

## Variation in Osteogenic Differentiation Capacities of Adipose-derived Stromal Cells by Anatomic Depot

Dalton Pham, Alan Nguyen, Jia Shen, Vi Nguyen, Michelle A. Scott, Aaron W. James

Dalton Pham, Alan Nguyen, Vi Nguyen, Aaron W. James, Department of Pathology and Laboratory Medicine, David Geffen School of Medicine, University of California, Los Angeles, California 90095, the United States

Alan Nguyen, Jia Shen, School of Dentistry, University of California, Los Angeles, California 90095, the United States

Michelle A. Scott, Nationwide Children's Hospital, Columbus, Ohio, the United States

Correspondence to: Aaron W. James, MD, Department of Pathology and Laboratory Medicine, David Geffen School of Medicine, University of California, Los Angeles, 10833 Le Conte Ave., 13-145 CHS, Los Angeles, California 90095, the United States.

Email: [Awjames@mednet.ucla.edu](mailto:Awjames@mednet.ucla.edu)

Telephone: +310-206-6754

Received: February 16, 2016

Revised: April 15, 2016

Accepted: April 17, 2016

Published online: June 23, 2016

tissue engineering. In summary, the current literature regarding ASC clearly shows that not all fat is created equally. Clear cut, and for the most part reproducible, differences exist in the osteogenic potential, adipogenic potential and proliferation of ASC depending on their site of origin. How this information should be used is still a matter of uncertainty.

**Key words:** Adipose-derived stromal cells; Anatomic depot

© 2016 The Authors. Published by ACT Publishing Group Ltd.

Pham D, Nguyen A, Shen J, Nguyen V, Scott MA, James AW. Variation in Osteogenic Differentiation Capacities of Adipose-derived Stromal Cells by Anatomic Depot. International Journal of Orthopaedics 2016; 3(3): 549-556 Available from: URL: <http://www.ghrnet.org/index.php/ijo/article/view/1608>

### ABSTRACT

Although bone marrow has long been studied as the primary source of mesenchymal stem cells (MSC), adipose tissue has been increasingly studied as a rich source of tissue resident MSC. Since the first description of adipose-derived MSC, or adipose-derived stromal cells (ASC), it has been clear that ASC have significant advantages for clinical translation, including increased cell frequency, high growth potential, and residence in a dispensable and accessible location (subcutaneous fat). Nevertheless, it is increasingly clear that ASC differ by anatomic location, although the exact differences between anatomic depot are not entirely agreed upon. Striking differences exist between subcutaneous and visceral fat depots, but even more interesting between various anatomic regions within subcutaneous fat. Understanding the specific manners in which ASC differ by location is especially important in the context of

### INTRODUCTION

Although bone marrow has long been studied as the primary source of mesenchymal stem cells (MSC), these bone marrow-derived stem cells (BMSC) present a number of drawbacks. Autologous tissues such as BMSC are often limited in availability, and require operation with a risk of morbidity<sup>[1,2]</sup>. By contrast, adipose-derived stromal cells can be isolated from the stromal vascular fraction of lipoaspirate, and has been increasingly studied as a rich source of tissue resident MSC. In 2001, Zuk *et al* described this population of culture-derived, multipotential MSC from subcutaneous human white adipose tissue, termed adipose-derived stromal cells (or ASC)<sup>[3]</sup>. Since this time, ASC have been of great interest to investigators due to their relative abundance and accessibility being derived from adiposetissue, in contrast to BMSC<sup>[3-7]</sup>. Unlike BMSC, ASC are also advantageous in that they present minimal morbidity<sup>[1]</sup>. Additionally, ASC proliferate easily, expand quickly, and possess stable multipotency through multiple passages<sup>[3,8-10]</sup>. To date, ASC have been studied extensively

in the context of bone tissue engineering, with the literature showing various strategies that have been implemented to better induce osteogenic differentiation in adipose tissue, including the introduction of growth factors, different substrates, and even genetic modification. For example, ASC can easily be directed towards osteogenic differentiation via growth factor or substrate treatment during cell culture with ascorbic acid,  $\beta$ -glycerophosphate, bone morphogenetic protein-2, and/or other osteoinductive factors to name a few<sup>[11-22]</sup>. Furthermore, there are a numerous strategies that can be employed to achieve genetic manipulation for the purposes of upregulating osteogenesis; specifically, the vector and the gene delivery product can be selected<sup>[23]</sup>. For example, among different vectors available, viral delivery is a well-studied platform for gene transfer, while non-integrating methods have also been explored as it addresses safety concerns from the former<sup>[24,25]</sup>. Furthermore, in terms of products for viral delivery, these can range from micro (mi)RNAs to bone morphogenetic protein (BMPs), some of which are potent inducers of osteogenesis<sup>[26,27]</sup>. Taken together, these studies validate the versatility and promising therapeutic potential of ASC in a number of applications.

Adipose tissue is distributed throughout the body and can be categorized into the subcutaneous and visceral adipose depots. The visceral depot can be further subdivided into omental, mesenteric, retroperitoneal, intrathoracic, and gonadal adipose depots, among others<sup>[28]</sup>. As a result of the numerous studies regarding osteogenic properties of ASC, findings have increasingly pointed to the idea that adipose tissue harvested from different adipose depots possess variable osteogenic capacities, which may be due to the inherent characteristics that differ based on anatomical locations such as vascularization and cell population heterogeneity<sup>[29-33]</sup>. While it would be most ideal to use a well-identified and homogenous population for regenerative therapy, the ASC population remains quite heterogeneous<sup>[34]</sup>. In fact, one of the major limiting factors in its clinical application, which includes its osteogenic potential for bone regeneration, is its lack of clear parameters to define this stem cell population<sup>[35]</sup>. One of the gold standard approaches currently utilized is fluorescent-activated cell sorting (FACS), which uses surface markers to select for CD34+ and CD31- for ASC in general<sup>[35]</sup>. This however can present complications since surface markers may change from passage to passage, thus lowering overall yield<sup>[36]</sup>. The marker profile may further change with differences in the depot of origin. For example, intrathoracic, subcutaneous, and omental ASC are strongly positive for CD44, CD73, CD90, and CD29 markers with low CD31 expression<sup>[32,36-38]</sup>. Subcutaneous and omental ASC also consistently express high levels of CD105<sup>[39,40]</sup>. However, ASC isolated from pericardial intrathoracic depots had a significantly higher proportion of CD34+ cells compared to both subcutaneous and omental ASC<sup>[32,40]</sup> (Please see Table 1 for a summary on cell surface markers). Thus, comparative studies have begun to investigate the differences in ASC osteogenic potential both within subcutaneous depots from varying locations as well as across adipose depots. The objective of this review aims to assess the current literature with a focus on differences in osteogenic differentiation capacities of ASC derived from a variety of adipose depots, among other differences including cellularity, gene expression, and cell systems<sup>[41]</sup>.

