lineage differentiation varied in each individual, particularly in females and at an age of >60 years. Another study investigated the differentiation potential of hASCs derived from nine young (<36 years) and nine old (>54 years) donors. After osteogenic induction for up to four weeks, no significant differences between hASCs of young and old donors in terms of matrix mineralization evaluated by OsteoImage™ Mineralization Assay and osteogenic genes (*RUNX2, CEBPA*) expression level by quantitative PCR were demonstrated [37].

In contrast, eight studies found a diminishing effect of donor age on the osteogenic function of hASCs. For instance, Alt et al. presented a correlation between age-related changes in the quality of

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stem cells and differentiation capabilities using hASCs from young (<20), middle-aged (30-40), and elderly (>50) healthy donors. They observed an age-dependent downregulation of miRNAs (*mir-27B and let-7G*) which regulate cell cycle, apoptosis, and inhibition of multilineage potential of hASCs [6]. Similarly, Choudhery et al. studied the influence of age on the in vitro differentiation of hASCs from young (<30), adult (35-50 years), and aged (>60 years) individuals. Cells from aged donors displayed higher cellular senescence (confirmed by increased SA-β-gal staining) that correlated with a lowered level of cell mineralization in von Kossa staining compared to their young counterparts [29]. The association between donor age and the differentiation potential of human orbital adipose-derived stem cells (OASCs) was investigated by Ye et al [31]. OASCs were isolated from the lower eyelid of young (20-38 years) and adult (50-67 years) female individuals who underwent routine blepharoplasty. OASCs from older donors displayed increased senescence-related gene (*p21, p53*) expression as well as decreased calcium deposition detected in the Alizarin Red assay [31]. Similarly, in another study, human eyelid adipose-derived stem cells showed decreased Alizarin Red staining for matrix calcification and less expression of osteoblastic gene (*OPN*) expression [36].

Marędziak and colleagues isolated hASCs from the subcutaneous fat of 28 healthy donors divided into four age groups, >20 years, >50 years, >60 years, and >70 years. They confirmed that the age group classified as younger (20-49 years) displayed a higher level of matrix calcification in Alizarin red assay and increased expression of osteogenic factors (*osteocalcin, BMP-2, osteopontin*) in RT-PCR and ELISA, compared to age group >50 years [32,39]. A similar observation was reported by Liu et al. in the following year on hASCs from adipose tissue of children (6-12 years), adults (22-27 years), and elderly (60-73 years) individuals [33]. They observed an age-associated increased cellular senescence manifested by an increase in SA-β-gal-positive cells, as well as a decline in osteogenic potential marked by down-regulation of osteogenic genes (*RUNX2, BMP-2, osteocalcin,* and *osteopontin*) in RT-PCR and decreased matrix calcification in Alizarin Red staining in hASC from elderly donors [33].

The regenerative potential of hASCs is predominantly attributed to their paracrine activity. A recent study illustrated that age altered secretory patterns of hASCs leading to a reduced release of vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), and stromal cell-derived factor 1-*α*. In addition, hASCs from elderly donors (>70 years) rarely differentiated into osteoblasts compared to hASCs derived from younger (<30 years) donors, as hASCs from the younger individuals revealed significantly higher calcium deposition in the Alizarin Red Assay. Although the secretion of BMP-2 protein was similar among both groups, the expression of its receptor (BMPR1A) was lower in the elderly group. Thus, the author postulated that elderly hASCs might exhibit a weaker response to the BMP-2 protein due to the reduced expression of its receptor [40]. Together, emerging evidence suggests that age impairs the osteogenic potentials of hASCs.

3. Discussion

As summarized in Table 1, there is conflicting evidence regarding the effect of age on the osteogenic differentiation potential of hASCs. Some studies found no significant effect of donor age on the osteogenic potential of the cells, while other studies depicted a deteriorating effect. This inconsistency could be dependent on several limitations in the included studies. For example, the sample size could have been too small to produce statistically significant data). In just one study, hASCs were isolated from a large number of donors (n=260 donors, aged 5-97 years) and demonstrated an age-dependent adverse effect on adipogenesis but not on osteogenesis or chondrogenesis [34]. As MSCs usually maintain a differentiation balance – if one differentiation lineage is favored, the other one is inhibited - the findings of the previously mentioned study partially support this paradigm [41].

Since there is no standard for age clustering, researchers grouped individuals in various ways. Often, the selected age range was not large enough to make a valid comparison. In other words, in many earlier observations, the age difference between young and old donors was small, which might have concealed a true age effect. Studies on hASCs isolated from donors with a narrower age range found no significant impact of age. For example, in a study by Horinouchi et al., the osteogenic

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potential of young (>34 years) and adults (<54 years) remained unaffected by age in terms of bone mineralization and osteogenic gene (*RUNX2*, *CEBPA*) expression [37]. In contrast, Park reported age-related alterations in bone mineralization and *BMP-2* gene expression in hASCs from two groups of donors with a wider age range (<30 years vs >70 years) [38]. Similarly, two studies included infants (<1 year) or children (6-12 years) and demonstrated that hASCs from infants and children have higher osteogenic potentials in comparison to hASCs from elderly people (>55 years) [28,33].

