




Review

Revisiting the Advances in Isolation, Characterization and Secretome of Adipose-Derived Stromal/Stem Cells

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Abstract: Adipose-derived stromal/stem cells (ASCs) seems to be a promising regenerative therapeutic agent due to the minimally invasive approach of their harvest and multi-lineage differentiation potential. The harvested adipose tissues are further digested to extract stromal vascular fraction (SVF), which is cultured, and the anchorage-dependent cells are isolated in order to characterize their stemness, surface markers, and multi-differentiation potential. The differentiation potential of ASCs is directed through manipulating culture medium composition with an introduction of growth factors to obtain the desired cell type. ASCs have been widely studied for its regenerative therapeutic solution to neurologic, skin, wound, muscle, bone, and other disorders. These therapeutic outcomes of ASCs are achieved possibly via autocrine and paracrine effects of their secretome comprising of cytokines, extracellular proteins and RNAs. Therefore, secretome-derivatives might offer huge advantages over cells through their synthesis and storage for long-term use. When considering the therapeutic significance and future prospects of ASCs, this review summarizes the recent developments made in harvesting, isolation, and characterization. Furthermore, this article also provides a deeper insight into secretome of ASCs mediating regenerative efficacy.

Keywords: adipose tissue; adipose-derived stem cells; secretome; regenerative therapy

1. Introduction

The self-renewal and differentiation potential of adipose-derived stromal/stem cells (ASCs) have accelerated the progress in regenerative therapy. In the previous literatures, a variety of terms have been used for these cells, such as adipose-derived adult stromal cells, adipose-derived adult stem (ADAS) cells, adipose-derived stromal cells (ADSC), adipose stromal cells (ASC), adipose mesenchymal stem cells (AdMSC), preadipocytes, processed lipoaspirate (PLA) cells, and adipose-derived stromal/stem cells (ASCs); however, to address this discrepancy, the International Fat Applied Technology Society (IFATS) reached a consensus to refer them as adipose-derived stromal/stem cells (ASC) [1]. These cells are mainly present in perivascular region of all tissue

and organs, including white adipose tissues [2–5]. The higher abundance of ASCs in these areas from which they could be easily harvested via minimally invasive procedures make them a suitable agent in cell-based therapy [5]. Aesthetic and economical liposuction surgeries are less painful and provide rich source of ASCs and progenitor cells in large quantity as compared to harvesting bone marrow stem cells (BMSCs) [6,7]. ASCs have been reported for its pluripotency/plasticity into various cells, such as chondrocytes, osteoblasts, myocytes, adipocytes, neural cells, and epithelial cells [8–10]. Therefore, their regenerative potential have been explored in the treatment of various diseases, such as diabetes and related complications, osteoarthritis, cardiovascular diseases [11–13], nerve regeneration and neurological disorders [10,14,15], skin aging [16], ischemic limb disease [17], skin burn, and wound healing [18–20]. Along with differentiation potential of ASCs, the exhibited paracrine activity, and secretion of growth and signaling factors enhance their clinical significance [21]. It is reported that ASCs maintain their phenotypic characteristics, differentiation potential, and proliferation capacity even after 25 passages [22]. This indicates their reduced frequency of passaging and hence the low risk of cellular senescence [23,24]. In the recent years, the intense research has focused on isolation and characterization of ASCs from various adipose tissue sources of animal models and human. These ASCs are present in stromal vascular fraction (SVF) along with other cells, such as endothelial, hematopoietic, and other cells [23,25]. After lipoaspiration, the adipose tissue is digested with collagenase and subcultured to obtain the sufficient number of cell populations [26]. Thereafter, the cell proliferation/viability is determined and the cells are further assessed for the presence of mesenchymal stem cell characteristics, such as cell surface markers in form of cluster of differentiation (CD) [5–7,25,27,28], and their multi-lineage differentiation potential, which is determined by culturing them in specific induction media.

Contemplating the importance of regenerative potential of ASCs; this review article comprehensively summarizes the isolation, characterization, and differentiation methodologies of ASCs from various sources for their possible use in regenerative therapy.

2. Adipose Tissues as Source of ASCs

Adipose tissues are a rich and popular source of adult stem cells [29,30]. They are also involved in homeostasis, metabolism regulation, and aging processes [31]. These tissues are derived from mesenchyme and mainly constitute stem cells, endothelial cells, collagen, resident monocytes/macrophages, lymphocytes, fibroblasts, vascular smooth muscle cells, preadipocytes, and adipocytes [26,32–35]. Adipose tissues are classified into three groups, namely, white adipose tissue (WAT), bone marrow adipose tissue (BMAT), and brown adipose tissue (BAT) in mammals [36–39]. Both, the BAT and WAT contain lipolytic and lipogenic functions and are involved in energy accumulation and dissipation, respectively [36]. However, BATs are larger in size and rich in mitochondria than WAT and possess a specific uncoupling protein-1 (UCP-1), a mitochondrial ion carrier [40]. The existing color of BAT is due to the high number of vascularization, cytochromes, and mitochondria, which is responsible for high energy dissipation along with other dedicated proteins, such as UCP-1 [41,42]. On the other hand, WAT is found as subcutaneous and visceral depots that are not involved in metabolic disorder due to high number of young adipocytes and adipose turnover [43,44]. The role of WAT in metabolism regulation is critical and has been shown that several metabolic disorders such as hyperglycemia, diabetes, hypertension, liver disease, hyperlipidemia, etc. are generally caused by an imbalance in adipocytes [45]. In addition, BAT is considered as source of heat as it catalyzes energy uncoupling, dissipation, and mitochondrial biogenesis [46]. Heterogeneity and plasticity are the characteristics of adipose tissues that depend on species and source of fat depots [42]. Adipose tissues are appealing due to their higher abundance in stem cells and ease of harvest when compared to bone marrow [42]. However, the ASCs yield is influenced by various factors, such as age, location of adipose tissue, harvesting techniques, and species [47–49]. BAT is mainly found in axillary, perirenal, periadrenal regions, and cervical in fetus and neonate; however, this tissue transformed mostly into WAT in adults [50,51]. Whereas, the human WAT is distributed at various

sites, such as subcutaneous region of abdomen, thighs and buttocks, intestines, perirenal, omentum, retro-orbital space, and bone marrow [52,53]. Subcutaneous depots are localized at superficial and deep region of abdomen and are considered superior source of stem cells when compared to other fat depots [52]; however, superficial abdominal adipose tissues has showed an enhanced multipotency and stemness characteristics [54]. Standard en bloc resection and liposuction are the two most common surgical procedures that are used to harvest adipose tissues [52]. Further, though the efficacy of adipose tissues site on ASC yield and its characteristics is not explicitly established, a few seminal studies demonstrated that abdominal adipose tissue is rich in ASCs as compared to other sites, such as hip, thigh, femoral, axilla, and flank regions [25,47,55]. Furthermore, another comparative study implied that superficial adipose tissue is a better source of stromal vascular fraction (SVF) [56]. These bodies of evidence indicate that superficial abdominal adipose tissue is a prospective source of ASCs. However, in contrast to the above studies, a recent report revealed a significantly higher yield of ASCs and SVF from adipose tissues of inner as well as outer thigh when compared to those of abdominal, waist, and inner knee regions [57]. In another study, Khojasteh et al. suggested that, when compared to abdomen and hip regions of both male and female donors, the buccal fat pad seems more promising source of ASCs for regenerating bone tissues [58]. On the other hand, no significant differences on yield characteristics and viability have also been observed among ASCs of abdomen, thigh, or hip [59]. Along with the donor site, the factors, such as gender and age, have also been extensively evaluated and need to be considered during harvesting of adipose tissues to isolate ASCs [60]. In a rabbit model, the aging induced suppression of ASCs yield and adipogenic potential was evident with no significant effect on their osteogenic and clonogenicity [61]. A recent systemic review has reported an inhibited proliferation and differentiation potential of ASCs with advancing age [62]; however, this phenomenon was not extensively uniform throughout. A comparative study concluded that yield and characteristics of human orbital adipose derived stem cells remain constant among young and aged donor [63]. Similarly, no significant effect of aging on ASC yield and therapeutic potential of ASCs was observed from adult to elderly stem cells [64]. This might be attributed to no influence of aging on the cellular senescence and ASC yield from subcutaneous adipose tissue, thereby gaining promising potential for regenerative therapy [65]. On contrary, Lee et al. found the higher cell population doubling levels and differentiation potential of ASCs among younger dog when compared to older ones, indicating that the age of donor is an important factor in cell-based therapy [66].

Coleman's technique, direct excision, and liposuction are the common harvesting techniques that are used in collection of adipose tissues [49]. The collection site and procedure followed in above techniques affects the yield and characteristics of ASCs [67]. However, no significant effect on ASCs yield and differentiation potential through direct resection and liposuction had also been reported. Further, the ASCs obtained through ultrasound-assisted liposuction are lower in yield and proliferative potential compared to resection and tumescent liposuction methods [59]. Notwithstanding, the liposuction seems better harvesting technique, and yield more homogenous ASCs than the resection technique [68]. However, the pattern of expressed genes from ASCs isolated by liposuction indicate their enhanced endodermal differentiation; whereas, ASCs isolated by resection had tendency of mesoderm and ectoderm differentiation [68]. Taken together, though the recent studies have established several factors that might affect ASCs yield, viability, and characteristics, an intense investigation is needed to gain a deeper insight on the role of factors on quality and quantity of ASCs.

2.1. Harvesting of Adipose Tissues

Harvesting adipose tissue is the first step in isolation of ASCs. The three general techniques liposuction, resection, and Coleman are used to harvest adipose tissues from human fat sites; of which, the liposuction results in better cellular yield and viability than others [69]. Liposuction is one of the most common and increasingly used surgical operation being carried out by plastic and reconstructive surgeons since several past years to restructure body contour to improve aesthetic looks and in treatment of pathologies in reconstructive surgery [70]. Further, though the various sites are targeted

to harvest adipose tissues, subcutaneous regions are considered as the most appropriate choice [70,71]. Liposuction is generally carried out Recently, Arpad and Giorgio Fischer has developed a novel method of liposuction in which a blunt hollow cannula is attached to a suction source to extract adipose tissues with lesser complication. This dry liposuction technique was further modified as wet/tumescent liposuction to decrease the effect of hemorrhagic risk and the associated complexity through bringing down the bleeding level <1% compared to 30% of dry liposuction [72]. In the wet/tumescent technique, firstly the Klein solution (0.05% lidocaine, 1:1,00,000 epinephrine, and 10 mL sodium bicarbonate per 1000 mL saline) or saline solution containing local anesthetic agent and/or epinephrine is injected at the target site to reduces blood loss and enhance the safety of the procedures [73]. Thereafter, the adipose tissues are harvested by using cannula and syringe of different sizes.

Besides, vacuum or syringe aspirations are the most commonly followed techniques during fat harvesting procedures [74,75]. The increased vacuum increases the aspiration rate; however, a very high pressure may disrupt structural integrity of ASCs and other cells [76,77]. Additionally, cannula size and types of syringe needles also might affect the cell yield, size, and viability of harvested fat [78–80]. However, a study by Campbell et al. reported that if the needle size is greater than 20 gauge, it exerts no significant effect on adipocyte morphology and metabolism [81]. In contrast to above studies, no significant effect of multi-perforated cannula with the Coleman 3 mm aspiration cannula was observed on cell viability or size of fat tissues [82,83]. Ultrasound and laser-assisted liposuction are the other two approaches to harvest fat with enhanced accuracy and safety during procedures [84–86]. Besides, the Coleman developed fat harvesting techniques using syringe, cannula, and centrifuge in which an incision is made at the target site and injected with 1 cc solution per cm³ of fat to be harvested [87].

2.2. Isolation of ASCs from Harvested Adipose Tissues

The first attempt to isolate ASCs is initiated by appropriate washing, followed by their digestion with collagenase and centrifugation to separate stromal vascular fraction (SVF). The SVF is considered as a source of adipocyte progenitors and ASCs along with other cells; iterative sub-culturing enriched the plastic adherent ASCs (Figure 1) [88–90].

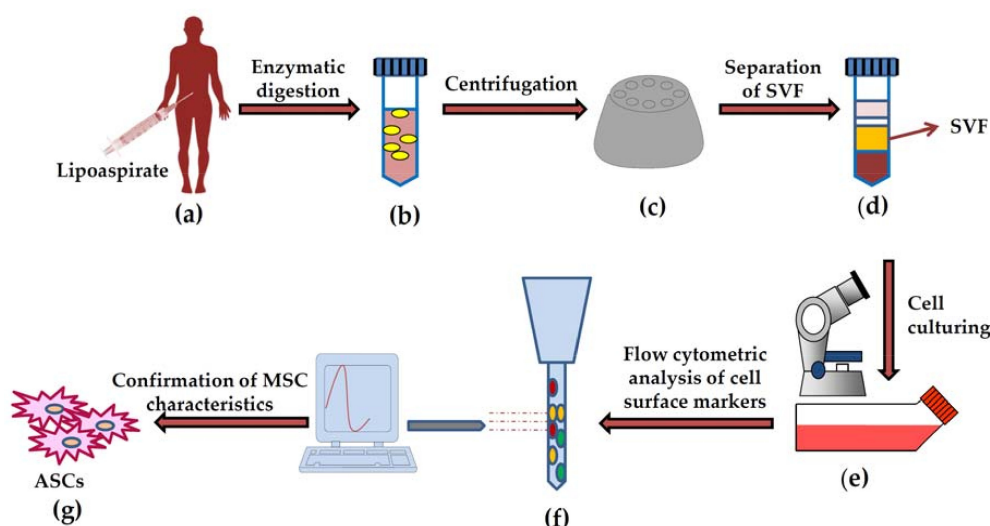


Figure 1. Schematic representation of process for harvesting, isolation and characterization of adipose derived stem cells (ASCs). Adipose tissues are harvested through liposuction, enzymatically digested; and centrifuged to isolate stromal vascular fraction (SVF). Finally, the SVF is cultured and adherent cells are analyzed for presence of cell surface markers through flow cytometric analysis to confirm the presence of mesenchymal stem cells characteristics.