## SUBCUTANEOUS ADIPOSE DEPOTS

A majority of the studies investigating the osteogenic potential of ASC have utilized subcutaneous adipose tissue as an ASC

source due to its relative abundance and accessibility<sup>[29,32,33,42-52]</sup>. Within the subcutaneous depot, a wide variety of ASC harvest sites have been assessed, including abdominal<sup>[29,32,33,42,45,47,48,51]</sup>, inguinal<sup>[49,50,52]</sup>, thigh<sup>[51]</sup>, dorsocervical<sup>[49]</sup>, flank<sup>[51]</sup>, gluteal<sup>[47]</sup>, arm<sup>[51]</sup>, and interscapular<sup>[46,52]</sup> regions. Even among subcutaneous adipose depots, there exists a number of differences including rates of proliferation and adipogenesis among other factors<sup>[41]</sup>. The emphasis of this review, however, is the osteogenic characteristics shared by these adipose depots. A study by Iwen *et al* comparing the osteogenic differentiation potential across subcutaneous adipose depots found that human gluteal subcutaneous (sc-)ASC exhibited higher alkaline phosphatase and proliferation rates than the donor-matched abdominal sc-ASC after 28 days<sup>[47]</sup>. Additionally, at this time point, mineralization was only present in gluteal sc-ASC, whereas it was absent in the abdominal sc-ASC<sup>[47]</sup>. Similarly, Levi *et al* found that abdominal sc-ASC had lesser osteogenic capacity compared to flank and thigh sc-ASC, with flank sc-ASC having the highest osteogenic potential, evidenced by alkaline phosphatase staining, bone nodule formation, and expression of a wide portfolio of osteogenic gene markers<sup>[51]</sup>. Another study comparing dorsocervical sc-ASC and inguinal sc-ASC discovered that inguinal sc-ASC demonstrated superior expression of alkaline phosphatase and osteocalcin<sup>[49]</sup>. In another bone regeneration study utilizing sc-ASC in a large animal model (canine), autologous-derived sc-ASC were expanded *ex vivo* and seeded onto a coral scaffold; the cranial bone defect was repaired with growth of typical bone tissue in the canine model used<sup>[53]</sup>. Another study tested the efficacy of undifferentiated sc-ASC, as most other studies use ASC differentiated *in vitro*; applied to a scaffold in athymic nude rat calvaria, it was shown that undifferentiated ASC could still differentiate into osteocytes and osteoblasts given a scaffolding material<sup>[54]</sup>. Overall, although differences in each study exist, sc-ASC taken from the lower extremity (inclusive of gluteus, flank, thigh, and inguinal region) seem to demonstrate enhanced *in vitro* osteogenic potential.

Compared to other adipose depots, including visceral<sup>[29,42]</sup>, omental<sup>[29,32,48]</sup>, and intrathoracic<sup>[32]</sup>, sc-ASC have consistently displayed greater osteogenic potential. Studies evaluating gene expression across multiple adipose depots showed that sc-ASC express significantly higher levels of the early osteogenic markers Runx2 and collagen I<sup>[29,32,42,45,46,48]</sup>. Furthermore, greater mineralization, osteocalcin, and osteonectin were observed in sc-ASC as well<sup>[29]</sup>. Not only did sc-ASC exhibit higher expression of osteogenic markers, but they also proliferated and differentiated into osteoblasts faster than ASC from other depots<sup>[29,33,42]</sup>. The more homogeneous cell population of spindle-shaped morphologies consistent with ASC from subcutaneous regions is hypothesized to underlie this difference in osteogenic differentiation rate, with the more heterogeneous visceral ASC depots exhibiting longer differentiation times<sup>[29]</sup>. In contrast, one study found that sc-ASC showed significantly lower alkaline phosphatase activity, proliferation rates, and mineralization than visceral (v-) ASC in rabbits<sup>[33]</sup>. However, it is important to note that sc-ASC from the same anatomic region in rabbits, rats, and humans did not display similar osteogenic potentials; rabbit sc-ASC displayed the lowest osteogenic potential out of the three model systems<sup>[50]</sup>. Thus, significant differences across species may complicate ASC comparisons. Remarkably, one clinical study has demonstrated successful reconstruction of craniomaxillofacial defects in 10 of 13 cases using human sc-ASC<sup>[55]</sup>. While sc-ASC show promise as a potential therapy for bone repair, further large-animal studies and pre-clinical trials are required before they can be reliably employed

**Table 1** Comparison of ASC depots of ease of accessibility, abundance of supply, cell surface markers and other notable features.