Notably, even though this review focuses on hASCs, some in-vivo mouse models also reported a similar age-dependent effect when comparing ASCs isolated from mice with large age differences. For example, age-related alterations in ASC proliferation and differentiation were reported by Li and Doshida et al. in the same species but with distinct age groups, i.e., 1-month-old vs 20-month-old, and 6-month-old vs 29-month-old, respectively [42,43]. [44]. However, Shi et al. observed no significant effect of age on the differentiation potential of ASCs from 6 days and 60 days old mice Thus, age-related changes in the osteogenic potential of hASC might be visible when hASCs from very young and very old donors with distinct age differences are compared. As younger individuals are less likely to undergo surgery, hASCs from young donors are significantly harder to recruit for experimental studies. This might be the reason why previous studies did not investigate hASCs from distinct age groups. For the same reason, hASCs were obtained predominantly from women than men because women are more likely to undergo plastic surgery [45]. Interestingly, some studies revealed that hASCs obtained from female individuals in their early 40's exhibited increased lipid accumulation and decreased potential to differentiate into osteogenic lineage compared to hASCs from younger (<30 years) and older (>55 years) women [25,30,37,46]. Menopause-related changes in estrogen levels could explain this transient effect of age on hASC function. Furthermore, osteoporosis after menopause is a predictor of declined osteogenic potential of hASCs [47]. Concluding, gender and menopausal status should be considered when grouping donors based on age and future studies should further explore the effects of hormonal changes and osteoporosis on ASCs properties.

Some authors reported high intragroup variability in hASC characterization and differentiation which could conceal age-dependent effects This apparent high donor-to-donor variability could be attributed to other demographic and lifestyle factors, e.g. general health status, medical and disease history, body mass index, or epigenetic patterns related to the environment, or donor habits may also influence experimental outcomes, as reviewed by Prieto González in 2019 [48]. These donor characteristics have been disregarded in the literature, and in many cases, BMI was used as the sole parameter to describe the obesity status of individuals [6,25–27,31,33,34,37,49]. Increased BMI as a marker of obesity is associated with a decreased osteogenic potential of hASCs [50]. However, the role of BMI in identifying people with obesity is controversial as it cannot distinguish from fat, muscle, or bone mass. Therefore, a more useful indicator of obesity should be used when defining non-obese donors of ASC.

Studies evaluating the effect of age on bone tissue engineering used hASCs from diverse anatomical sites including the abdomen, the epididymis and the eyelidSurgical methods of fat harvesting also varied between the presented studies. Differences in the anatomical origin of adipose tissue and surgical procedures may be the underlying confounder since hASCs from different donor sites and methods of extraction exhibit distinct biological properties [51]. For instance, Requicha et al. assessed the expression profile of osteogenic genes (*COLIA1*, *RUNX2*, and *Osteocalcin*) of hASCs from the canine subcutaneous and omental origin by RT-PCR analysis. While *RUNX2* expression did not differ between the two fat depots, *COLIA1* was significantly higher expressed subcutaneous hASC whereas *osteocalcin* displayed an inverse expression pattern[52].

Apart from donor-related factors, the proliferation and differentiation potential of hASCs is also influenced by long-term passage, cryopreservation, and culture conditions (Figure 2), as these parameters varied in previous reports [48,53]. Often, the osteogenic induction was performed on cryopreserved cells, after passages 1 to 5 by using osteogenic induction media with different compositions. Furthermore, previous studies selected different endpoints as the marker of osteogenic differentiation with various readout methods. Growth factors and serum supplementation also

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greatly differ between laboratories. These experimental variations may influence hASC stemness, proliferation, and differentiation [48].

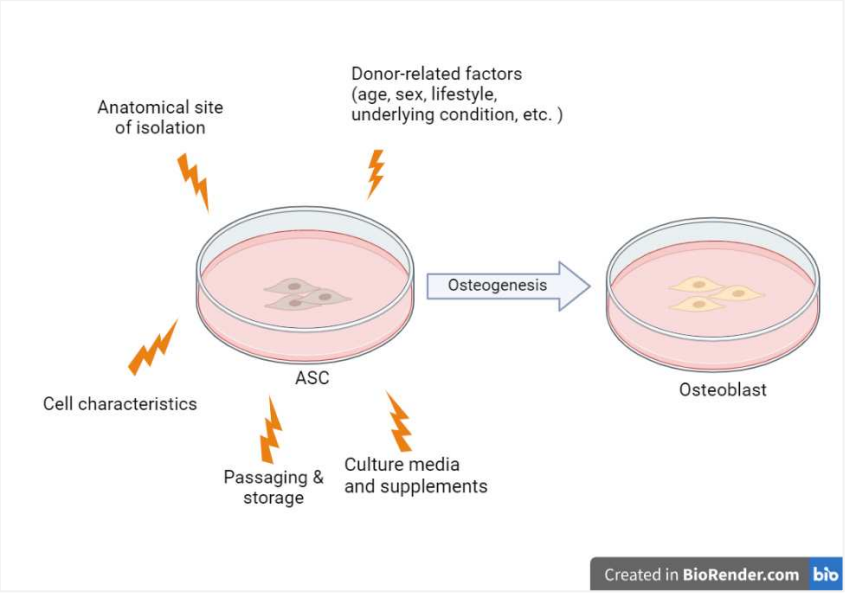

Figure 2. Osteogenic potential of ASC is influenced by both donor-related and experimental factors.

4. Conclusions

The effect of age on the osteogenic differentiation potential of hASCs has been highly debated in the literature and hitherto poor agreement has been achieved in previous studies. Factors that might contribute to a disagreement in previous research include experimental variables such as small sample size, lack of standard age grouping, differences in protocols for osteogenic differentiation and readout methods, as well as donor-related factors, for instance, hormonal status, underlying disease conditions, and metabolic status of hASC donors. Apart from the effect of age, future studies should also consider the intrinsic and extrinsic factors that may influence the osteogenic potential of hASCs.

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