Additionally, this process has been further modified to recover ASCs from human adipose tissues [91–95]. Centrifugation speed affects the cell yield and $1200\times g$ has been observed as optimal centrifugation speed for sufficient recovery of cells [96]. The general procedure of isolation of ASCs initiates from fragmenting large adipose tissues into smaller tissue chips and to avoid connective tissues as they might become source of contamination; this is followed by washing adipose tissues with phosphate-buffered saline (PBS)/Dulbecco's phosphate buffered saline or saline to remove blood; wash buffers can be supplemented with antibiotic/antimycotic [97]. The properly rinsed tissue is further minced in sterile condition and then washed again with PBS to remove any traces of blood. The minced tissue is incubated with 0.075–0.5% collagenase type IA at 37 °C for 30 min [68,97]. Another study used collagenase type I (0.5 mg/mL) in equal volume of adipose tissues to digest adipose tissue [98]. Collagenase type II and type IV might also be used; however, optimum concentration of enzyme depends upon quality of enzyme [97]. In addition to collagenase a recent study showed that trypsin can be a cheaper alternative for digesting adipose tissues [99]. Enzymatic activity of collagenase or trypsin is negated by supplementing digested tissue sample with DMEM or α -MEM supplemented with 10% or 20% inactivated fetal bovine serum (FBS) [53,97]. Notwithstanding the enzymatic digestion is a costly method for extraction of ASCs and might affect efficacy and safety [100–102]. Therefore, the recent study has explored the economical non-enzymatic method for standardization of ASCs isolation [103]. In another study, lipoaspirate was cultured without enzymatic digestion and sub-cultured after five days; suspension cells were removed from culture flasks by washing and only adherent cells were further analyzed for mesenchymal stem cells characteristics [104]. Similar to this study, another attempt was made to develop non-enzymatic method by simple washing and excessive and repeated shaking of adipose tissues to collect infranatant, which was further centrifuged and collected SVF was cultured to grow ASCs [105]. Notably, this study reported no major differences in cell characteristics isolated from enzymatic and non-enzymatic methods; however, cellular yield was higher in the enzymatically digested method. In another recent study, the mesenchymal stem cells (MSCs) obtained from harvested adipose tissue of animal or human were pluripotent and successfully differentiated into adipocyte and osteoblasts [106]. Various commercial mechanical devices have been developed to process adipose tissue; which uses forces, such as pressure, centrifugal force, shear force, radiation, and ultrasound, etc. to disintegrate the tissues [107]. To maintain sterility, safety, and quality of ASCs and to fulfill the regulatory requirements, various attempts have been made to develop closed and sterile isolation system to reduce uncertainty [107]. However, more extensive studies are required to set standard protocol to fulfill the clinical regulation to explore real-time therapeutic effectiveness of ASCs.

3. Characterization of ASCs

Ability of colony formation of stem cells is an indicator of potency and proliferation [108,109]. When stem cells are cultured in low density, each cell have capacity to form individual colonies [110]; however, stem cells that are isolated from rat or mouse may form more than one colony, as the cells may disintegrate from colony and regenerate another cell colony [111–113]. CFU can be determined by culturing the cells in medium for 10–14 days, after which thier colonies are visualized and counted using crystal-violet stain. Similarly, cells are also characterized based on expression of their surface markers by using flow cytometry [114]. Characterization of surface markers of ASC is generally carried out by incubating subcultured cells with primary monoclonal and secondary antibodies that are labeled with dyes, such as fluorescein isothiocyanate (FITC), texas red, allophycocyanin (APC), or phycoerythrin (PE) [115,116]. Further, these cells incubated with labeled dye conjugated secondary antibodies are washed. The minimum suggested for positive markers are represented in Table 1.

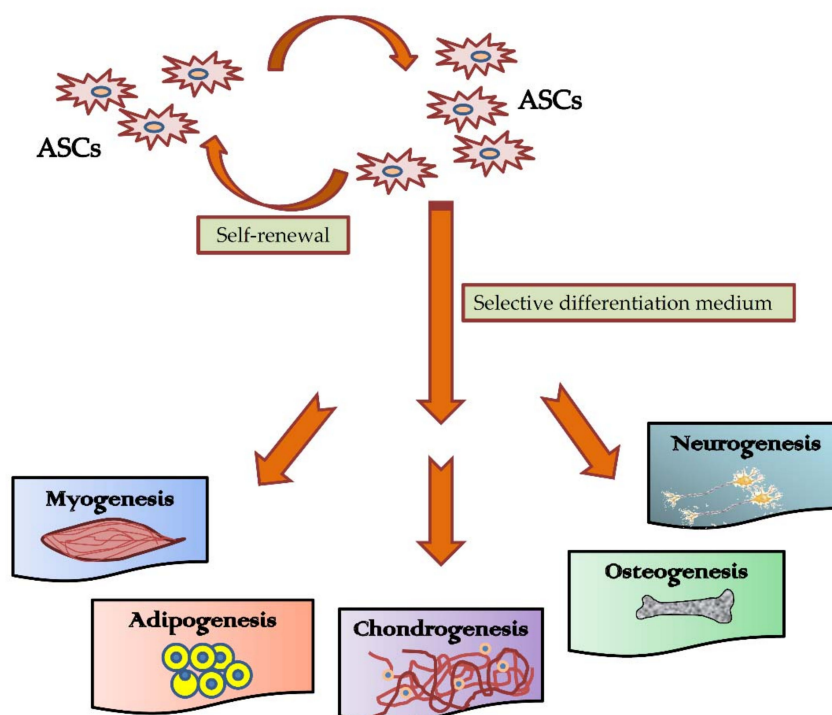
Table 1. List of minimum mesenchymal stem cells (MSC) immunophenotypic markers on ASC [117].

ASC Immunophenotypic Surface Markers	
Positive (+ve)	CD90, CD44, CD29, CD105, CD13, CD73, CD166, CD10, CD49e and CD59
Negative (–ve)	CD31, CD34, CD45, CD14, CD11b, CD19, CD56 and CD146

However, there is a great discrepancy and inconsistency in the data available about expression of CD34 in ASCs. It has been widely accepted that CD34 is not present on the surface of cells; however, this observation is largely based on cultured MSCs, not the tissue-resident MSCs [118]. The evidences have shown that CD34 is present in freshly isolated ASCs and disappear after several passages [30,118]. Notably, the presence of other surface markers, such as HLA-ABC, HLA-DR, SH2, SH3, STRO-1, VEGF2, vWF, ABCG2, SSEA-1 (CD15), PDGFR, α -SMA, c-Kit (CD117), OCT4⁺, and CCR5X (CD195) have also been reported [117]. Further, after corroborating the surface markers, cells are characterized based on their differentiation potential in chemically-induced medium.

3.1. Differentiation Potential of Adipose-Derived Stem Cells

Multilineage differentiation potential of ASCs towards both mesenchymal and non-mesenchymal lineage cells have been reported [119]. This may be achieved by the introduction of factors promoting specific lineage (Figure 2) [53].

**Figure 2.** Multi-differentiation potential of ASCs.

3.1.1. Osteogenic Differentiation of ASCs

ASCs has potential to differentiate into osteoblasts in presence of limited number of cytokines; which provides opportunity to address bone related disorders within short-time period [120]. Osteogenic medium contains inducing factors, such as dexamethasone, ascorbic acid/ascorbate 2-phosphate, cholecalciferol, and β -glycerophosphates [121–124] in combination with factor, such as transforming growth factor- β (TGF- β), vitamin D3, and bone morphogenetic proteins (BMPs) [125,126]. Recent studies have described role of quercetin, a natural flavonoid, in up-regulating *Osx*, *Runx2*,

BMP-2, *Col-1*, *OPN*, and *OCN* genes, promoting the osteogenesis of mouse and human ASCs [127,128]. Osteogenic differentiation is regulated by transcription factors, such as core binding factor-1 alpha (CBF-1 α), runt-related transcription factor 2 (Runx2), osterix, homeobox protein Hox-B7 (HOXB7), Hoxa2, Hoxa9, core binding factor- β (Cbf- β), olmya enhancer binding protein 2 β (Pebp2 β), Sox9, TNF- α , FOXC2, PPAR γ , YAP, MyoD, BMP9, β -catenin GATA4, and GATA6 [129]. Moreover, two factors HIF-1 α and TWIST have been reported for their inhibitory effect on osteogenic differentiation through their interaction with Runx2. Transforming growth factor- β (TGF- β)/bone morphogenetic proteins (BMPs), Wnt/ β -Catenin, Notch, Hedgehog, and fibroblast growth Factor (FGF), etc. are reported as major signaling pathways in regulating osteogenic potential of ASCs [121]. Dexamethasone activates FHL2/ β -catenin-pathway to induce over-expression of RunX2 and collagen type I alpha 1 (COL1A1); whereas, ascorbic acid promotes the secretion of collagen type I to increase the activation of integrin-mediated signaling and β -glycerophosphate provide phosphate resources to up-regulate the expression of osteogenic gene [130]. Vascular endothelial growth factor A (VEGF-A) plays a crucial role in bone regeneration due to its potential to promote both angiogenesis and osteogenesis in human ASCs [131]. A combined treatment of ASCs with VEGF and BMP-2, -4, -6, and -9 have demonstrated to promote osteogenesis through over-expressing osteogenic alkaline phosphatase gene [132,133]. Moreover, a recent in vitro study reported that BMP2 exert no significant and constant effect in the promotion of osteogenesis [134]. Similarly, no catalyzing effect of BMP2 have been reported on osteogenesis of hASC in presence of ascorbic acid and β -glycerophosphate [135]. On contrary, BMP2 has been reported for its synergistic effect on vitamin D3 in the promotion of osteogenesis of ASCs [136]. Interestingly, hypoxia in addition to promoting angiogenesis [137], has also been reported to enhance osteogenic potential and up-regulate the expression of octamer-binding transcription factor 4 (OCT4), Kruppel-like factor 4 (KLF4) and NANOG [138–140]. However, the inhibitory activity of hypoxia against mineralization and osteogenic potential of ASCs via IGFBP3 up-regulation have also been documented [141]. The hypoxia also inhibit the alkaline phosphatase activity, expression of core binding factor α -1 (CBFA-1), and osteopontin leading to negative regulation of osteogenic potential of ASC [142]. During osteogenic differentiation, the mitochondria get activated to fulfill high energy demands in necessary biochemical reactions [143]. Sirtuin, such as Sirt1 and Sirt 6, also plays a crucial role in osteogenic and chondrogenic potential of MSCs [144,145]. Additionally, bone morphogenetic protein (BMP), a cytokine inducer is promptly used to direct osteogenic differentiation among ASCs [146], and the significance of BMP-2 and BMP-7 have been clinically accepted in Australia, United States, and Europe [147]. BMP-2, BMP-6, and BMP-14 are considered as major factors in osteogenic differentiation of ASCs [148,149]; whereas, BMP-7 promotes both chondrogenesis as well as osteogenesis [150]. The osteogenic potential of ASCs is affected by the concentration of BMP and nature of differentiation medium [151–153]. A notable osteogenesis promoting effect of combined retinoic acid and BMP2 in murine ASCs have also been evidenced [154].

It has also been postulated that BMP alone is insufficient to direct MSCs to differentiate into osteogenic lineage; as it triggers both adipogenesis and osteogenesis at an equal rate [146]. Specifically, BMP signaling pathway activates with binding of ligand to heterodimeric serine/threonine kinase BMP receptor, which triggers the activation of Smad-dependent signaling pathway (Smad1/5/8) and Smad-independent signaling pathway (JNK, p38); mediating both adipo- and osteogenesis [146]. However, the heterodimer of Smad4 with phosphorylated transcription factors Smad1, Smad5, or Smad8 activates the expression of osteogenic promoting genes of ASCs [121]. BMP also regulates expression level of other osteogenic factors, such as core-binding factor-1/Runt-related family 2 (Cbfa1/Runx2) [155]. Besides, the Wnt5a directs osteogenic differentiation through Wnt signaling pathway and suppress PPAR- γ in ASCs [156]. This pathway activates β -Catenin-T-cell factor/lymphoid enhancer factor (TCF)/Lef transcription factors (Lef) which further enhances osteogenesis [121]. A similar behavior of endogenous cytokine, such as tumor necrosis factor-alpha (TNF- α) has also been observed [157], where it mediates its effect through activation of nuclear factor- κ B (NF- κ B) and inhibit PPAR- γ function; TNF- α also promotes expression of TAZ

(transcriptional coactivator with PDZ-binding motif) leading to osteogenic differentiation of ASCs. Beside BMP and Wnt signaling pathway, the notch signaling route has also been reported for its role in osteogenic differentiation of stem cells through sequential release, nuclear transportation, and assembly of Notch intracellular domain (NICD) into nuclear transcription factor, leading to cascade of events regulating the expression of osteogenic genes [158]. Apart from these pathways, accumulation of reactive oxygen species (ROS), an indicator of oxidative stress, have also been reported to suppress the osteogenic potential of MSCs.

3.1.2. Chondrogenic Differentiation of ASCs

Adipose-derived stem cells (ASCs) have been shown to exhibit similar chondrogenic potential as bone-marrow derived stem cells [28,159]. However, recent studies have suggested that inclusion of cytokine, such as BMP-6 and a higher concentration of other growth factors in culture medium, improve the chondrogenic potential of ASCs [160,161]. Furthermore, the presence of ascorbic acid phosphate, dexamethasone, bovine serum albumin, linoleic acid, sodium pyruvate, transferrin, selenous acid, proline, L-glutamine, and TGF- β 1 have also been reported for their chondrogenic promoting activity in vitro [126,129,162,163]. In addition to this, the transcription factors, such as SRY-related high mobility group-box gene 9 (*Sox9*), Zinc-finger protein 145 (ZNF145), HOXD9/10/11/13, FOXO3 A, Wnt 11, and STAT3 play an active role in chondrogenesis [129]. However, some other transcription factors such as HOX2a, Smad3 and YAP down regulate the chondrogenic differentiation potential of MSCs. Scaffold- and pellet-based culture systems provide three-dimensional (3D) support, high culture density, and microenvironment for chondrocytes differentiation, leading to cartilage generation [164,165]. Micropellets are used as high-density culture system (2.5×10^6 cells/pellet) to promote cellular interaction for the development of cartilage like structure [164].

The ASCs tend to grow as a monolayer in vitro and avoid cell-cell contact by growth inhibition. However, excessive cell accumulation, as occurring in high-density micropellets, is a fundamental prerequisite for chondrogenic differentiation. In recent years, three-dimensional (3D) constructs, such as scaffolds, various hydrogels, alginate gels, and matrices, have been developed to mimic the physiological milieu and overcome growth inhibition [12]. Similarly, scaffolds that are covered with different chemotactic agents, as well as matrices of varying stiffness values, have been designed to achieve the directional migration of cell cultures. In 2007, Xu et al. were among the first groups to focus on mechanical properties of chondrocyte differentiation in a 3D mass model [46]. Hydrogels of polymers, such as agar, alginate, and agarose are also used to provide structural support, mechanical stimuli and micro-environment to direct chondrogenesis [166–168]; however, continuous interaction of cells with hydrogels may cause cellular sensation [169]. The Dulbecco's modified Eagle's medium (DMEM) is generally used as basal medium that is used in cell culture, which is supplemented with ascorbate-2-phosphate, insulin, TGF- β 1 and 1% FCS [28]. However, even in absence of FCS, the DMEM when supplemented with TGF- β 3, insulin, transferrin, albumin, dexamethasone, and ascorbic acid promoted chondrogenic differentiation of ASCs [170]. Besides, the effect of oxygen concentration seems confounding; still, its concentration needs to be properly regulated to direct chondrogenesis in ASCs [166]. Molecular techniques, such as real-time PCR, western blot analysis, ELISA, and RNA microarray are used to study the expression of chondrogenic genes, such as *collagen I/II/VI/IX/X*, *COMP*, *HAPLN1*, *SOX 9*, *matrilin 3*, *Indian hedgehog*, *homeobox 7*, *chondroadherin*, *WNT 11*, *aggrecan*, *alkaline phosphatase*, *fibromodulin*, *osteocalcin*, and *PTHrP* during osteogenesis of ASCs [166,167]. Additionally, staining of ECM with alcian blue, toluidine blue, or safranin-o are simple methods to determine the chondrogenic potential of ASCs.