ASC Depot	Access	Supply	Notable Features	Cell Surface Markers
Subcutaneous [21566786, 22628159, 17654479, 11573204, 24361924, 24669358]	Easy	Abundant	High osteogenic potential Greater mineralization Faster proliferation and osteoblastic differentiation Relatively homogeneous cell population Resilient against lipolysis	CD3- / CD7- / CD10- / CD11b- (99%) / CD13+ (99% ± 2%) / CD14- (100%) / CD16- / CD17- / CD19- / CD20- / CD22- / CD25- / CD29+ (98% ± 1%) / CD31- (99% ± 1%) / CD33- / CD34- (72% ± 13%) / CD44+ (60% ± 15%) / CD45- (100%) / CD73+ (89.1%) / CD90+ (96.1% ± 4.2%) / CD105+ (36% ± 9%) / CD106+ (3% ± 0.7%) / CD166+ (82% ± 18%) / CD195-
Visceral	Moderate	Moderate	Heterogeneous cell population containing fibroblasts and mesothelial cells Express longer telomere lengths	CD29+ / CD44+ / CD73+ / CD90+
Omental [22167410, 24361924]	Moderate	Moderate	Contain mesothelial cells Less stiff tissue structure More vascularized Higher SVF yield	CD29+ / CD34- / CD44+ / CD73+ / CD90+ / CD105+ / CD166+ (37% ± 17%)
Intrathoracic [24361924]	Difficult	Scarce	Spontaneous intracellular lipid accumulation Inferior osteogenic differentiation Inclined to adipogenic differentiation	CD29+ / CD34+ (pericardial 82% ± 18%; thymic remnant 47% ± 25%) / CD44+ / CD73+ / CD90+ / CD166+ (pericardial 60% ± 25%; thymic remnant 72% ± 20%)

clinically for bone repair.

## VISCERAL ADIPOSE DEPOTS

An alternative source of ASC that has been well studied with respect to osteogenic capacity includes visceral adipose depots. In a recent study conducted by Jung *et al* involving visceral abdominal human ASC, the visceral ASC (v-ASC) were observed to have a more heterogeneous morphological makeup<sup>[29]</sup>. In addition to the spindle-shaped cells consistent with ASC, these cell cultures also contained contaminating cells, especially fibroblasts and mesothelial cells. These other cell types, however, were found to be transient in v-ASC cultures, decaying after only four passages. As a result of the heterogeneity of v-ASC, osteogenic differentiation was observed to be markedly lower than for abdominal human sc-ASC<sup>[29]</sup>.

Studies have indicated that the specific tissue source indeed plays a role in determining the characteristics for that extracted cell population. Specifically, observed variations in proliferation, and regulation of adipogenic and osteogenic regulation are notable<sup>[42,56]</sup>, and functional differences are hypothesized to be due to differences in adipose stem cell. For instance, in terms of adiposity and proliferation, the subcutaneous depot exhibited a significantly higher level than that for visceral depots, thus yielding better organized adipocytes<sup>[42]</sup>. The human sc-ASC showed increased secretion of adiponectin and were more resilient against lipolysis<sup>[42]</sup>. It is further hypothesized that such functional differences may be accounted for by metabolic differences observed between subcutaneous and visceral sources through a ‘memory’ of the fat pad of origin. Interestingly too, this difference does show significant correlation to variations in telomere length; in comparison to sc-ASC, v-ASC actually have been shown to express significantly longer telomere lengths which was strongly correlated to patient age. However, the differences in depot may be a reflection of the shorter telomere length of ASC cells<sup>[57]</sup>. Finally, one study observed a statistically significant difference in fibroblast colony-forming unit CFU-F ability between sc-ASC and v-ASC<sup>[58]</sup>; tissue harvested from subcutaneous sites had a yield of 2.3-fold more CFU-F/unit volume than those from visceral donor sites, thus indicating differences in

stem and progenitor cell content<sup>[58]</sup>. Taken together, in contrast to sc-ASC, v-ASC exhibit lower levels of proliferation, adiposity, and osteogenic potential along with increased cellular heterogeneity, all of which may potentially be attributed to differences in ASC profile dependent on the site of origin. With a relatively high heterogeneity and markedly lower osteogenic capacity compared to sc-ASC, among other drawbacks, v-ASC do not appear to be as promising for clinical applications given other sources of ASC available.

## OMENTAL ADIPOSE DEPOTS

The omental adipose depots are a subset of visceral adipose depots, which also include mesenteric, retroperitoneal, intrathoracic, and gonadal adipose depots<sup>[28]</sup>. Similar to the visceral abdominal human ASC cultures, the omental abdominal human ASC cultures in the study done by Jung *et al* also contained contaminating mesothelial cells at earlier time points in culture<sup>[29]</sup>. In fact, the majority of the cell cultures were mesothelial cells, with only a few spindle-shaped visceral-type ASC. The presence of the mesothelial cells in omental ASC cultures led to lower osteogenic differentiation, consistent with the idea that heterogeneous populations of different ratios of cell types may affect osteogenic differentiation potentials<sup>[29-31]</sup>. The mesothelial cells proved to be short-lived, as evidenced by the predominantly visceral-type ASC in culture by the fourth and fifth passage; however, despite still exhibiting osteogenic differentiation potential, past studies have shown that osteogenic differentiation potential actually decreases as the number of cell passages increases<sup>[29,48]</sup>.

In comparative studies against other sources of fat depots, ASC isolated from the omentum exhibit several notable characteristics. For example, in contrast to intrathoracic depots which show greater levels of adipogenic markers, ASC isolated from the omentum demonstrate greater osteogenic markers in culture<sup>[56]</sup>. Investigators believe that this difference is due to the inherent properties of such adipose cell progenitors. To account for differences of cell types from different depots, flow cytometry analysis was used to demonstrate that subcutaneous adipose tissue contained more adipose tissue-derived cells while the omentum contained increased blood derived cells<sup>[43]</sup>. Proliferation rates were noted to be similar

from both depots, although the findings suggest an overall, lower differentiation capacity for the omental depot<sup>[43]</sup>. These findings have been confirmed via electrophysiological analysis testing for membrane potential and capacitance, again comparing donor-matched subcutaneous adipose tissue to donor-matched omental pads. In another study, a significant difference in proliferation and adipogenesis was found in the subcutaneous-derived population<sup>[59]</sup>. Interestingly, in regards to tissue ultrastructure, the omentum depot was found to be macroscopically less stiff and even more vascularized than those from the abdominal subcutaneous depot. Furthermore, omentum provided for a significantly higher stromal vascular fraction (SVF) yield<sup>[56]</sup>, which may be explained by the presence of more endothelial cells in the vascularized omentum. Taken together, the differences in proliferation, adiposity and osteogenic potential between omentum derived ASC and other depots suggest that the origin of the depot has a profound effect on the predilection and characteristics of the ASC.

## OTHER ADIPOSE DEPOTS

The buccal fat pad in the oral cavity, also known as Bichat's fat pad, has also been identified as a viable source of ASC<sup>[60]</sup>. For example, Niada *et al* assessed the osteogenic potential of porcine ASC from the buccal fat pad compared to sc-ASC and found comparable increases in collagen production, calcified extracellular matrix, alkaline phosphatase, and osteonectin<sup>[46]</sup>. These findings are consistent with the findings of another study involving human ASC from the same region<sup>[45]</sup>. Nevertheless, the buccal fat pad presents the challenge of being limited in supply, along with difficulty of access being in proximity to important neurovasculature.

Aside from omental depots, other smaller depots of visceral adipose depots, such as retroperitoneal and intrathoracic, have not been studied as extensively. One study comparing sc-ASC to retroperitoneal perinephric ASC found that the retroperitoneal ASC had significantly lesser osteogenic capacity<sup>[49]</sup>. Similarly, regarding intrathoracic adipose depots, human pericardial ASC possessed superior osteogenic potential than thymic remnant ASC, though both were of inferior osteogenic differentiation capacity compared to omental and sc-ASC<sup>[32]</sup>. Interestingly, ASC from intrathoracic depots seemed to be oriented more towards adipogenic differentiation even in osteogenic conditions, as seen by spontaneous intracellular lipid accumulation, a phenomenon less commonly observed in the subcutaneous or omental ASC<sup>[32]</sup>.

It is important to note that while retroperitoneal and intrathoracic adipose sources may function in theory, this does not hold practical clinical applications outside of basic biology. A feasible tissue engineering approach would not likely utilize such a source that is both difficult to access and limited in supply.

## DISCUSSION

The current literature regarding adipose tissue resident MSC (or ASC) clearly shows that not all fat is created equally. Clear cut, and for the most part reproducible, differences exist in the osteogenic potential, adipogenic potential and proliferation of ASC depending on their site of origin. How this information should be used is still a matter of uncertainty.

Firstly, differences between sc-ASC clearly exist between anatomic sites, with sc-ASC from the thigh/gluteus/flank appearing to be most primed to undergo osteogenesis. The reasons for this variation by anatomic site are still unclear. Differences in adiposity, vascularity,

and relative isolation of superficial versus deep adipose tissue may play a role. Nevertheless, larger scale studies are needed to verify these findings. In the end, such information is clinically relevant, for example, in that a surgeon may have a better foundation for choosing materials for tissue engineering applications.

Even more striking are the differences in differentiation potential between sc-ASC and v-ASC. These comparisons are more meaningful from a basic biologic perspective, rather than a tissue engineering perspective. Certainly, sc-ASC are an obvious first choice for any tissue engineering application given their abundance and accessibility. Nevertheless, some reproducible differences do exist, including lower levels of proliferation, adiposity, and osteogenic potential along with increased cellular heterogeneity in v-ASC compared to sc-ASC. Furthermore, while studies exploring the effects of paracrine and endocrine signaling by ASC are few, one study did indicate that adipose tissue do secrete endocrine/paracrine activity to elicit effects on the skeletal regeneration<sup>[61]</sup>. Specifically, the endocrine/paracrine activity of some adipose tissue consists of bone anabolic factors, which include insulin like growth factor binding protein (IGFBP)-2 and WNT10b<sup>[61]</sup>. Furthermore, between omental and preadipocytes, the secretomes have been found to be distinct; the former has been found to induce more macrophage and monocyte chemoattraction through Interleukin 6 (IL-6) and Janus kinase (JAK)-mediated signaling<sup>[62]</sup>. Subcutaneous adipose tissue also had higher production of pro-inflammatory cytokines, including IL-6 and IL-8, in contrast to that of abdominal adipose tissue<sup>[63]</sup>. Another study evaluating the effect of origin site on angiogenic growth factor gene expression found that there was a specific correlation between adipocyte size, which was a function of depot location, and vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), fibroblast growth factor-10 (FGF-10), and leptin mRNA levels<sup>[64]</sup>. In both subcutaneous and omental human adipose tissue derived, comparing obese patients versus non-obese patients, there was increased expression of tumor necrosis factor alpha receptor (TNFR)-60 in the former, which correlated with BMI and fat cell size<sup>[65-67]</sup>. Similarly, it has further been hypothesized that this balance between subcutaneous fat and visceral fat may influence systemic insulin resistance, likely through regulation of TNF<sup>[68]</sup>. Potential explanations for these differences have included the degree of vascularity, composition of ASC, presence of contaminating mesothelial cells, and endocrine differences with adiposity highly regulated by glucocorticoids<sup>[69,70]</sup>.

While several studies have investigated the immunomodulatory capacity of ASC, particularly their role in increasing regulatory T cells<sup>[71]</sup>, suppressing T cell function<sup>[72-75]</sup> and natural killer cell cytotoxicity<sup>[76]</sup>, the exact sources of ASC used in these studies remain unspecified. While few studies exist elucidating the differences in immunomodulatory effects of origin-specific ASC, one study by Zhu *et al* recently demonstrated that ASC derived from omental and subcutaneous differ significantly in their inflammatory effects due to greater production of chemokines by omental ASC, which is responsible for inducing migration of macrophages and monocytes, likely through Interleukin 6 (IL-6) and Janus kinase (JAK)-mediated signaling<sup>[62]</sup>. The study also suggests ASC secretion of exosomes via identification of typically intracytoplasmic proteins in the culture medium<sup>[62]</sup>. This hypothesis would be consistent with prior findings of human ASC-derived exosomes' immunomodulatory role via inhibition of T cell proliferation, differentiation, and activation<sup>[72]</sup>. Similarly, in another study by Cohen *et al*, various intra-abdominal fat depots were found to have distinct immunomodulatory micro-environments<sup>[77]</sup>. Specifically, the leukocyte and progenitor

populations were compared, and omental adipose tissue had nearly two-fold more leukocytes and a significantly higher proportion of T-cells than its parametrial and retroperitoneal counterparts<sup>[77]</sup>.