3.1.3. Adipogenic Differentiation of ASCs

Adipogenic potential is considered as an exclusive characteristic to determine the quality of ASCs. The adipogenesis is directed by using differential medium enriched with isobutylmethylxanthine

(IBMX), indomethacin, 3-Isobutyl-1-methylxanthine (IDII), insulin, and dexamethasone at varying concentrations [53,171,172]. During adipogenic differentiation, MSCs are firstly directed to differentiate into preadipocytes and then to adipocytes [173]. The effect of dexamethasone on adipogenic differentiation depends upon factors, such as time and concentration [171]. The prolonged exposure of dexamethasone promotes adipogenesis and curtails osteogenesis in MSCs [174]. At high concentration, insulin behaves like insulin growth factor 1 (IGF-1) and it promotes differentiation and the proliferation of preadipocytes [175]. Hydrocortisone is another glucocorticoid agonist along with dexamethasone which initiate the signal cascade to activate preadipocyte receptors and their differentiation into adipocytes in the presence of insulin [176]. IBMX along with dexamethasone activates protein kinase A (PKA) signaling pathway directing the transcription of PPAR γ , and finally leading to adipogenic differentiation [177,178]. The ASCs differentiation is primarily regulated through receptor tyrosine kinases (RTKs) by Akt and extracellular ERK-1) signaling pathways; in which Akt activity promotes adipogenesis; whereas, ERK-1 negatively regulates adipogenesis [179]. It has been also reported that high cell density and structural support also promotes adipogenic differentiation through paracrine and autocrine actions [180,181]. Similarly, obestatin mediates its adipogenic differentiation via autocrine and paracrine activities [177]. PPAR γ agonist, such as rosiglitazone, troglitazone, pioglitazone thiazolidinediones, or glitazones might also be useful to enhance the adipogenesis in vitro [53,171]. In addition to transcription factors, such as PPAR γ 1, PPAR γ 2, and EBF-1; other factors, such as PRDM16, Twist-1, Dermo-1, COUP-II, Sox2, and Oct4 promote adipogenic differentiation; whereas, GATA2, Foxa1, and HOXC8 downregulate the adipogenesis of MSCs [129]. Furthermore, C/EBP- α , C/EBP- β , and C/EBP- δ regulate the transcription of PPAR γ to modulate adipogenic differentiation of ASCs [182]. Cell culture models have indicated that BMP4, Wnt signaling, cell shape, and density also induce adipogenesis in MSCs [183,184]. On the other hand, though previous studies have reported potential of BMP2 and BMP-7 to form fat cells, the role of BMP in induction of adipogenesis is not well understood, and thus, it is not considered as an integral component of adipogenic differentiation medium [171]. After the cells grown in adipogenic differentiation medium, they are fixed in 10% formalin solution or 70% ethanol to determine their lipid content by staining with dyes, such as Oil Red-O, neutral lipid fluorescent dye, or nuclear fluorescent dye at room temperature [53].

4. ASC Secretome and Its Therapeutic Effect

ASCs regeneration potential and therapeutic values also lies in its secretome, which is rich in extracellular proteins and growth factors (Figure 3) [185].

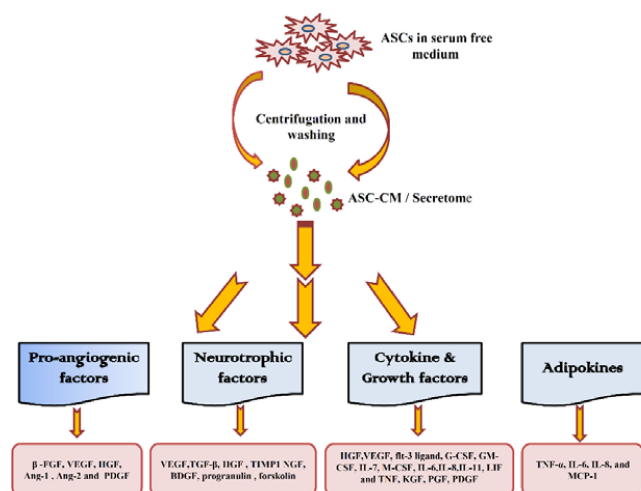


Figure 3. ASC-secretome. The Secretome is highly rich in cytokines, growth factors, angiogenic factors, adipokines and neurotrophic factors, which enables ASCs to regenerate and repair injured/diseased tissues.

This secretome exerts varying beneficial effects through the paracrine activity of ASCs [186]. The pro-angiogenic factors in secretome mainly include PDGF, FGF, VEGF, HGF, angiopoietin (Ang-1 and Ang-2), of which PDGF is present in higher concentration [187,188]. Cell secretome is harvested from the cells that were cultured in serum-free medium for 12 h to 48 h [189]; and their level is determined using techniques, such as two-dimensional (2D) and difference gel electrophoresis, mass spectrometry and ELISA [190]. Other techniques, like stable isotope labeling by amino acid in cell culture (SILAC), isobaric tags for relative and absolute quantitation labeling (iTRAQ), western blot, 2D planar arrays or 3D bead systems have also been employed [191]. Proteins of secretomes are mainly associated with cytoplasm, nucleus, endoplasmic reticulum, and ECM [185]. These proteins assist and regulate cellular metabolic activity, cell signaling, DNA repair, cytoskeletal development, and mitosis. In the mouse model, the secretome of human ASCs conditioned medium (hASCs-CM) restored cytokine balance and reduced the diabetic pain [192]. Another study reported that ASCs-CM enhances the collagen synthesis and migration of dermal fibroblast to improve wrinkling and wound healing in the animal model [193]. The hypoxic condition has also been known to influence the characteristics of stem cells, including their secretome and efficacy. In the interesting reports, hypoxia increased the rate of proliferation of ASCs and accelerated their wound-healing function through the up-regulation of VEGF and bFGF [194,195]. Wang et al. documented that hypoxic condition (5%) increased the differentiation of ASCs toward the smooth muscle phenotype [196]. Hypoxia also augmented the migration potential of ASC by enhancing the expression of stromal cell-derived factor (SDF)-1 [197]. Besides, the other secreted growth factors, like keratinocyte growth factor (KGF), TGF- β 1, HGF, and VEGF of conditioned medium also might play a crucial role in wound healing. Ribeiro et al. revealed an increased neuronal cell density and its metabolic activity by introducing ASCs secretome supplemented with growth factor bFGF and B27 [198]. A recent study has reported that sphingosine-1-phosphate (S1P) and cytokine of ASCs secretome control the inflammation of central nervous system [199]. According to Constantin et al. ASCs secretome containing bFGF, PDGF-AB, and brain-derived growth factor controlled the experimental autoimmune encephalomyelitis (EAE) [200]. Reports have also evidenced that secretion of VEGF, TGF- β , and hepatocyte growth factor (HGF) promote angiogenic and neurogenic responses [185,201]. Further, the released tissue inhibitor of metalloproteinase-1 (TIMP-1) and progranulin provide neuroprotection potential to ASCs [202]. In this concord, IGF-1 and BDNF have been shown to improve the functional recovery in learning and behavior in rat model [203]. The *in vitro* study also indicates that ASCs play a crucial role in tissue regeneration through NGF-induced activation of 5' AMP-activated protein kinase (AMPK) [204]. A recent study has demonstrated that the BDNF upregulated the axonal growth in CNS [205]. Besides, the ASC-CM mitigated the oxidative stress in stressed SH-SY5Y neuron-like cells and restored cell morphology, viability, and electrophysiological activity [206]. This restructuring activity was linked with the presence of antioxidant and growth factors, like BDNF, glial cell line-derived neurotrophic factor, and TGF- β 1. Another study indicated that VEGF-A and VEGF165b derived from ASCs and ADSC-CM were effective in reducing the pain level in oxaliplatin-treated neuropathic rats [207]. It has been reported that mechanical stress enhances the secretion of VEGF, G-CSF, HGF, Leptin, IL-8, PDGF-BB, Angiopoietin-2, human umbilical vein endothelial cell (HUVEC) migration-stimulating factors, and follistatin [208]. Further, the oxidative stress and hypoxia also increased the level of VEGF, IL-8, leptin, angiopoietin-2, and PDGF-BB in cell culture medium.

The cytokines in human ASCs secretome mainly include, angiogenic, hematopoietic, and proinflammatory cytokines, like HGF, VEGF, flt-3 ligand, G-CSF, GM-CSF, IL-7, M-CSF, IL-6, IL-8, IL-11, LIF, and TNF α [209]. ASCs also secrete adipokines such as FGF, ILs, IGF-binding protein, PDGF, TGF- β , TNF- α , and VEGF [210]. However, the adipokines like TNF- α , IL-6, IL-8, and MCP-1 have been reported to promote tumor growth [211]. The role of ASCs in regulating breast cancer is confounding due to varied nature of secreted adipokines, such as CCL5, which enhances the motility of MCF-7 breast cancer cell *in vitro* [212]. In contrast to this, another study reported that high density

ADSC-CM inhibited the MCF-7 [213]. Though the contradictory impact of ASCs and its secretome is wide in literature, it has been proposed that ASCs might only promotes cancer in active breast cancer cells [214]. Wang et al. reported that ASC-CM significantly improved cellular proliferation, regulated apoptosis and cellular senescence in UVB irradiated human dermal fibroblasts (HDFs); which indicates the protective role of secretome against damages that are caused due to aging [215]. Similarly, TGF- β 1-treated ASCs-CM upregulated type I collagen and promoted proliferation and mobility of skin fibroblasts in mice model indicating the role ASCs-CM in wound healing [216]. The ASCs-CM human antimicrobial peptide LL-37 treatment also improved the migration of HDF [217]. The presence of VEGF, bFGF, TGF- β 1, TGF- β 2, HGF, keratinocyte growth factor (KGF), PDGF-AA, placenta growth factor (PGF), type I collagen, fibronectin, and superoxide dismutase (SOD) in ASCs seretome was effective in improving skin texture and wrinkle in micro pig model [218]. In addition, another study evaluated the potential of secretome (concentrated ASCs-CM) in controlling ischemia reperfusion (IR) injury in mice model indicating the potential ASC-secretome in providing therapeutic option for treatment of IR injury [219]. The ASCs-CM has also recovered gastric wound in rat model through promoting angiogenesis and re-epithelization [220].

Along with growth factors and cytokines, ASCs also secrete exosomes of 30–150 nm size [221]; which mediate the signaling effects and mimic the functional characteristic of cells [222,223]. Studies revealed that the exosomes of ASCs (over-expressing Nrf2) have improved wound healing in diabetic foot ulcer rat model [224]. In a seminal report, the exosomes derived from ASC isolated from cancer patient have been partly attributed for their therapeutic effect, indicating that expanded ASC remain unaffected by patient condition [225]. Choi et al. demonstrated that the ASCs-exosomes enriched in micro-RNA improvised the regeneration of human dermal fibroblasts [221]. Similarly, ASCs-exosomes when engulfed by fibroblasts promoted soft tissue repair and cutaneous wound healing [226]. Furthermore, the intravenously administered ASCs-exosome regulated the ratios of collagen type III: type I, TGF- β 3:TGF- β 1 MMP3:TIMP1, fibroblast differentiation, and thereby reduced scar size in the murine model [227]. In a report by Lee et al., ASCs-secreted exosomes demonstrated therapeutic potential against Huntington's disease by considerably reducing the aggregation of mutant Huntingtin protein, mitochondrial dysfunction, and cellular apoptosis in R6/2 mice-derived neuronal cells [228]. These exosomes have also improved the efficacy of anti-cancer drug in mouse model of hepatocellular carcinoma and promoted the migration of breast cancer cell line (MCF7) [229]. Similarly, various studies have documented the therapeutic activities of ASC released exosomes against neurodegenerative and vascular diseases [230].

Based on above body of evidence, the ASCs-derived secretome seems to be a potential agent for the treatment of various disorders.

5. Conclusions

Adipose tissues are considered as most promising and enriched source of ASCs, and the easy harvesting procedure and less ethical complexities, makes ASCs the most appropriate stem cell source in development of regenerative therapeutic approaches. These cells exert their beneficial effect not only through differentiation, but also through the paracrine effect of secretome. However, the extensive studies are needed to understand the nature of secretome of ASC and their specific role in regeneration and repair of damaged/diseased tissues.