Furthermore, several limitations exist regarding the current literature in comparing ASC depots. For example, there is disagreement in which subset of omental depot is most osteogenic or adipogenic. Not only is there a lack of consensus in terms of clear trends, there is also the underlying problem of a lack of standardization in methods<sup>[32]</sup>. Specifically, extraction protocols, culture conditions, number of passages, and donor demographics are all variables to consider in comparing studies<sup>[32]</sup>. Furthermore, species of ASC derivation is a potential complication factor, as ASC do differ based on species. Several investigators have postulated that anatomical characteristics of each adipose depot such as heterogeneity of cell populations and vasculature may affect the corresponding ASC osteogenic differentiation potentials<sup>[33,43]</sup>. Some studies, on the other hand, have found conflicting results, where non-subcutaneous ASC exhibited either stronger or similar osteogenic indicators compared to ASC from subcutaneous adipose depots<sup>[32,33,44,46]</sup>. In fact, one study found that all ASC possessed the same osteogenic differentiation potential within an individual donor<sup>[78]</sup>. Culture methods too, along with cell isolation techniques and degree of cellular heterogeneity may differ between studies. Considering that the present studies reviewed have also used donor-matched ASC comparisons, this finding brings up an essential point of consideration in the complexity of assessing the osteogenic potential of ASC. Not only does depot-dependent variability exist for osteogenic capacity of ASC, but donor-dependent differences are also a large contributing factor as well. A myriad of donor-dependent factors have been documented as affecting osteogenic potential of ASC, including obesity/body mass index<sup>[79-82]</sup>, osteoarthritis<sup>[81,83]</sup>, and gender<sup>[84]</sup>. Interestingly, donor age did not seem to have a significant effect<sup>[85,86]</sup>. Additionally, the complexity of drawing comparisons between various ASC populations for consensus is further exacerbated by the absence of standardization in research methods and practices pertaining to ASC extraction, culture conditions, and especially the number of passages ASC undergo before being evaluated, which has been found to have a significant impact on osteogenic potential<sup>[48]</sup>. In aggregate, researchers must take into consideration this multitude of contributing factors while evaluating a wide array of osteogenic indicators to obtain a better representation of the efficacy of certain ASC in bone-forming applications.

In terms of clinical applications, there are some concerns regarding the safety of using ASC in regenerative therapy. For one, the literature has shown that excessive adiposity has been associated with increased incidence of breast cancer in post-menopausal women, likely due to IL-6 secretion by ASC which is hypothesized to promote migration and invasion of breast cancer cells<sup>[87,88]</sup>. Another study performed *ex vivo* detected chromosomal abnormalities after extended ASC expansion in culture<sup>[89]</sup>, similar to the findings of other groups that culture propagation of MSC introduces genetic instability and tumorigenicity<sup>[90]</sup>. Furthermore, one ASC study pertaining to bone formation in canine by Kang *et al* found cytologic abnormalities along with increased proliferation associated with ASC<sup>[91,92]</sup>. With regards to muscle formation, human-derived ASC applied to a murine model demonstrated increased inflammation in cardiac muscle<sup>[93]</sup>. Lastly, in the context of cartilage repair, Jung *et al* demonstrated that human-derived ASC applied in a murine model did not trigger any changes in proliferation rate, inflammation, tumor formation, or cytologic

abnormalities<sup>[94]</sup>. On the contrary, an increasing number of recent Phase I and IIb studies focusing on the safety of ASC clinical application do not identify any significant risks or adverse effects in the short term<sup>[95]</sup>. However, it is important to note that the standardization of laboratory practices for ASC is lacking; even for MSC, cell proliferation and local inflammation are assessed in less than one-third of the manuscripts, and of the safety assessment manuscripts, MSC comprise of 85% while ASC constitutes less than 15%<sup>[96]</sup>. Taken together, while some studies report that ASC in clinical applications have not shown any significant risks, others have shown effects on increased inflammation and cytologic abnormalities which would require further investigation.

In terms of future direction of ASC therapy, with its discovery nearly 16 years ago, research in ASC application has made significant progress to provide for a promising stem cell therapy tool<sup>[95]</sup> and interest continues to increase. Regenerative applications of ASC are not limited to bone; ASC have been applied to cartilage repair<sup>[97]</sup>, cardiovascular regeneration<sup>[98]</sup>, and even hair<sup>[99]</sup> among other tissue types. With such versatility to repair and regenerate various tissue types, therapies using ASC has offered a paradigm shift that may provide an alternative solution to numerous diseases. With their relative abundance and ease of harvest from adipose tissue, ASC when combined with other tissue engineering techniques such as scaffolds and growth factors provide great promise as a therapeutic tool for the future<sup>[100]</sup>, and will hopefully overcome the existing limitations surrounding the conventional approaches used in reconstruction surgery.

## CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

## REFERENCES

1. Fang TD, Nacamuli RP, Song HJ, Fong KD, Shi YY, Longaker MT. Guided Tissue Regeneration Enhances Bone Formation in a Rat Model of Failed Osteogenesis. *Plastic and Reconstructive Surgery* 2006; **117**: 1177-1185.
2. Chim H, Schantz JT. New Frontiers in Calvarial Reconstruction: Integrating Computer-Assisted Design and Tissue Engineering in Cranioplasty. *Plastic and Reconstructive Surgery* 2005; **116**: 1726-1741.
3. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, Benhaim P, Lorenz HP, Hedrick MH. Multilineage Cells from Human Adipose Tissue: Implications for Cell-Based Therapies. *Tissue Engineering* 2001; **7**: 211-228.
4. Zielins ER, Tevlin R, Hu MS, Chung MT, McArdele A, Paik KJ, Atashroo D, Duldulao CR, Luan A, Senarath-Yapa K, Walmsley GG, Wearda T, Longaker MT, Wan DC. Isolation and Enrichment of Human Adipose-Derived Stromal Cells for Enhanced Osteogenesis. *J Vis Exp* 2015: 52181.
5. Marble HD, Sutermaster BA, Kanthilal M, Fonseca VC, Darling EM. Gene Expression-Based Enrichment of Live Cells from Adipose Tissue Produces Subpopulations with Improved Osteogenic Potential. *Stem Cell Research & Therapy* 2014; **5**: 145.
6. Chung CG, James AW, Asatrian G, Chang L, Nguyen A, Le K, Bayani G, Lee R, Stoker D, Zhang X, Ting K, Peault B, Soo C. Human Perivascular Stem Cell-Based Bone Graft Substitute Induces Rat Spinal Fusion. *Stem Cells Transl Med* 2014; **3**: 1231-1241.
7. Arana M, Mazo M, Aranda P, Pelacho B, Prosper F. Adipose Tissue-Derived Mesenchymal Stem Cells: Isolation, Expansion, and Characterization. *Methods in Molecular Biology* 2013; **1036**: 47-61.