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References

1. Mitchell, J.B.; McIntosh, K.; Zvonic, S.; Garrett, S.; Floyd, Z.E.; Kloster, A.; Di Halvorsen, Y.; Storms, R.W.; Goh, B.; Kilroy, G.; et al. Immunophenotype of human adipose-derived cells: Temporal changes in stromal-associated and stem cell-associated markers. *Stem Cells* **2006**, *24*, 376–385. [[CrossRef](#)] [[PubMed](#)]
2. Wankhade, U.D.; Shen, M.; Kolhe, R.; Fulzele, S. Advances in Adipose-Derived Stem Cells Isolation, Characterization, and Application in Regenerative Tissue Engineering. *Stem Cells Int.* **2016**, *2016*, 3206807. [[CrossRef](#)] [[PubMed](#)]
3. Crisan, M.; Yap, S.; Casteilla, L.; Chen, C.-W.; Corselli, M.; Park, T.S.; Andriolo, G.; Sun, B.; Zheng, B.; Zhang, L.; et al. A Perivascular Origin for Mesenchymal Stem Cells in Multiple Human Organs. *Cell Stem Cell* **2008**, *3*, 301–313. [[CrossRef](#)] [[PubMed](#)]
4. Kishi, K.; Imanishi, N.; Ohara, H.; Ninomiya, R.; Okabe, K.; Hattori, N.; Kubota, Y.; Nakajima, H.; Nakajima, T. Distribution of adipose-derived stem cells in adipose tissues from human cadavers. *J. Plast. Reconstr. Aesthet. Surg.* **2010**, *63*, 1717–1722. [[CrossRef](#)] [[PubMed](#)]
5. Baer, P.C.; Geiger, H. Adipose-Derived Mesenchymal Stromal/Stem Cells: Tissue Localization, Characterization, and Heterogeneity. *Stem Cells Int.* **2012**, *2012*, 812693. [[CrossRef](#)] [[PubMed](#)]
6. Zuk, P.A.; Zhu, M.; Ashjian, P.; De Ugarte, D.A.; Huang, J.I.; Mizuno, H.; Alfonso, Z.C.; Fraser, J.K.; Benhaim, P.; Hedrick, M.H. Human Adipose Tissue Is a Source of Multipotent Stem Cells. *Mol. Biol. Cell* **2002**, *13*, 4279–4295. [[CrossRef](#)] [[PubMed](#)]
7. Bajek, A.; Gurtowska, N.; Gackowska, L.; Kubiszewska, I.; Bodnar, M.; Marszałek, A.; Januszewski, R.; Michalkiewicz, J.; Drewa, T. Does the liposuction method influence the phenotypic characteristic of human adipose-derived stem cells? *Biosci. Rep.* **2015**, *35*, e00212. [[CrossRef](#)] [[PubMed](#)]
8. Liu, H.-Y.; Chiou, J.-F.; Wu, A.T.H.; Tsai, C.-Y.; Leu, J.-D.; Ting, L.-L.; Wang, M.-F.; Chen, H.-Y.; Lin, C.-T.; Williams, D.F.; et al. The effect of diminished osteogenic signals on reduced osteoporosis recovery in aged mice and the potential therapeutic use of adipose-derived stem cells. *Biomaterials* **2012**, *33*, 6105–6112. [[CrossRef](#)] [[PubMed](#)]
9. Brzoska, M.; Geiger, H.; Gauer, S.; Baer, P. Epithelial differentiation of human adipose tissue-derived adult stem cells. *Biochem. Biophys. Res. Commun.* **2005**, *330*, 142–150. [[CrossRef](#)] [[PubMed](#)]
10. Gao, S.; Zhao, P.; Lin, C.; Sun, Y.; Wang, Y.; Zhou, Z.; Yang, D.; Wang, X.; Xu, H.; Zhou, F.; et al. Differentiation of human adipose-derived stem cells into neuron-like cells which are compatible with photocurable three-dimensional scaffolds. *Tissue Eng. Part A* **2014**, *20*, 1271–1284. [[CrossRef](#)] [[PubMed](#)]
11. Ma, T.; Sun, J.; Zhao, Z.; Lei, W.; Chen, Y.; Wang, X.; Yang, J.; Shen, Z. A brief review: Adipose-derived stem cells and their therapeutic potential in cardiovascular diseases. *Stem Cell Res. Ther.* **2017**, *8*, 124. [[CrossRef](#)] [[PubMed](#)]
12. Peng, B.-Y.; Dubey, N.K.; Mishra, V.K.; Tsai, F.-C.; Dubey, R.; Deng, W.-P.; Wei, H.-J. Addressing Stem Cell Therapeutic Approaches in Pathobiology of Diabetes and Its Complications. *J. Diabetes Res.* **2018**, *2018*, 7806435. [[CrossRef](#)]
13. Dubey, N.K.; Mishra, V.K.; Dubey, R.; Syed-Abdul, S.; Wang, J.R.; Wang, P.D.; Deng, W.-P. Combating Osteoarthritis through Stem Cell Therapies by Rejuvenating Cartilage: A Review. *Stem Cells Int.* **2018**, *2018*, 5421019. [[CrossRef](#)] [[PubMed](#)]
14. Erba, P.; Terenghi, G.; Kingham, P.J. Neural differentiation and therapeutic potential of adipose tissue derived stem cells. *Curr. Stem Cell Res. Ther.* **2010**, *5*, 153–160. [[CrossRef](#)] [[PubMed](#)]
15. Huang, T.; He, D.; Kleiner, G.; Kuluz, J. Neuron-like Differentiation of Adipose-Derived Stem Cells From Infant Piglets In Vitro. *J. Spinal Cord Med.* **2007**, *30* (Suppl. 1), S35–S40. [[CrossRef](#)] [[PubMed](#)]
16. Kim, J.-H.; Jung, M.; Kim, H.-S.; Kim, Y.-M.; Choi, E.-H. Adipose-derived stem cells as a new therapeutic modality for ageing skin. *Exp. Dermatol.* **2011**, *20*, 383–387. [[CrossRef](#)] [[PubMed](#)]
17. Nakagami, H.; Maeda, K.; Morishita, R.; Iguchi, S.; Nishikawa, T.; Takami, Y.; Kikuchi, Y.; Saito, Y.; Tamai, K.; Ogihara, T.; et al. Novel Autologous Cell Therapy in Ischemic Limb Disease Through Growth Factor Secretion by Cultured Adipose Tissue-Derived Stromal Cells. *Arterioscler. Thromb. Vasc. Biol.* **2005**, *25*, 2542–2547. [[CrossRef](#)] [[PubMed](#)]
18. Zeppieri, M.; Salvatat, M.; Beltrami, A.; Cesselli, D.; Russo, R.; Alcalde, I.; Merayo-Llodes, J.; Brusini, P.; Parodi, P. Adipose Derived Stem Cells for Corneal Wound Healing after Laser Induced Corneal Lesions in Mice. *J. Clin. Med.* **2017**, *6*, 115. [[CrossRef](#)] [[PubMed](#)]

19. Chen, Y.-W.; Scutaru, T.T.; Ghetu, N.; Carasevici, E.; Lupascu, C.D.; Ferariu, D.; Pieptu, D.; Coman, C.-G.; Danciu, M. The Effects of Adipose-Derived Stem Cell-Differentiated Adipocytes on Skin Burn Wound Healing in Rats. *J. Burn Care Res.* **2017**, *38*, 1–10. [[CrossRef](#)] [[PubMed](#)]
20. Kim, W.-S.; Park, B.-S.; Sung, J.-H. The wound-healing and antioxidant effects of adipose-derived stem cells. *Expert Opin. Biol. Ther.* **2009**, *9*, 879–887. [[CrossRef](#)] [[PubMed](#)]
21. Frese, L.; Dijkman, P.E.; Hoerstrup, S.P. Adipose Tissue-Derived Stem Cells in Regenerative Medicine. *Transfus. Med. Hemother.* **2016**, *43*, 268–274. [[CrossRef](#)] [[PubMed](#)]
22. Zhu, Y.; Liu, T.; Song, K.; Fan, X.; Ma, X.; Cui, Z. Adipose-derived stem cell: A better stem cell than BMSC. *Cell Biochem. Funct.* **2008**, *26*, 664–675. [[CrossRef](#)] [[PubMed](#)]
23. Casteilla, L.; Planat-Benard, V.; Laharrague, P.; Cousin, B. Adipose-derived stromal cells: Their identity and uses in clinical trials, an update. *World J. Stem Cells* **2011**, *3*, 25–33. [[CrossRef](#)] [[PubMed](#)]
24. Tarte, K.; Gaillard, J.; Lataillade, J.-J.; Fouillard, L.; Becker, M.; Mossafa, H.; Tchirkov, A.; Rouard, H.; Henry, C.; Springard, M.; et al. Clinical-grade production of human mesenchymal stromal cells: Occurrence of aneuploidy without transformation. *Blood* **2010**, *115*, 1549–1553. [[CrossRef](#)] [[PubMed](#)]
25. Jurgens, W.J.; Oedayrajsingh-Varma, M.J.; Helder, M.N.; Zandiehoulabi, B.; Schouten, T.E.; Kuik, D.J.; Ritt, M.J.; van Milligen, F.J. Effect of tissue-harvesting site on yield of stem cells derived from adipose tissue: Implications for cell-based therapies. *Cell Tissue Res.* **2008**, *332*, 415–426. [[CrossRef](#)] [[PubMed](#)]
26. Schäffler, A.; Büchler, C. Concise Review: Adipose Tissue-Derived Stromal Cells—Basic and Clinical Implications for Novel Cell-Based Therapies. *Stem Cells* **2007**, *25*, 818–827. [[CrossRef](#)] [[PubMed](#)]
27. Tobita, M.; Tajima, S.; Mizuno, H. Adipose tissue-derived mesenchymal stem cells and platelet-rich plasma: Stem cell transplantation methods that enhance stemness. *Stem Cell Res. Ther.* **2015**, *6*, 215. [[CrossRef](#)] [[PubMed](#)]
28. Zuk, P.A.; Zhu, M.; Mizuno, H.; Huang, J.; Futrell, J.W.; Katz, A.J.; Benhaim, P.; Lorenz, H.P.; Hedrick, M.H. Multilineage cells from human adipose tissue: Implications for cell-based therapies. *Tissue Eng.* **2001**, *7*, 211–228. [[CrossRef](#)] [[PubMed](#)]
29. Zuk, P.A. The Adipose-derived Stem Cell: Looking Back and Looking Ahead. *Mol. Biol. Cell* **2010**, *21*, 1783–1787. [[CrossRef](#)] [[PubMed](#)]
30. Lin, C.S.; Xin, Z.C.; Deng, C.H.; Ning, H.; Lin, G.; Lue, T.F. Defining adipose tissue-derived stem cells in tissue and in culture. *Histol. Histopathol.* **2010**, *25*, 807–815. [[PubMed](#)]
31. Lafontan, M. Historical perspectives in fat cell biology: The fat cell as a model for the investigation of hormonal and metabolic pathways. *Am. J. Physiol. Cell Physiol.* **2012**, *302*, C327–C359. [[CrossRef](#)] [[PubMed](#)]
32. Hsu, V.M.; Stransky, C.A.; Bucky, L.P.; Percec, I. Fat Grafting's Past, Present, and Future: Why Adipose Tissue Is Emerging as a Critical Link to the Advancement of Regenerative Medicine. *Aesthet. Surg. J.* **2012**, *32*, 892–899. [[CrossRef](#)] [[PubMed](#)]
33. Laharrague, P.; Casteilla, L. The emergence of adipocytes. *Endocr. Dev.* **2010**, *19*, 21–30. [[PubMed](#)]
34. Bai, X.; Alt, E. Myocardial regeneration potential of adipose tissue-derived stem cells. *Biochem. Biophys. Res. Commun.* **2010**, *401*, 321–326. [[CrossRef](#)] [[PubMed](#)]
35. Vaquero, A.; Sternglanz, R.; Reinberg, D. NAD⁺-dependent deacetylation of H4 lysine 16 by class III HDACs. *Oncogene* **2007**, *26*, 5505–5520. [[CrossRef](#)] [[PubMed](#)]
36. Casteilla, L.; Dani, C. Adipose tissue-derived cells: From physiology to regenerative medicine. *Diabetes Metab.* **2006**, *32 Pt 1*, 393–401. [[CrossRef](#)]
37. Himms-Hagen, J. Brown adipose tissue thermogenesis: Interdisciplinary studies. *FASEB J.* **1990**, *4*, 2890–2898. [[CrossRef](#)] [[PubMed](#)]
38. Ailhaud, G.; Grimaldi, P.; Negrel, R. Cellular and molecular aspects of adipose tissue development. *Annu. Rev. Nutr.* **1992**, *12*, 207–233. [[CrossRef](#)] [[PubMed](#)]
39. Gimble, J.M.; Robinson, C.E.; Wu, X.; Kelly, K.A. The function of adipocytes in the bone marrow stroma: An update. *Bone* **1996**, *19*, 421–428. [[CrossRef](#)]
40. Klaus, S.; Casteilla, L.; Bouillaud, F.; Ricquier, D. The uncoupling protein UCP: A membraneous mitochondrial ion carrier exclusively expressed in brown adipose tissue. *Int. J. Biochem.* **1991**, *23*, 791–801. [[CrossRef](#)]
41. Cannon, B.; Nedergaard, J. Brown adipose tissue: Function and physiological significance. *Physiol. Rev.* **2004**, *84*, 277–359. [[CrossRef](#)] [[PubMed](#)]

42. Casteilla, L.; Penicaud, L.; Cousin, B.; Calise, D. Choosing an adipose tissue depot for sampling: Factors in selection and depot specificity. *Methods Mol. Biol.* **2008**, *456*, 23–38. [[PubMed](#)]
43. Bjorntorp, P.; Bengtsson, C.; Blohme, G.; Jonsson, A.; Sjoström, L.; Tibblin, E.; Tibblin, G.; Wilhelmsen, L. Adipose tissue fat cell size and number in relation to metabolism in randomly selected middle-aged men and women. *Metabolism* **1971**, *20*, 927–935. [[CrossRef](#)]
44. Salans, L.B.; Cushman, S.W.; Weismann, R.E. Studies of Human Adipose Tissue Adipose Cell Size and Number in Nonobese and Obese Patients. *J. Clin. Investig.* **1973**, *52*, 929–941. [[CrossRef](#)] [[PubMed](#)]
45. Berry, D.C.; Stenesen, D.; Zeve, D.; Graff, J.M. The developmental origins of adipose tissue. *Development* **2013**, *140*, 3939–3949. [[CrossRef](#)] [[PubMed](#)]
46. Kajimura, S.; Seale, P.; Spiegelman, B.M. Transcriptional Control of Brown Fat Development. *Cell Metab.* **2010**, *11*, 257–262. [[CrossRef](#)] [[PubMed](#)]
47. Iyyanki, T.; Hubenak, J.; Liu, J.; Chang, E.I.; Beahm, E.K.; Zhang, Q. Harvesting Technique Affects Adipose-Derived Stem Cell Yield. *Aesthet. Surg. J.* **2015**, *35*, 467–476. [[CrossRef](#)] [[PubMed](#)]
48. Kornicka, K.; Marycz, K.; Tomaszewski, K.A.; Maređziak, M.; Śmieszek, A. The Effect of Age on Osteogenic and Adipogenic Differentiation Potential of Human Adipose Derived Stromal Stem Cells (hASCs) and the Impact of Stress Factors in the Course of the Differentiation Process. *Oxid. Med. Cell. Longev.* **2015**, *2015*, 309169. [[CrossRef](#)] [[PubMed](#)]
49. Dai, R.; Wang, Z.; Samanipour, R.; Koo, K.-I.; Kim, K. Adipose-Derived Stem Cells for Tissue Engineering and Regenerative Medicine Applications. *Stem Cells Int.* **2016**, *2016*, 6737345. [[CrossRef](#)] [[PubMed](#)]
50. Nedergaard, J.; Bengtsson, T.; Cannon, B. Unexpected evidence for active brown adipose tissue in adult humans. *Am. J. Physiol. Endocrinol. Metab.* **2007**, *293*, E444–E452. [[CrossRef](#)] [[PubMed](#)]
51. Gesta, S.; Tseng, Y.-H.; Kahn, C.R. Developmental Origin of Fat: Tracking Obesity to Its Source. *Cell* **2007**, *131*, 242–256. [[CrossRef](#)] [[PubMed](#)]
52. Kocan, B.; Maziarz, A.; Tabarkiewicz, J.; Ochiya, T.; Banaś-Ząbczyk, A. Trophic Activity and Phenotype of Adipose Tissue-Derived Mesenchymal Stem Cells as a Background of Their Regenerative Potential. *Stem Cells Int.* **2017**, *2017*, 1653254. [[CrossRef](#)] [[PubMed](#)]
53. Bunnell, B.A.; Flaate, M.; Gagliardi, C.; Patel, B.; Ripoll, C. Adipose-derived stem cells: Isolation, expansion and differentiation. *Methods* **2008**, *45*, 115–120. [[CrossRef](#)] [[PubMed](#)]
54. Fraser, J.K.; Wulur, I.; Alfonso, Z.; Hedrick, M.H. Fat tissue: An underappreciated source of stem cells for biotechnology. *Trends in Biotechnol.* **2006**, *24*, 150–154. [[CrossRef](#)] [[PubMed](#)]
55. Hauner, H.; Entenmann, G. Regional variation of adipose differentiation in cultured stromal-vascular cells from the abdominal and femoral adipose tissue of obese women. *Int. J. Obes.* **1991**, *15*, 121–126. [[PubMed](#)]
56. Di Taranto, G.; Cicione, C.; Visconti, G.; Isgrò, M.A.; Barba, M.; Di Stasio, E.; Stigliano, E.; Bernardini, C.; Michetti, F.; Salgarello, M.; et al. Qualitative and quantitative differences of adipose-derived stromal cells from superficial and deep subcutaneous lipoaspirates: A matter of fat. *Cytotherapy* **2015**, *17*, 1076–1089. [[CrossRef](#)] [[PubMed](#)]
57. Tsekouras, A.; Mantas, D.; Tsilimigras, I.D.; Moris, D.; Kontos, M.; Zografos, C.G. Comparison of the Viability and Yield of Adipose-Derived Stem Cells (ASCs) from Different Donor Areas. *In Vivo* **2017**, *31*, 1229–1234. [[PubMed](#)]
58. Rezai Rad, M.; Bohlooli, M.; Akhavan Rahnema, M.; Anbarlou, A.; Nazeman, P.; Khojasteh, A. Impact of Tissue Harvesting Sites on the Cellular Behaviors of Adipose-Derived Stem Cells: Implication for Bone Tissue Engineering. *Stem Cells Int.* **2017**, *2017*, 2156478. [[CrossRef](#)] [[PubMed](#)]
59. Oedayrajsingh-Varma, M.J.; van Ham, S.M.; Knippenberg, M.; Helder, M.N.; Klein-Nulend, J.; Schouten, T.E.; Ritt, M.J.; van Milligen, F.J. Adipose tissue-derived mesenchymal stem cell yield and growth characteristics are affected by the tissue-harvesting procedure. *Cytotherapy* **2006**, *8*, 166–177. [[CrossRef](#)] [[PubMed](#)]
60. Van Vollenstee, F.A.; Hoffmann, D.; Pepper, M.S. Harvesting and Collection of Adipose Tissue for the Isolation of Adipose-Derived Stromal/Stem Cells. In *Stem Cell Processing*; Pham, P.V., Ed.; Springer International Publishing: Cham, Switzerland, 2016; pp. 199–220.
61. Beane, O.S.; Fonseca, V.C.; Cooper, L.L.; Koren, G.; Darling, E.M. Impact of Aging on the Regenerative Properties of Bone Marrow-, Muscle-, and Adipose-Derived Mesenchymal Stem/Stromal Cells. *PLoS ONE* **2014**, *9*, e115963. [[CrossRef](#)] [[PubMed](#)]