8. Gaiba S, Franca LP, Franca JP, Ferreira LM. Characterization of Human Adipose-Derived Stem Cells. *Acta Cirurgica Brasileira* 2012; **27**: 471-476.
9. Lee MJ, Wu Y, Fried SK. A Modified Protocol to Maximize Differentiation of Human Preadipocytes and Improve Metabolic Phenotypes. *Obesity (Silver Spring)* 2012; **20**: 2334-2340.
10. Park E, Patel AN. Changes in the Expression Pattern of Mesenchymal and Pluripotent Markers in Human Adipose-Derived Stem Cells. *Cell Biology International* 2010; **34**: 979-984.
11. Schaffler A, Buchler C. Concise Review: Adipose Tissue-Derived Stromal Cells--Basic and Clinical Implications for Novel Cell-Based Therapies. *Stem Cells* 2007; **25**: 818-827.
12. Dragoo JL, Choi JY, Lieberman JR, Huang J, Zuk PA, Zhang J, Hedrick MH, Benhaim P. Bone Induction by Bmp-2 Transduced Stem Cells Derived from Human Fat. *Journal of Orthopaedic Research* 2003; **21**: 622-629.
13. Knippenberg M, Helder MN, Zandieh Doulabi B, Wuisman PI, Klein-Nulend J. Osteogenesis Versus Chondrogenesis by Bmp-2 and Bmp-7 in Adipose Stem Cells. *Biochemical and Biophysical Research Communications* 2006; **342**: 902-908.
14. de Girolamo L, Sartori MF, Albiseti W, Brini AT. Osteogenic Differentiation of Human Adipose-Derived Stem Cells: Comparison of Two Different Inductive Media. *Journal of Tissue Engineering and Regenerative Medicine* 2007; **1**: 154-157.
15. Fang X, Murakami H, Demura S, Hayashi K, Matsubara H, Kato S, Yoshioka K, Inoue K, Ota T, Shinmura K, Tsuchiya H. A Novel Method to Apply Osteogenic Potential of Adipose Derived Stem Cells in Orthopaedic Surgery. *PLoS One* 2014; **9**: e88874.
16. Ogawa R, Mizuno H, Watanabe A, Migita M, Shimada T, Hyakusoku H. Osteogenic and Chondrogenic Differentiation by Adipose-Derived Stem Cells Harvested from Gfp Transgenic Mice. *Biochemical and Biophysical Research Communications* 2004; **313**: 871-877.
17. Frohlich M, Grayson WL, Marolt D, Gimble JM, Kregar-Velikonja N, Vunjak-Novakovic G. Bone Grafts Engineered from Human Adipose-Derived Stem Cells in Perfusion Bioreactor Culture. *Tissue Eng Part A* 2010; **16**: 179-189.
18. Lee JA, Parrett BM, Conejero JA, Laser J, Chen J, Kogon AJ, Nanda D, Grant RT, Breitbart AS. Biological Alchemy: Engineering Bone and Fat from Fat-Derived Stem Cells. *Annals of Plastic Surgery* 2003; **50**: 610-617.
19. James AW, Pang S, Askarinam A, Corselli M, Zara JN, Goyal R, Chang L, Pan A, Shen J, Yuan W, Stoker D, Zhang X, Adams JS, Ting K, Soo C. Additive Effects of Sonic Hedgehog and NELL-1 Signaling in Osteogenic Versus Adipogenic Differentiation of Human Adipose-Derived Stromal Cells. *Stem Cells Dev* 2012; **21**: 2170-2178.
20. Levi B, James AW, Nelson ER, Li S, Peng M, Commons GW, Lee M, Wu B, Longaker MT. Human Adipose-Derived Stromal Cells Stimulate Autogenous Skeletal Repair Via Paracrine Hedgehog Signaling with Calvarial Osteoblasts. *Stem Cells Dev* 2011; **20**: 243-257.
21. James AW, Leucht P, Levi B, Carre AL, Xu Y, Helms JA, Longaker MT. Sonic Hedgehog Influences the Balance of Osteogenesis and Adipogenesis in Mouse Adipose-Derived Stromal Cells. *Tissue Eng Part A* 2010; **16**: 2605-2616.
22. Senarath-Yapa K, McArdle A, Renda A, Longaker MT, Quarto N. Adipose-Derived Stem Cells: A Review of Signaling Networks Governing Cell Fate and Regenerative Potential in the Context of Craniofacial and Long Bone Skeletal Repair. *Int J Mol Sci* 2014; **15**: 9314-9330.
23. Giatsidis G, Dalla Venezia E, Bassetto F. The Role of Gene Therapy in Regenerative Surgery: Updated Insights. *Plastic and Reconstructive Surgery* 2013; **131**: 1425-1435.
24. Heyde M, Partridge KA, Oreffo RO, Howdle SM, Shakesheff KM, Garnett MC. Gene Therapy Used for Tissue Engineering Applications. *Journal of Pharmacy and Pharmacology* 2007; **59**: 329-350.
25. Goessler UR, Riedel K, Hormann K, Riedel F. Perspectives of Gene Therapy in Stem Cell Tissue Engineering. *Cells Tissues Organs* 2006; **183**: 169-179.
26. Eskildsen T, Taipaleenmaki H, Stenvang J, Abdallah BM, Ditzel N, Nossent AY, Bak M, Kauppinen S, Kassem M. MicroRNA-138 Regulates Osteogenic Differentiation of Human Stromal (Mesenchymal) Stem Cells in Vivo. *Proceedings of the National Academy of Sciences of the United States of America* 2011; **108**: 6139-6144.
27. Park J, Ries J, Gelse K, Kloss F, von der Mark K, Wiltfang J, Neukam FW, Schneider H. Bone Regeneration in Critical Size Defects by Cell-Mediated Bmp-2 Gene Transfer: A Comparison of Adenoviral Vectors and Liposomes. *Gene Therapy* 2003; **10**: 1089-1098.
28. Bjorndal B, Burri L, Staalesen V, Skorve J, Berge RK. Different Adipose Depots: Their Role in the Development of Metabolic Syndrome and Mitochondrial Response to Hypolipidemic Agents. *Journal of Obesity* 2011; **2011**: 490650.
29. Jung S, Kleineidam B, Kleinheinz J. Regenerative Potential of Human Adipose-Derived Stromal Cells of Various Origins. *Journal of Cranio-Maxillo-Facial Surgery* 2015; **43**: 2144-2151.
30. Pettersson P, Cigolini M, Sjoström L, Smith U, Bjorntorp P. Cells in Human Adipose Tissue Developing into Adipocytes. *Acta Medica Scandinavica* 1984; **215**: 447-451.
31. Lennon DP, Haynesworth SE, Arm DM, Baber MA, Caplan AI. Dilution of Human Mesenchymal Stem Cells with Dermal Fibroblasts and the Effects on in Vitro and in Vivo Osteochondrogenesis. *Developmental Dynamics* 2000; **219**: 50-62.
32. Russo V, Yu C, Belliveau P, Hamilton A, Flynn LE. Comparison of Human Adipose-Derived Stem Cells Isolated from Subcutaneous, Omental, and Intrathoracic Adipose Tissue Depots for Regenerative Applications. *Stem Cells Transl Med* 2014; **3**: 206-217.
33. Peptan IA, Hong L, Mao JJ. Comparison of Osteogenic Potentials of Visceral and Subcutaneous Adipose-Derived Cells of Rabbits. *Plastic and Reconstructive Surgery* 2006; **117**: 1462-1470.
34. Johal KS, Lees VC, Reid AJ. Adipose-Derived Stem Cells: Selecting for Translational Success. *Regenerative Medicine* 2015; **10**: 79-96.
35. Kanthilal M, Darling EM. Characterization of Mechanical and Regenerative Properties of Human, Adipose Stromal Cells. *Cell Mol Bioeng* 2014; **7**: 585-597.
36. Gronthos S, Franklin DM, Leddy HA, Robey PG, Storms RW, Gimble JM. Surface Protein Characterization of Human Adipose Tissue-Derived Stromal Cells. *Journal of Cellular Physiology* 2001; **189**: 54-63.
37. Mitchell JB, McIntosh K, Zvonic S, Garrett S, Floyd ZE, Kloster A, Di Halvorsen Y, Storms RW, Goh B, Kilroy G, Wu X, Gimble JM. Immunophenotype of Human Adipose-Derived Cells: Temporal Changes in Stromal-Associated and Stem Cell-Associated Markers. *Stem Cells* 2006; **24**: 376-385.
38. Tucker HA, Bunnell BA. Characterization of Human Adipose-Derived Stem Cells Using Flow Cytometry. *Methods in Molecular Biology* 2011; **702**: 121-131.
39. Yu G, Wu X, Dietrich MA, Polk P, Scott LK, Ptiysyn AA, Gimble JM. Yield and Characterization of Subcutaneous Human Adipose-Derived Stem Cells by Flow Cytometric and Adipogenic Mrna Analyses. *Cytotherapy* 2010; **12**: 538-546.
40. Klopp AH, Zhang Y, Solley T, Amaya-Manzanares F, Marini F, Andreeff M, Debeb B, Woodward W, Schmandt R, Broaddus R, Lu K, Kolonin MG. Omental Adipose Tissue-Derived Stromal Cells Promote Vascularization and Growth of Endometrial Tumors. *Clinical Cancer Research* 2012; **18**: 771-782.
41. Dodson MV, Du M, Wang S, Bergen WG, Fernyhough-Culver M, Basu U, Poulos SP, Hausman GJ. Adipose Depots Differ in Cellularity, Adipokines Produced, Gene Expression, and Cell Systems. *Adipocyte* 2014; **3**: 236-241.
42. Baglioni S, Cantini G, Poli G, Francalanci M, Squecco R, Di Franco A, Borgogni E, Frontera S, Nesi G, Liotta F, Lucchese M,