62. Varghese, J.; Griffin, M.; Mosahebi, A.; Butler, P. Systematic review of patient factors affecting adipose stem cell viability and function: Implications for regenerative therapy. *Stem Cell Res. Ther.* **2017**, *8*, 45. [[CrossRef](#)] [[PubMed](#)]
63. Ye, X.; Liao, C.; Liu, G.; Xu, Y.; Tan, J.; Song, Z. Age-Related Changes in the Regenerative Potential of Adipose-Derived Stem Cells Isolated from the Prominent Fat Pads in Human Lower Eyelids. *PLoS ONE* **2016**, *11*, e0166590. [[CrossRef](#)] [[PubMed](#)]
64. Wu, W.; Niklason, L.; Steinbacher, D.M. The effect of age on human adipose-derived stem cells. *Plast. Reconstr. Surg.* **2013**, *131*, 27–37. [[CrossRef](#)] [[PubMed](#)]
65. Dufrane, D. Impact of Age on Human Adipose Stem Cells for Bone Tissue Engineering. *Cell Transplant.* **2017**, *26*, 1496–1504. [[CrossRef](#)] [[PubMed](#)]
66. Lee, J.; Lee, K.S.; Kim, C.-L.; Byeon, J.S.; Gu, N.-Y.; Cho, I.-S.; Cha, S.-H. Effect of donor age on the proliferation and multipotency of canine adipose-derived mesenchymal stem cells. *J. Vet. Sci.* **2017**, *18*, 141–148. [[CrossRef](#)] [[PubMed](#)]
67. Schreml, S.; Babilas, P.; Fruth, S.; Orsó, E.; Schmitz, G.; Mueller, M.B.; Nerlich, M.; Prantl, L. Harvesting human adipose tissue-derived adult stem cells: Resection versus liposuction. *Cytotherapy* **2009**, *11*, 947–957. [[CrossRef](#)] [[PubMed](#)]
68. Gnanasegaran, N.; Govindasamy, V.; Musa, S.; Kasim, N.H.A. Different Isolation Methods Alter the Gene Expression Profiling of Adipose Derived Stem Cells. *Int. J. Med. Sci.* **2014**, *11*, 391–403. [[CrossRef](#)] [[PubMed](#)]
69. Dominici, M.; Le Blanc, K.; Mueller, I.; Slaper-Cortenbach, I.; Marini, F.; Krause, D.; Deans, R.; Keating, A.; Prockop, D.; Horwitz, E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* **2006**, *8*, 315–317. [[CrossRef](#)] [[PubMed](#)]
70. Bellini, E.; Grieco, M.P.; Rapisio, E. A journey through liposuction and liposculpture: Review. *Ann. Med. Surg.* **2017**, *24*, 53–60. [[CrossRef](#)] [[PubMed](#)]
71. Katz, A.J.; Llull, R.; Hedrick, M.H.; Futrell, J.W. Emerging approaches to the tissue engineering of fat. *Clin. Plast. Surg.* **1999**, *26*, 587–603. [[PubMed](#)]
72. Agostini, T.; Lazzeri, D.; Pini, A.; Marino, G.; Li Quattrini, A.; Bani, D.; Dini, M. Wet and Dry Techniques for Structural Fat Graft Harvesting: Histomorphometric and Cell Viability Assessments of Lipoaspirated Samples. *Plast. Reconstr. Surg.* **2012**, *130*, 331e–339e. [[CrossRef](#)] [[PubMed](#)]
73. Klein, J.A. The Tumescence Technique for Lipo-Suction Surgery. *Am. J. Cosmet. Surg.* **1987**, *4*, 263–267. [[CrossRef](#)]
74. Simonacci, F.; Bertozzi, N.; Grieco, M.P.; Grignaffini, E.; Rapisio, E. Procedure, applications, and outcomes of autologous fat grafting. *Ann. Med. Surg.* **2017**, *20*, 49–60. [[CrossRef](#)] [[PubMed](#)]
75. Illouz, Y. Une nouvelle technique pour les lipodystrophies localisées. *Rev. Chir. Esthet. Fr.* **1980**, *4*, 19.
76. Kakagia, D.; Pallua, N. Autologous fat grafting: In search of the optimal technique. *Surg. Innov.* **2014**, *21*, 327–336. [[CrossRef](#)] [[PubMed](#)]
77. Pu, L.L.; Coleman, S.R.; Cui, X.; Ferguson, R.E., Jr.; Vasconez, H.C. Autologous fat grafts harvested and refined by the Coleman technique: A comparative study. *Plast. Reconstr. Surg.* **2008**, *122*, 932–937. [[CrossRef](#)] [[PubMed](#)]
78. Ozsoy, Z.; Kul, Z.; Bilir, A. The role of cannula diameter in improved adipocyte viability: A quantitative analysis. *Aesthet. Surg. J.* **2006**, *26*, 287–289. [[CrossRef](#)] [[PubMed](#)]
79. Erdim, M.; Tezel, E.; Numanoglu, A.; Sav, A. The effects of the size of liposuction cannula on adipocyte survival and the optimum temperature for fat graft storage: An experimental study. *J. Plast. Reconstr. Aesthet. Surg.* **2009**, *62*, 1210–1214. [[CrossRef](#)] [[PubMed](#)]
80. Gimble, J.M.; Katz, A.J.; Bunnell, B.A. Adipose-Derived Stem Cells for Regenerative Medicine. *Circ. Res.* **2007**, *100*, 1249–1260. [[CrossRef](#)] [[PubMed](#)]
81. Campbell, G.L.; Laudenslager, N.; Newman, J. The Effect of Mechanical Stress on Adipocyte Morphology and Metabolism. *Am. J. Cosmet. Surg.* **1987**, *4*, 89–94. [[CrossRef](#)]
82. Alharbi, Z.; Oplander, C.; Almakadi, S.; Fritz, A.; Vogt, M.; Pallua, N. Conventional vs. micro-fat harvesting: How fat harvesting technique affects tissue-engineering approaches using adipose tissue-derived stem/stromal cells. *J. Plast. Reconstr. Aesthet. Surg.* **2013**, *66*, 1271–1278. [[CrossRef](#)] [[PubMed](#)]

83. Nguyen, P.S.; Desouches, C.; Gay, A.M.; Hautier, A.; Magalon, G. Development of micro-injection as an innovative autologous fat graft technique: The use of adipose tissue as dermal filler. *J. Plast. Reconstr. Aesthet. Surg.* **2012**, *65*, 1692–1699. [[CrossRef](#)] [[PubMed](#)]
84. Zocchi, M. Ultrasonic liposculpturing. *Aesthet. Plast. Surg.* **1992**, *16*, 287–298. [[CrossRef](#)]
85. Apfelberg, D.B.; Rosenthal, S.; Hunstad, J.P.; Achauer, B.; Fodor, P.B. Progress report on multicenter study of laser-assisted liposuction. *Aesthet. Plast. Surg.* **1994**, *18*, 259–264. [[CrossRef](#)]
86. Apfelberg, D. Laser-assisted liposuction may benefit surgeons, patients. *Clin. Laser Mon.* **1992**, *10*, 193–194. [[PubMed](#)]
87. Coleman, S.R. Hand rejuvenation with structural fat grafting. *Plast. Reconstr. Surg.* **2002**, *110*, 1731–1744. [[CrossRef](#)] [[PubMed](#)]
88. Rodbell, M. Metabolism of isolated fat cells. II. The similar effects of phospholipase C (*Clostridium perfringens* alpha toxin) and of insulin on glucose and amino acid metabolism. *J. Biol. Chem.* **1966**, *241*, 130–139. [[PubMed](#)]
89. Rodbell, M. The metabolism of isolated fat cells. IV. Regulation of release of protein by lipolytic hormones and insulin. *J. Biol. Chem.* **1966**, *241*, 3909–3917. [[PubMed](#)]
90. Rodbell, M.; Jones, A.B. Metabolism of isolated fat cells. 3. The similar inhibitory action of phospholipase C (*Clostridium perfringens* alpha toxin) and of insulin on lipolysis stimulated by lipolytic hormones and theophylline. *J. Biol. Chem.* **1966**, *241*, 140–142. [[PubMed](#)]
91. Van, R.L.; Bayliss, C.E.; Roncari, D.A. Cytological and enzymological characterization of adult human adipocyte precursors in culture. *J. Clin. Investig.* **1976**, *58*, 699–704. [[CrossRef](#)] [[PubMed](#)]
92. Bjorntorp, P.; Karlsson, M.; Pertoft, H.; Pettersson, P.; Sjostrom, L.; Smith, U. Isolation and characterization of cells from rat adipose tissue developing into adipocytes. *J. Lipid Res.* **1978**, *19*, 316–324. [[PubMed](#)]
93. Deslex, S.; Negrel, R.; Vannier, C.; Etienne, J.; Ailhaud, G. Differentiation of human adipocyte precursors in a chemically defined serum-free medium. *Int. J. Obes.* **1987**, *11*, 19–27. [[PubMed](#)]
94. Hauner, H.; Wabitsch, M.; Pfeiffer, E.F. Differentiation of adipocyte precursor cells from obese and nonobese adult women and from different adipose tissue sites. *Horm. Metab. Res. Suppl.* **1988**, *19*, 35–39. [[PubMed](#)]
95. Hauner, H.; Entenmann, G.; Wabitsch, M.; Gaillard, D.; Ailhaud, G.; Negrel, R.; Pfeiffer, E.F. Promoting effect of glucocorticoids on the differentiation of human adipocyte precursor cells cultured in a chemically defined medium. *J. Clin. Investig.* **1989**, *84*, 1663–1670. [[CrossRef](#)] [[PubMed](#)]
96. Kurita, M.; Matsumoto, D.; Shigeura, T.; Sato, K.; Gonda, K.; Harii, K.; Yoshimura, K. Influences of Centrifugation on Cells and Tissues in Liposuction Aspirates: Optimized Centrifugation for Lipotransfer and Cell Isolation. *Plast. Reconstr. Surg.* **2008**, *121*, 1033–1041. [[CrossRef](#)] [[PubMed](#)]
97. Zhu, M.; Heydarkhan-Hagvall, S.; Hedrick, M.; Benhaim, P.; Zuk, P. Manual Isolation of Adipose-derived Stem Cells from Human Lipoaspirates. *J. Vis. Exp.* **2013**, e50585. [[CrossRef](#)] [[PubMed](#)]
98. Schneider, S.; Unger, M.; van Griensven, M.; Balmayor, E.R. Adipose-derived mesenchymal stem cells from liposuction and resected fat are feasible sources for regenerative medicine. *Eur. J. Med. Res.* **2017**, *22*, 17. [[CrossRef](#)] [[PubMed](#)]
99. Markarian, C.F.; Frey, G.Z.; Silveira, M.D.; Milani, A.R.; Ely, P.B.; Horn, A.P.; Nardi, N.B.; Camassola, M. Isolation of adipose-derived stem cells: A comparison among different methods. *Biotechnol. Lett.* **2014**, *36*, 693–702. [[CrossRef](#)] [[PubMed](#)]
100. Carvalho, P.P.; Gimble, J.M.; Dias, I.R.; Gomes, M.E.; Reis, R.L. Xenofree enzymatic products for the isolation of human adipose-derived stromal/stem cells. *Tissue Eng. Part C Methods* **2013**, *19*, 473–478. [[CrossRef](#)] [[PubMed](#)]
101. Kirkpatrick, C.; Melzner, I.; Göller, T. Comparative effects of trypsin, collagenase and mechanical harvesting on cell membrane lipids studied in monolayer-cultured endothelial cells and a green monkey kidney cell line. *Biochim. Biophys. Acta-Mol. Cell Res.* **1985**, *846*, 120–126. [[CrossRef](#)]
102. Stadler, G.; Hennerbichler, S.; Lindenmair, A.; Peterbauer, A.; Hofer, K.; Van Griensven, M.; Gabriel, C.; Redl, H.; Wolbank, S. Phenotypic shift of human amniotic epithelial cells in culture is associated with reduced osteogenic differentiation in vitro. *Cytotherapy* **2008**, *10*, 743–752. [[CrossRef](#)] [[PubMed](#)]
103. Bellei, B.; Migliano, E.; Tedesco, M.; Caputo, S.; Picardo, M. Maximizing non-enzymatic methods for harvesting adipose-derived stem from lipoaspirate: Technical considerations and clinical implications for regenerative surgery. *Sci. Rep.* **2017**, *7*, 10015. [[CrossRef](#)] [[PubMed](#)]

104. Hélène, B.; Cécile, D.B.; Frédéric, U.; Mehdi, N.; Karlien, P.; Gordana, R.; Nathalie, M.; Dominique, B.; Laurence, L. Isolation of Adipose-Derived Stromal Cells Without Enzymatic Treatment: Expansion, Phenotypical, and Functional Characterization. *Stem Cells Dev.* **2014**, *23*, 2390–2400.
105. Shah, F.S.; Wu, X.; Dietrich, M.; Rood, J.; Gimble, J.M. A non-enzymatic method for isolating human adipose tissue-derived stromal stem cells. *Cytotherapy* **2013**, *15*, 979–985. [[CrossRef](#)] [[PubMed](#)]
106. Ghorbani, A.; Jalali, S.A.; Varedi, M. Isolation of adipose tissue mesenchymal stem cells without tissue destruction: A non-enzymatic method. *Tissue Cell* **2014**, *46*, 54–58. [[CrossRef](#)] [[PubMed](#)]
107. Oberbauer, E.; Steffenhagen, C.; Wurzer, C.; Gabriel, C.; Redl, H.; Wolbank, S. Enzymatic and non-enzymatic isolation systems for adipose tissue-derived cells: Current state of the art. *Cell Regen.* **2015**, *4*, 7. [[CrossRef](#)] [[PubMed](#)]
108. Pamphilon, D.; Selogie, E.; McKenna, D.; Cancelas-Peres, J.; Szczepiorkowski, Z.M.; Sacher, R.; McMannis, J.; Eichler, H.; Garritsen, H.; Takanashi, M.; et al. Current practices and Prospects for Standardization of the Hematopoietic Colony-Forming-Unit (CFU) assay: A Report by the Cellular Therapy Team of the Biomedical Excellence for Safer Transfusion (BEST) Collaborative. *Cytotherapy* **2013**, *15*, 255–262. [[CrossRef](#)] [[PubMed](#)]
109. Sarma, N.J.; Takeda, A.; Yaseen, N.R. Colony Forming Cell (CFC) Assay for Human Hematopoietic Cells. *J. Vis. Exp.* **2010**, 2195. [[CrossRef](#)] [[PubMed](#)]
110. Friedenstein, A.J.; Chailakhyan, R.K.; Latsinik, N.V.; Panasyuk, A.F.; Keiliss-Borok, I.V. Stromal cells responsible for transferring the microenvironment of the hemopoietic tissues. Cloning in vitro and retransplantation in vivo. *Transplantation* **1974**, *17*, 331–340. [[CrossRef](#)] [[PubMed](#)]
111. Pochampally, R. Colony forming unit assays for MSCs. *Methods Mol. Biol.* **2008**, *449*, 83–91. [[PubMed](#)]
112. Javazon, E.H.; Colter, D.C.; Schwarz, E.J.; Prockop, D.J. Rat marrow stromal cells are more sensitive to plating density and expand more rapidly from single-cell-derived colonies than human marrow stromal cells. *Stem Cells* **2001**, *19*, 219–225. [[CrossRef](#)] [[PubMed](#)]
113. Peister, A.; Mellad, J.A.; Larson, B.L.; Hall, B.M.; Gibson, L.F.; Prockop, D.J. Adult stem cells from bone marrow (MSCs) isolated from different strains of inbred mice vary in surface epitopes, rates of proliferation, and differentiation potential. *Blood* **2004**, *103*, 1662–1668. [[CrossRef](#)] [[PubMed](#)]
114. Rodeheffer, M.S.; Birsoy, K.; Friedman, J.M. Identification of white adipocyte progenitor cells in vivo. *Cell* **2008**, *135*, 240–249. [[CrossRef](#)] [[PubMed](#)]
115. Lin, G.; Garcia, M.; Ning, H.; Banie, L.; Guo, Y.-L.; Lue, T.F.; Lin, C.-S. Defining Stem and Progenitor Cells within Adipose Tissue. *Stem Cells Dev.* **2008**, *17*, 1053–1063. [[CrossRef](#)] [[PubMed](#)]
116. Gaiba, S.; França, L.P.D.; França, J.P.D.; Ferreira, L.M. Characterization of human adipose-derived stem cells. *Acta Cir. Bras.* **2012**, *27*, 471–476. [[CrossRef](#)] [[PubMed](#)]
117. Mildmay-White, A.; Khan, W. Cell Surface Markers on Adipose-Derived Stem Cells: A Systematic Review. *Curr. Stem Cell Res. Ther.* **2017**, *12*, 484–492. [[CrossRef](#)] [[PubMed](#)]
118. Lin, C.-S.; Ning, H.; Lin, G.; Lue, T.F. Is CD34 Truly a Negative Marker for Mesenchymal Stem Cells? *Cytotherapy* **2012**, *14*. [[CrossRef](#)] [[PubMed](#)]
119. Strem, B.M.; Hicok, K.C.; Zhu, M.; Wulur, I.; Alfonso, Z.; Schreiber, R.E.; Fraser, J.K.; Hedrick, M.H. Multipotential differentiation of adipose tissue-derived stem cells. *Keio J. Med.* **2005**, *54*, 132–141. [[CrossRef](#)] [[PubMed](#)]
120. Cowan, C.M.; Shi, Y.-Y.; Aalami, O.O.; Chou, Y.-F.; Mari, C.; Thomas, R.; Quarto, N.; Contag, C.H.; Wu, B.; Longaker, M.T. Adipose-derived adult stromal cells heal critical-size mouse calvarial defects. *Nat. Biotechnol.* **2004**, *22*, 560. [[CrossRef](#)] [[PubMed](#)]
121. Grottkau, B.E.; Lin, Y. Osteogenesis of Adipose-Derived Stem Cells. *Bone Res.* **2013**, *1*, 133. [[CrossRef](#)] [[PubMed](#)]
122. De Girolamo, L.; Sartori, M.F.; Albisetti, W.; Brini, A.T. Osteogenic differentiation of human adipose-derived stem cells: Comparison of two different inductive media. *J. Tissue Eng. Regen. Med.* **2007**, *1*, 154–157. [[CrossRef](#)] [[PubMed](#)]
123. De Girolamo, L.; Sartori, M.F.; Arrigoni, E.; Rimondini, L.; Albisetti, W.; Weinstein, R.L.; Brini, A.T. Human adipose-derived stem cells as future tools in tissue regeneration: Osteogenic differentiation and cell-scaffold interaction. *Int. J. Artif. Organs* **2008**, *31*, 467–479. [[CrossRef](#)] [[PubMed](#)]
124. Arrigoni, E.; Lopa, S.; de Girolamo, L.; Stanco, D.; Brini, A.T. Isolation, characterization and osteogenic differentiation of adipose-derived stem cells: From small to large animal models. *Cell Tissue Res.* **2009**, *338*, 401. [[CrossRef](#)] [[PubMed](#)]

125. Friedenstein, A.J.; Chailakhyan, R.K.; Gerasimov, U.V. Bone marrow osteogenic stem cells: In vitro cultivation and transplantation in diffusion chambers. *Cell Prolif.* **1987**, *20*, 263–272. [[CrossRef](#)]
126. Okamoto, T.; Aoyama, T.; Nakayama, T.; Nakamata, T.; Hosaka, T.; Nishijo, K.; Nakamura, T.; Kiyono, T.; Toguchida, J. Clonal heterogeneity in differentiation potential of immortalized human mesenchymal stem cells. *Biochem. Biophys. Res. Commun.* **2002**, *295*, 354–361. [[CrossRef](#)]
127. Zhou, C.; Lin, Y. Osteogenic differentiation of adipose-derived stem cells promoted by quercetin. *Cell Prolif.* **2014**, *47*, 124–132. [[CrossRef](#)] [[PubMed](#)]
128. Kim, Y.J.; Bae, Y.C.; Suh, K.T.; Jung, J.S. Quercetin, a flavonoid, inhibits proliferation and increases osteogenic differentiation in human adipose stromal cells. *Biochem. Pharmacol.* **2006**, *72*, 1268–1278. [[CrossRef](#)] [[PubMed](#)]
129. Almalki, S.G.; Agrawal, D.K. Key Transcription Factors in the Differentiation of Mesenchymal Stem Cells. *Differ. Res. Biol. Divers.* **2016**, *92*, 41–51. [[CrossRef](#)] [[PubMed](#)]
130. Langenbach, F.; Handschel, J. Effects of dexamethasone, ascorbic acid and β -glycerophosphate on the osteogenic differentiation of stem cells in vitro. *Stem Cell Res. Ther.* **2013**, *4*, 117. [[CrossRef](#)] [[PubMed](#)]
131. Behr, B.; Tang, C.; Germann, G.; Longaker, M.T.; Quarto, N. Locally applied VEGFA increases the osteogenic healing capacity of human adipose derived stem cells by promoting osteogenic and endothelial differentiation. *Stem Cells* **2011**, *29*, 286–296. [[CrossRef](#)] [[PubMed](#)]
132. Li, X.L.; Liu, Y.B.; Ma, E.G.; Shen, W.X.; Li, H.; Zhang, Y.N. Synergistic effect of BMP9 and TGF- β in the proliferation and differentiation of osteoblasts. *Genet. Mol. Res.* **2015**, *14*, 7605–7615. [[CrossRef](#)] [[PubMed](#)]
133. Zhang, Y.; Madhu, V.; Dighe, A.S.; Irvine, J.N., Jr.; Cui, Q. Osteogenic response of human adipose-derived stem cells to BMP-6, VEGF, and combined VEGF plus BMP-6 in vitro. *Growth Factors* **2012**, *30*, 333–343. [[CrossRef](#)] [[PubMed](#)]
134. Zuk, P.; Chou, Y.F.; Mussano, F.; Benhaim, P.; Wu, B.M. Adipose-derived stem cells and BMP2: Part 2. BMP2 may not influence the osteogenic fate of human adipose-derived stem cells. *Connect. Tissue Res.* **2011**, *52*, 119–132. [[CrossRef](#)] [[PubMed](#)]
135. Cruz, A.C.C.; Silva, M.L.; Caon, T.; Simões, C.M.O. Addition of bone morphogenetic protein type 2 to ascorbate and β -glycerophosphate supplementation did not enhance osteogenic differentiation of human adipose-derived stem cells. *J. Appl. Oral Sci.* **2012**, *20*, 628–635. [[CrossRef](#)] [[PubMed](#)]
136. Song, I.; Kim, B.S.; Kim, C.S.; Im, G.I. Effects of BMP-2 and vitamin D3 on the osteogenic differentiation of adipose stem cells. *Biochem. Biophys. Res. Commun.* **2011**, *408*, 126–131. [[CrossRef](#)] [[PubMed](#)]
137. Abdollahi, H.; Harris, L.J.; Zhang, P.; McIlhenny, S.; Tulenko, T.; DiMuzio, P.J. The Role of Hypoxia in Stem Cell Differentiation and Therapeutics. *J. Surg. Res.* **2011**, *165*, 112–117. [[CrossRef](#)] [[PubMed](#)]
138. Valorani, M.G.; Montelatici, E.; Germani, A.; Biddle, A.; D'Alessandro, D.; Strollo, R.; Patrizi, M.P.; Lazzari, L.; Nye, E.; Otto, W.R.; et al. Pre-culturing human adipose tissue mesenchymal stem cells under hypoxia increases their adipogenic and osteogenic differentiation potentials. *Cell Prolif.* **2012**, *45*, 225–238. [[CrossRef](#)] [[PubMed](#)]
139. Xu, L.; Sun, X.; Cao, K.; Wu, Y.; Zou, D.; Liu, Y.; Zhang, X.; Zhang, X.; Wang, G.; Huang, Q.; et al. Hypoxia induces osteogenesis in rabbit adipose-derived stem cells overexpressing bone morphogenetic protein-2. *Oral Dis.* **2014**, *20*, 430–439. [[CrossRef](#)] [[PubMed](#)]
140. Skubis, A.; Sikora, B.; Zmarzły, N.; Wojdas, E.; Mazurek, U. Adipose-derived stem cells: A review of osteogenesis differentiation. *Folia Biol. Oecol.* **2016**, *12*, 38–47. [[CrossRef](#)]
141. Kim, J.H.; Yoon, S.M.; Song, S.U.; Park, S.G.; Kim, W.-S.; Park, I.G.; Lee, J.; Sung, J.-H. Hypoxia Suppresses Spontaneous Mineralization and Osteogenic Differentiation of Mesenchymal Stem Cells via IGFBP3 Up-Regulation. *Int. J. Mol. Sci.* **2016**, *17*, 1389. [[CrossRef](#)] [[PubMed](#)]
142. Hsu, S.H.; Chen, C.T.; Wei, Y.H. Inhibitory effects of hypoxia on metabolic switch and osteogenic differentiation of human mesenchymal stem cells. *Stem Cells* **2013**, *31*, 2779–2788. [[CrossRef](#)] [[PubMed](#)]
143. Li, Q.; Gao, Z.; Chen, Y.; Guan, M.-X. The role of mitochondria in osteogenic, adipogenic and chondrogenic differentiation of mesenchymal stem cells. *Protein Cell* **2017**, *8*, 439–445. [[CrossRef](#)] [[PubMed](#)]
144. Pan, H.; Guan, D.; Liu, X.; Li, J.; Wang, L.; Wu, J.; Zhou, J.; Zhang, W.; Ren, R.; Zhang, W.; et al. SIRT6 safeguards human mesenchymal stem cells from oxidative stress by coactivating NRF2. *Cell Res.* **2016**, *26*, 190–205. [[CrossRef](#)] [[PubMed](#)]
145. Min-Wen, J.C.; Jun-Hao, E.T.; Shyh-Chang, N. Stem cell mitochondria during aging. *Semin. Cell Dev. Biol.* **2016**, *52*, 110–118. [[CrossRef](#)] [[PubMed](#)]

146. Zhang, X.; Guo, J.; Zhou, Y.; Wu, G. The Roles of Bone Morphogenetic Proteins and Their Signaling in the Osteogenesis of Adipose-Derived Stem Cells. *Tissue Eng. Part B Rev.* **2014**, *20*, 84–92. [[CrossRef](#)] [[PubMed](#)]
147. Reddi, A.H. BMPs: From bone morphogenetic proteins to body morphogenetic proteins. *Cytokine Growth Factor Rev.* **2005**, *16*, 249–250. [[CrossRef](#)] [[PubMed](#)]
148. Levi, B.; Hyun, J.S.; Nelson, E.R.; Li, S.; Montoro, D.T.; Wan, D.C.; Jia, F.J.; Glotzbach, J.C.; James, A.W.; Lee, M.; et al. Nonintegrating Knockdown and Customized Scaffold Design Enhances Human Adipose-Derived Stem Cells in Skeletal Repair. *Stem Cells* **2011**, *29*, 2018–2029. [[CrossRef](#)] [[PubMed](#)]
149. Mizrahi, O.; Sheyn, D.; Tawackoli, W.; Kallai, I.; Oh, A.; Su, S.; Da, X.; Zarrini, P.; Cook-Wiens, G.; Gazit, D.; et al. BMP-6 is more efficient in bone formation than BMP-2 when overexpressed in mesenchymal stem cells. *Gene Ther.* **2013**, *20*, 370–377. [[CrossRef](#)] [[PubMed](#)]
150. Knippenberg, M.; Helder, M.N.; Zandieh Doulabi, B.; Wuisman, P.I.; Klein-Nulend, J. Osteogenesis versus chondrogenesis by BMP-2 and BMP-7 in adipose stem cells. *Biochem. Biophys. Res. Commun.* **2006**, *342*, 902–908. [[CrossRef](#)] [[PubMed](#)]
151. Zheng, Y.; Wu, G.; Zhao, J.; Wang, L.; Sun, P.; Gu, Z. rhBMP2/7 heterodimer: An osteoblastogenesis inducer of not higher potency but lower effective concentration compared with rhBMP2 and rhBMP7 homodimers. *Tissue Eng. Part A* **2010**, *16*, 879–887. [[CrossRef](#)] [[PubMed](#)]
152. Vicente Lopez, M.A.; Vazquez Garcia, M.N.; Entrena, A.; Olmedillas Lopez, S.; Garcia-Arranz, M.; Garcia-Olmo, D.; Zapata, A. Low doses of bone morphogenetic protein 4 increase the survival of human adipose-derived stem cells maintaining their stemness and multipotency. *Stem Cells Dev.* **2011**, *20*, 1011–1019. [[CrossRef](#)] [[PubMed](#)]
153. Panetta, N.J.; Gupta, D.M.; Lee, J.K.; Wan, D.C.; Commons, G.W.; Longaker, M.T. Human adipose-derived stromal cells respond to and elaborate bone morphogenetic protein-2 during in vitro osteogenic differentiation. *Plast. Reconstr. Surg.* **2010**, *125*, 483–493. [[CrossRef](#)] [[PubMed](#)]
154. Wan, D.C.; Shi, Y.-Y.; Nacamuli, R.P.; Quarto, N.; Lyons, K.M.; Longaker, M.T. Osteogenic differentiation of mouse adipose-derived adult stromal cells requires retinoic acid and bone morphogenetic protein receptor type IB signaling. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 12335–12340. [[CrossRef](#)] [[PubMed](#)]
155. Ducy, P.; Zhang, R.; Geoffroy, V.; Ridall, A.L.; Karsenty, G. Osf2/Cbfa1: A transcriptional activator of osteoblast differentiation. *Cell* **1997**, *89*, 747–754. [[CrossRef](#)]
156. Santos, A.; Bakker, A.D.; de Blicke-Hogervorst, J.M.; Klein-Nulend, J. WNT5A induces osteogenic differentiation of human adipose stem cells via rho-associated kinase ROCK. *Cytotherapy* **2010**, *12*, 924–932. [[CrossRef](#)] [[PubMed](#)]
157. Takada, I.; Kouzmenko, A.P.; Kato, S. Molecular switching of osteoblastogenesis versus adipogenesis: Implications for targeted therapies. *Expert Opin. Ther. Targets* **2009**, *13*, 593–603. [[CrossRef](#)] [[PubMed](#)]
158. Chillakuri, C.R.; Sheppard, D.; Lea, S.M.; Handford, P.A. Notch receptor–ligand binding and activation: Insights from molecular studies. *Semin. Cell Dev. Biol.* **2012**, *23*, 421–428. [[CrossRef](#)] [[PubMed](#)]
159. Gimble, J.; Guilak, F. Adipose-derived adult stem cells: Isolation, characterization, and differentiation potential. *Cytotherapy* **2003**, *5*, 362–369. [[CrossRef](#)] [[PubMed](#)]
160. Estes, B.T.; Wu, A.W.; Guilak, F. Potent induction of chondrocytic differentiation of human adipose-derived adult stem cells by bone morphogenetic protein 6. *Arthritis Rheum.* **2006**, *54*, 1222–1232. [[CrossRef](#)] [[PubMed](#)]
161. Kim, H.J.; Im, G.I. Chondrogenic differentiation of adipose tissue-derived mesenchymal stem cells: Greater doses of growth factor are necessary. *J. Orthop. Res.* **2009**, *27*, 612–619. [[CrossRef](#)] [[PubMed](#)]
162. Sottile, V.; Halleux, C.; Bassilana, F.; Keller, H.; Seuwen, K. Stem cell characteristics of human trabecular bone-derived cells. *Bone* **2002**, *30*, 699–704. [[CrossRef](#)]
163. Suva, D.; Garavaglia, G.; Menetrey, J.; Chapuis, B.; Hoffmeyer, P.; Bernheim, L.; Kindler, V. Non-hematopoietic human bone marrow contains long-lasting, pluripotential mesenchymal stem cells. *J. Cell. Physiol.* **2004**, *198*, 110–118. [[CrossRef](#)] [[PubMed](#)]
164. Puetzer, J.L.; Petite, J.N.; Lobo, E.G. Comparative review of growth factors for induction of three-dimensional in vitro chondrogenesis in human mesenchymal stem cells isolated from bone marrow and adipose tissue. *Tissue Eng. Part B Rev.* **2010**, *16*, 435–444. [[CrossRef](#)] [[PubMed](#)]
165. Wei, Y.; Sun, X.; Wang, W.; Hu, Y. Adipose-derived stem cells and chondrogenesis. *Cytotherapy* **2007**, *9*, 712–716. [[CrossRef](#)] [[PubMed](#)]

166. Stromps, J.P.; Paul, N.E.; Rath, B.; Nourbakhsh, M.; Bernhagen, J.; Pallua, N. Chondrogenic Differentiation of Human Adipose-Derived Stem Cells: A New Path in Articular Cartilage Defect Management? *BioMed Res. Int.* **2014**, *2014*, 740926. [[CrossRef](#)] [[PubMed](#)]
167. Xu, J.; Wang, W.; Ludeman, M.; Cheng, K.; Hayami, T.; Lotz, J.C.; Kapila, S. Chondrogenic differentiation of human mesenchymal stem cells in three-dimensional alginate gels. *Tissue Eng. Part A* **2008**, *14*, 667–680. [[CrossRef](#)] [[PubMed](#)]
168. Erickson, G.R.; Gimble, J.M.; Franklin, D.M.; Rice, H.E.; Awad, H.; Guilak, F. Chondrogenic potential of adipose tissue-derived stromal cells in vitro and in vivo. *Biochem. Biophys. Res. Commun.* **2002**, *290*, 763–769. [[CrossRef](#)] [[PubMed](#)]
169. Awad, H.A.; Wickham, M.Q.; Leddy, H.A.; Gimble, J.M.; Guilak, F. Chondrogenic differentiation of adipose-derived adult stem cells in agarose, alginate, and gelatin scaffolds. *Biomaterials* **2004**, *25*, 3211–3222. [[CrossRef](#)] [[PubMed](#)]
170. Baptista, L.S.; Silva, K.R.; Pedrosa, C.S.; Amaral, R.J.; Belizário, J.V.; Borojevic, R.; Granjeiro, J.M. Bioengineered Cartilage in a Scaffold-Free Method by Human Cartilage-Derived Progenitor Cells: A Comparison With Human Adipose-Derived Mesenchymal Stromal Cells. *Artif. Organs* **2013**, *37*, 1068–1075. [[CrossRef](#)] [[PubMed](#)]
171. Scott, M.A.; Nguyen, V.T.; Levi, B.; James, A.W. Current Methods of Adipogenic Differentiation of Mesenchymal Stem Cells. *Stem Cells Dev.* **2011**, *20*, 1793–1804. [[CrossRef](#)] [[PubMed](#)]
172. Higuchi, M.; Dusting, G.J.; Peshavariya, H.; Jiang, F.; Hsiao, S.T.-F.; Chan, E.C.; Liu, G.-S. Differentiation of Human Adipose-Derived Stem Cells into Fat Involves Reactive Oxygen Species and Forkhead Box O1 Mediated Upregulation of Antioxidant Enzymes. *Stem Cells Dev.* **2013**, *22*, 878–888. [[CrossRef](#)] [[PubMed](#)]
173. Cristancho, A.G.; Lazar, M.A. Forming functional fat: A growing understanding of adipocyte differentiation. *Nat. Rev. Mol. Cell Biol.* **2011**, *12*, 722–734. [[CrossRef](#)] [[PubMed](#)]
174. Yin, L.; Li, Y.B.; Wang, Y.S. Dexamethasone-induced adipogenesis in primary marrow stromal cell cultures: Mechanism of steroid-induced osteonecrosis. *Chin. Med. J.* **2006**, *119*, 581–588. [[PubMed](#)]
175. Ailhaud, G. Adipose cell differentiation in culture. *Mol. Cell. Biochem.* **1982**, *49*, 17–31. [[CrossRef](#)] [[PubMed](#)]
176. Rosen, E.D.; Walkey, C.J.; Puigserver, P.; Spiegelman, B.M. Transcriptional regulation of adipogenesis. *Genes Dev.* **2000**, *14*, 1293–1307. [[PubMed](#)]
177. Gurriarán-Rodríguez, U.; Al-Massadi, O.; Roca-Rivada, A.; Crujeiras, A.B.; Gallego, R.; Pardo, M.; Seoane, L.M.; Pazos, Y.; Casanueva, F.F.; Camiña, J.P. Obestatin as a regulator of adipocyte metabolism and adipogenesis. *J. Cell. Mol. Med.* **2011**, *15*, 1927–1940. [[CrossRef](#)] [[PubMed](#)]
178. Kim, S.P.; Ha, J.M.; Yun, S.J.; Kim, E.K.; Chung, S.W.; Hong, K.W.; Kim, C.D.; Bae, S.S. Transcriptional activation of peroxisome proliferator-activated receptor-gamma requires activation of both protein kinase A and Akt during adipocyte differentiation. *Biochem. Biophys. Res. Commun.* **2010**, *399*, 55–59. [[CrossRef](#)] [[PubMed](#)]
179. Scioli, M.G.; Bielli, A.; Gentile, P.; Mazzaglia, D.; Cervelli, V.; Orlandi, A. The Biomolecular Basis of Adipogenic Differentiation of Adipose-Derived Stem Cells. *Int. J. Mol. Sci.* **2014**, *15*, 6517–6526. [[CrossRef](#)] [[PubMed](#)]
180. Hemmingsen, M.; Vedel, S.; Skafté-Pedersen, P.; Sabourin, D.; Collas, P.; Bruus, H.; Dufva, M. The Role of Paracrine and Autocrine Signaling in the Early Phase of Adipogenic Differentiation of Adipose-derived Stem Cells. *PLoS ONE* **2013**, *8*, e63638. [[CrossRef](#)] [[PubMed](#)]
181. Miyamoto, Y.; Ikeuchi, M.; Noguchi, H.; Yagi, T.; Hayashi, S. Enhanced Adipogenic Differentiation of Human Adipose-Derived Stem Cells in an in vitro Microenvironment: The Preparation of Adipose-Like Microtissues Using a Three-Dimensional Culture. *Cell Med.* **2017**, *9*, 35–44. [[CrossRef](#)] [[PubMed](#)]
182. White, U.A.; Tchoukalova, Y.D. Adipose Stem Cells and Adipogenesis. In *Adipose Tissue and Adipokines in Health and Disease*; Fantuzzi, G., Braunschweig, C., Eds.; Humana Press: Totowa, NJ, USA, 2014; pp. 15–32.
183. Otto, T.C.; Lane, M.D. Adipose development: From stem cell to adipocyte. *Crit. Rev. Biochem. Mol. Biol.* **2005**, *40*, 229–242. [[CrossRef](#)] [[PubMed](#)]
184. Yuan, Z.; Li, Q.; Luo, S.; Liu, Z.; Luo, D.; Zhang, B.; Zhang, D.; Rao, P.; Xiao, J. PPARgamma and Wnt Signaling in Adipogenic and Osteogenic Differentiation of Mesenchymal Stem Cells. *Curr. Stem Cell Res. Ther.* **2016**, *11*, 216–225. [[CrossRef](#)] [[PubMed](#)]
185. Salgado, A.J.; Reis, R.L.; Sousa, N.; Gimble, J.M. Adipose tissue derived stem cells secretome: Soluble factors and their roles in regenerative medicine. *Curr. Stem Cell Res. Ther.* **2010**, *5*, 103–110. [[CrossRef](#)] [[PubMed](#)]

186. Maumus, M.; Jorgensen, C.; Noël, D. Mesenchymal stem cells in regenerative medicine applied to rheumatic diseases: Role of secretome and exosomes. *Biochimie* **2013**, *95*, 2229–2234. [[CrossRef](#)] [[PubMed](#)]
187. Nakanishi, C.; Nagaya, N.; Ohnishi, S.; Yamahara, K.; Takabatake, S.; Konno, T.; Hayashi, K.; Kawashiri, M.A.; Tsubokawa, T.; Yamagishi, M. Gene and protein expression analysis of mesenchymal stem cells derived from rat adipose tissue and bone marrow. *Circ. J.* **2011**, *75*, 2260–2268. [[CrossRef](#)] [[PubMed](#)]
188. Kachgal, S.; Putnam, A.J. Mesenchymal stem cells from adipose and bone marrow promote angiogenesis via distinct cytokine and protease expression mechanisms. *Angiogenesis* **2011**, *14*, 47–59. [[CrossRef](#)] [[PubMed](#)]
189. Kapur, S.K.; Katz, A.J. Review of the adipose derived stem cell secretome. *Biochimie* **2013**, *95*, 2222–2228. [[CrossRef](#)] [[PubMed](#)]
190. Helena, S.; Jan, M.; Jivan, G.S.; Hana, K. Mapping of the secretome of primary isolates of mammalian cells, stem cells and derived cell lines. *Proteomics* **2011**, *11*, 691–708.
191. Xu, D.; Suenaga, N.; Edelman, M.J.; Fridman, R.; Muschel, R.J.; Kessler, B.M. Novel MMP-9 Substrates in Cancer Cells Revealed by a Label-free Quantitative Proteomics Approach. *Mol. Cell. Proteom.* **2008**, *7*, 2215–2228. [[CrossRef](#)] [[PubMed](#)]
192. Brini, A.T.; Amodeo, G.; Ferreira, L.M.; Milani, A.; Niada, S.; Moschetti, G.; Franchi, S.; Borsani, E.; Rodella, L.F.; Panerai, A.E.; et al. Therapeutic effect of human adipose-derived stem cells and their secretome in experimental diabetic pain. *Sci. Rep.* **2017**, *7*, 9904. [[CrossRef](#)] [[PubMed](#)]
193. Kim, W.-S.; Park, B.-S.; Sung, J.-H. Protective role of adipose-derived stem cells and their soluble factors in photoaging. *Arch. Dermatol. Res.* **2009**, *301*, 329–336. [[CrossRef](#)] [[PubMed](#)]
194. Lee, E.Y.; Xia, Y.; Kim, W.S.; Kim, M.H.; Kim, T.H.; Kim, K.J.; Park, B.S.; Sung, J.H. Hypoxia-enhanced wound-healing function of adipose-derived stem cells: Increase in stem cell proliferation and up-regulation of VEGF and bFGF. *Wound Repair Regen.* **2009**, *17*, 540–547. [[CrossRef](#)] [[PubMed](#)]
195. Kakudo, N.; Morimoto, N.; Ogawa, T.; Taketani, S.; Kusumoto, K. Hypoxia Enhances Proliferation of Human Adipose-Derived Stem Cells via HIF-1 α Activation. *PLoS ONE* **2015**, *10*, e0139890. [[CrossRef](#)] [[PubMed](#)]
196. Wang, F.; Zachar, V.; Pennisi, C.P.; Fink, T.; Maeda, Y.; Emmersen, J. Hypoxia Enhances Differentiation of Adipose Tissue-Derived Stem Cells toward the Smooth Muscle Phenotype. *Int. J. Mol. Sci.* **2018**, *19*, 517. [[CrossRef](#)] [[PubMed](#)]
197. Thangarajah, H.; Vial, I.N.; Chang, E.; El-Ftesi, S.; Januszyk, M.; Chang, E.I.; Paterno, J.; Neofytou, E.; Longaker, M.T.; Gurtner, G.C. IFATS collection: Adipose stromal cells adopt a proangiogenic phenotype under the influence of hypoxia. *Stem Cells* **2009**, *27*, 266–274. [[CrossRef](#)] [[PubMed](#)]
198. Ribeiro, C.A.; Fraga, J.S.; Grãos, M.; Neves, N.M.; Reis, R.L.; Gimble, J.M.; Sousa, N.; Salgado, A.J. Thesecretome of stem cells isolated from the adipose tissue and Wharton jelly acts differently on central nervous system derived cell populations. *Stem Cell Res. Ther.* **2012**, *3*, 18. [[CrossRef](#)] [[PubMed](#)]
199. Marfia, G.; Navone, S.E.; Hadi, L.A.; Paroni, M.; Berno, V.; Beretta, M.; Gualtierotti, R.; Ingegnoli, F.; Levi, V.; Miozzo, M.; et al. The Adipose Mesenchymal Stem Cell Secretome Inhibits Inflammatory Responses of Microglia: Evidence for an Involvement of Sphingosine-1-Phosphate Signalling. *Stem Cells Dev.* **2016**, *25*, 1095–1107. [[CrossRef](#)] [[PubMed](#)]
200. Constantin, G.; Marconi, S.; Rossi, B.; Angiari, S.; Calderan, L.; Anghileri, E.; Gini, B.; Bach, S.D.; Martinello, M.; Bifari, F.; et al. Adipose-derived mesenchymal stem cells ameliorate chronic experimental autoimmune encephalomyelitis. *Stem Cells* **2009**, *27*, 2624–2635. [[CrossRef](#)] [[PubMed](#)]
201. Rehman, J.; Traktuev, D.; Li, J.; Merfeld-Clauss, S.; Temm-Grove, C.J.; Bovenkerk, J.E.; Pell, C.L.; Johnstone, B.H.; Considine, R.V.; March, K.L. Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. *Circulation* **2004**, *109*, 1292–1298. [[CrossRef](#)] [[PubMed](#)]
202. Egashira, Y.; Sugitani, S.; Suzuki, Y.; Mishiro, K.; Tsuruma, K.; Shimazawa, M.; Yoshimura, S.; Iwama, T.; Hara, H. The conditioned medium of murine and human adipose-derived stem cells exerts neuroprotective effects against experimental stroke model. *Brain Res.* **2012**, *1461*, 87–95. [[CrossRef](#)] [[PubMed](#)]
203. Wei, X.; Du, Z.; Zhao, L.; Feng, D.; Wei, G.; He, Y.; Tan, J.; Lee, W.H.; Hampel, H.; Dodel, R.; et al. IFATS collection: The conditioned media of adipose stromal cells protect against hypoxia-ischemia-induced brain damage in neonatal rats. *Stem Cells* **2009**, *27*, 478–488. [[CrossRef](#)] [[PubMed](#)]
204. Tan, B.; Luan, Z.; Wei, X.; He, Y.; Wei, G.; Johnstone, B.H.; Farlow, M.; Du, Y. AMP-activated kinase mediates adipose stem cell-stimulated neuritogenesis of PC12 cells. *Neuroscience* **2011**, *181*, 40–47. [[CrossRef](#)] [[PubMed](#)]

205. Martins, L.F.; Costa, R.O.; Pedro, J.R.; Aguiar, P.; Serra, S.C.; Teixeira, F.G.; Sousa, N.; Salgado, A.J.; Almeida, R.D. Mesenchymal stem cells secretome-induced axonal outgrowth is mediated by BDNF. *Sci. Rep.* **2017**, *7*, 4153. [[CrossRef](#)] [[PubMed](#)]
206. Palomares, T.; Cordero, M.; Bruzos-Cidon, C.; Torrecilla, M.; Ugedo, L.; Alonso-Varona, A. The Neuroprotective Effect of Conditioned Medium from Human Adipose-Derived Mesenchymal Stem Cells is Impaired by *N*-acetyl Cysteine Supplementation. *Mol. Neurobiol.* **2018**, *55*, 13–25. [[CrossRef](#)] [[PubMed](#)]
207. Di Cesare Mannelli, L.; Tenci, B.; Micheli, L.; Vona, A.; Corti, F.; Zanardelli, M.; Lapucci, A.; Clemente, A.M.; Failli, P.; Ghelardini, C. Adipose-derived stem cells decrease pain in a rat model of oxaliplatin-induced neuropathy: Role of VEGF-A modulation. *Neuropharmacology* **2018**, *131*, 166–175. [[CrossRef](#)] [[PubMed](#)]
208. Bravo, B.; de Durango, C.G.; González, Á.; Gortázar, A.R.; Santos, X.; Forteza-Vila, J.; Vidal-Vanaclocha, F. Opposite Effects of Mechanical Action of Fluid Flow on Proangiogenic Factor Secretion From Human Adipose-Derived Stem Cells with and without Oxidative Stress. *J. Cell. Physiol.* **2017**, *232*, 2158–2167. [[CrossRef](#)] [[PubMed](#)]
209. Kilroy, G.E.; Foster, S.J.; Wu, X.; Ruiz, J.; Sherwood, S.; Heifetz, A.; Ludlow, J.W.; Stricker, D.M.; Potiny, S.; Green, P.; et al. Cytokine profile of human adipose-derived stem cells: Expression of angiogenic, hematopoietic, and pro-inflammatory factors. *J. Cell. Physiol.* **2007**, *212*, 702–709. [[CrossRef](#)] [[PubMed](#)]
210. Krumboeck, A.; Giovanoli, P.; Plock, J.A. Fat grafting and stem cell enhanced fat grafting to the breast under oncological aspects—recommendations for patient selection. *Breast* **2013**, *22*, 579–584. [[CrossRef](#)] [[PubMed](#)]
211. O'Halloran, N.; Courtney, D.; Kerin, M.J.; Lowery, A.J. Adipose-Derived Stem Cells in Novel Approaches to Breast Reconstruction: Their Suitability for Tissue Engineering and Oncological Safety. *Breast Cancer Basic Clin. Res.* **2017**, *11*. [[CrossRef](#)] [[PubMed](#)]
212. Zhang, Y.; Yao, F.; Yao, X.; Yi, C.; Tan, C.; Wei, L.; Sun, S. Role of CCL5 in invasion, proliferation and proportion of CD44+/CD24− phenotype of MCF-7 cells and correlation of CCL5 and CCR5 expression with breast cancer progression. *Oncol. Rep.* **2009**, *21*, 1113–1121. [[PubMed](#)]
213. Ryu, H.; Oh, J.-E.; Rhee, K.-J.; Baik, S.K.; Kim, J.; Kang, S.J.; Sohn, J.H.; Choi, E.; Shin, H.C.; Kim, Y.M. Adipose tissue-derived mesenchymal stem cells cultured at high density express IFN- β and suppress the growth of MCF-7 human breast cancer cells. *Cancer Lett.* **2014**, *352*, 220–227. [[CrossRef](#)] [[PubMed](#)]
214. Zimmerlin, L.; Donnenberg, A.D.; Rubin, J.P.; Basse, P.; Landreneau, R.J.; Donnenberg, V.S. Regenerative therapy and cancer: In vitro and in vivo studies of the interaction between adipose-derived stem cells and breast cancer cells from clinical isolates. *Tissue Eng. Part A* **2010**, *17*, 93–106. [[CrossRef](#)] [[PubMed](#)]
215. Wang, T.; Guo, S.; Liu, X.; Xu, N.; Zhang, S. Protective effects of adipose-derived stem cells secretome on human dermal fibroblasts from ageing damages. *Int. J. Clin. Exp. Pathol.* **2015**, *8*, 15739–15748. [[PubMed](#)]
216. Cho, J.W.; Kang, M.C.; Lee, K.S. TGF- β 1-treated ADSCs-CM promotes expression of type I collagen and MMP-1, migration of human skin fibroblasts, and wound healing in vitro and in vivo. *Int. J. Mol. Med.* **2010**, *26*, 901–906. [[PubMed](#)]
217. Yang, E.-J.; Bang, S.-I. Effects of conditioned medium from LL-37 treated adipose stem cells on human fibroblast migration. *Exp. Ther. Med.* **2017**, *14*, 723–729. [[CrossRef](#)] [[PubMed](#)]
218. Park, B.S.; Jang, K.A.; Sung, J.H.; Park, J.S.; Kwon, Y.H.; Kim, K.J.; Kim, W.S. Adipose-derived stem cells and their secretory factors as a promising therapy for skin aging. *Dermatol. Surg.* **2008**, *34*, 1323–1326. [[PubMed](#)]
219. Lee, S.C.; Kim, J.O.; Kim, S.-J. Secretome from human adipose-derived stem cells protects mouse liver from hepatic ischemia-reperfusion injury. *Surgery* **2015**, *157*, 934–943. [[CrossRef](#)] [[PubMed](#)]
220. Xia, X.; Chiu, P.W.Y.; Lam, P.K.; Chin, W.C.; Ng, E.K.W.; Lau, J.Y.W. Secretome from hypoxia-conditioned adipose-derived mesenchymal stem cells promotes the healing of gastric mucosal injury in a rodent model. *Biochim. Biophys. Acta-Mol. Basis Dis.* **2018**, *1864*, 178–188. [[CrossRef](#)] [[PubMed](#)]
221. Choi, E.W.; Seo, M.K.; Woo, E.Y.; Kim, S.H.; Park, E.J.; Kim, S. Exosomes from human adipose-derived stem cells promote proliferation and migration of skin fibroblasts. *Exp. Dermatol.* **2017**. [[CrossRef](#)] [[PubMed](#)]
222. Lai, R.C.; Arslan, F.; Lee, M.M.; Sze, N.S.; Choo, A.; Chen, T.S.; Salto-Tellez, M.; Timmers, L.; Lee, C.N.; El Oakley, R.M.; et al. Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury. *Stem Cell Res.* **2010**, *4*, 214–222. [[CrossRef](#)] [[PubMed](#)]
223. Vyas, N.; Dhawan, J. Exosomes: Mobile platforms for targeted and synergistic signaling across cell boundaries. *Cell. Mol. Life Sci.* **2017**, *74*, 1567–1576. [[CrossRef](#)] [[PubMed](#)]

224. Li, X.; Xie, X.; Lian, W.; Shi, R.; Han, S.; Zhang, H.; Lu, L.; Li, M. Exosomes from adipose-derived stem cells overexpressing Nrf2 accelerate cutaneous wound healing by promoting vascularization in a diabetic foot ulcer rat model. *Exp. Mol. Med.* **2018**, *50*, 29. [[CrossRef](#)] [[PubMed](#)]
225. García-Contreras, M.; Vera-Donoso, C.D.; Hernández-Andreu, J.M.; García-Verdugo, J.M.; Oltra, E. Therapeutic Potential of Human Adipose-Derived Stem Cells (ADSCs) from Cancer Patients: A Pilot Study. *PLoS ONE* **2014**, *9*, e113288. [[CrossRef](#)] [[PubMed](#)]
226. Hu, L.; Wang, J.; Zhou, X.; Xiong, Z.; Zhao, J.; Yu, R.; Huang, F.; Zhang, H.; Chen, L. Exosomes derived from human adipose mesenchymal stem cells accelerates cutaneous wound healing via optimizing the characteristics of fibroblasts. *Sci. Rep.* **2016**, *6*, 32993. [[CrossRef](#)] [[PubMed](#)]
227. Wang, L.; Hu, L.; Zhou, X.; Xiong, Z.; Zhang, C.; Shehada, H.M.A.; Hu, B.; Song, J.; Chen, L. Exosomes secreted by human adipose mesenchymal stem cells promote scarless cutaneous repair by regulating extracellular matrix remodelling. *Sci. Rep.* **2017**, *7*, 13321. [[CrossRef](#)] [[PubMed](#)]
228. Lee, M.; Liu, T.; Wooseok, I.; Manho, K. Exosomes from adipose-derived stem cells ameliorate phenotype of Huntington's disease in vitro model. *Eur. J. Neurosci.* **2016**, *44*, 2114–2119. [[CrossRef](#)] [[PubMed](#)]
229. Lou, G.; Song, X.; Yang, F.; Wu, S.; Wang, J.; Chen, Z.; Liu, Y. Exosomes derived from miR-122-modified adipose tissue-derived MSCs increase chemosensitivity of hepatocellular carcinoma. *J. Hematol. Oncol.* **2015**, *8*, 122. [[CrossRef](#)] [[PubMed](#)]
230. Gao, X.; Salomon, C.; Freeman, D.J. Extracellular Vesicles from Adipose Tissue—A Potential Role in Obesity and Type 2 Diabetes? *Front. Endocrinol.* **2017**, *8*, 202. [[CrossRef](#)] [[PubMed](#)]



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