- Perigli G, Francini F, Forti G, Serio M, Luconi M. Functional Differences in Visceral and Subcutaneous Fat Pads Originate from Differences in the Adipose Stem Cell. *PLoS One* 2012; **7**: e36569.
43. Toyoda M, Matsubara Y, Lin K, Sugimachi K, Furue M. Characterization and Comparison of Adipose Tissue-Derived Cells from Human Subcutaneous and Omental Adipose Tissues. *Cell Biochemistry and Function* 2009; **27**: 440-447.
  44. Shah FS, Li J, Dietrich M, Wu X, Hausmann MG, LeBlanc KA, Wade JW, Gimble JM. Comparison of Stromal/Stem Cells Isolated from Human Omental and Subcutaneous Adipose Depots: Differentiation and Immunophenotypic Characterization. *Cells Tissues Organs* 2014; **200**: 204-211.
  45. Broccaioli E, Niada S, Rasperini G, Ferreira LM, Arrigoni E, Yenagi V, Brini AT. Mesenchymal Stem Cells from Bichat's Fat Pad: In Vitro Comparison with Adipose-Derived Stem Cells from Subcutaneous Tissue. *Biores Open Access* 2013; **2**: 107-117.
  46. Niada S, Ferreira LM, Arrigoni E, Addis A, Campagnol M, Broccaioli E, Brini AT. Porcine Adipose-Derived Stem Cells from Buccal Fat Pad and Subcutaneous Adipose Tissue for Future Preclinical Studies in Oral Surgery. *Stem Cell Research & Therapy* 2013; **4**: 148.
  47. Iwen KA, Prieue AC, Winnefeld M, Rose C, Siemers F, Rohwedel J, Cakiroglu F, Lehnert H, Schepky A, Klein J, Kramer J. Gluteal and Abdominal Subcutaneous Adipose Tissue Depots as Stroma Cell Source: Gluteal Cells Display Increased Adipogenic and Osteogenic Differentiation Potentials. *Experimental Dermatology* 2014; **23**: 395-400.
  48. Requiça JF, Viegas CA, Albuquerque CM, Azevedo JM, Reis RL, Gomes ME. Effect of Anatomical Origin and Cell Passage Number on the Stemness and Osteogenic Differentiation Potential of Canine Adipose-Derived Stem Cells. *Stem Cell Rev* 2012; **8**: 1211-1222.
  49. Chen L, Peng EJ, Zeng XY, Zhuang QY, Ye ZQ. Comparison of the Proliferation, Viability, and Differentiation Capacity of Adipose-Derived Stem Cells from Different Anatomic Sites in Rabbits. *Cells Tissues Organs* 2012; **196**: 13-22.
  50. Ni YW, Zhou YS, Liu YS, Zeng BJ, Xu YW. [Comparison of Biological Characteristics of Human, Rabbit and Rat Adipose Tissue-Derived Stromal Cells in Vitro]. *Beijing Da Xue Xue Bao* 2009; **41**: 95-99.
  51. Levi B, James AW, Glotzbach JP, Wan DC, Commons GW, Longaker MT. Depot-Specific Variation in the Osteogenic and Adipogenic Potential of Human Adipose-Derived Stromal Cells. *Plastic and Reconstructive Surgery* 2010; **126**: 822-834.
  52. Arrigoni E, Lopa S, de Girolamo L, Stanco D, Brini AT. Isolation, Characterization and Osteogenic Differentiation of Adipose-Derived Stem Cells: From Small to Large Animal Models. *Cell and Tissue Research* 2009; **338**: 401-411.
  53. Cui L, Liu B, Liu G, Zhang W, Cen L, Sun J, Yin S, Liu W, Cao Y. Repair of Cranial Bone Defects with Adipose Derived Stem Cells and Coral Scaffold in a Canine Model. *Biomaterials* 2007; **28**: 5477-5486.
  54. Choi JW, Park EJ, Shin HS, Shin IS, Ra JC, Koh KS. In Vivo Differentiation of Undifferentiated Human Adipose Tissue-Derived Mesenchymal Stem Cells in Critical-Sized Calvarial Bone Defects. *Annals of Plastic Surgery* 2014; **72**: 225-233.
  55. Sandor GK, Numminen J, Wolff J, Thesleff T, Miettinen A, Tuovinen VJ, Mannerstrom B, Patrikoski M, Seppanen R, Miettinen S, Rautiainen M, Ohman J. Adipose Stem Cells Used to Reconstruct 13 Cases with Cranio-Maxillofacial Hard-Tissue Defects. *Stem Cells Transl Med* 2014; **3**: 530-540.
  56. Cecio A, Vittoria A, Budetta G, Corona M. Staining of Neuroendocrine Cells by Linder's Argyrophil Method. *Journal of Anatomy* 1988; **157**: 197-201.
  57. Lakowa N, Trieu N, Flehmig G, Lohmann T, Schon MR, Dietrich A, Zeplin PH, Langer S, Stumvoll M, Blüher M, Klötting N. Telomere Length Differences between Subcutaneous and Visceral Adipose Tissue in Humans. *Biochemical and Biophysical Research Communications* 2015; **457**: 426-432.
  58. Fraser J, Wulur I, Alfonso Z, Zhu M, Wheeler E. Differences in Stem and Progenitor Cell Yield in Different Subcutaneous Adipose Tissue Depots. *Cytotherapy* 2007; **9**: 459-467.
  59. Barczewski RA, Kornegay ET, Notter DR, Veit HP, Wright ME. Effects of Feeding Restricted Energy and Elevated Calcium and Phosphorus During Growth on Gait Characteristics of Culled Sows and Those Surviving Three Parities. *Journal of Animal Science* 1990; **68**: 3046-3055.
  60. Farre-Guasch E, Marti-Page C, Hernandez-Alfaro F, Klein-Nulend J, Casals N. Buccal Fat Pad, an Oral Access Source of Human Adipose Stem Cells with Potential for Osteochondral Tissue Engineering: An in Vitro Study. *Tissue Eng Part C Methods* 2010; **16**: 1083-1094.
  61. Rahman S, Lu Y, Czernik PJ, Rosen CJ, Enerback S, Lecka-Czernik B. Inducible Brown Adipose Tissue, or Beige Fat, Is Anabolic for the Skeleton. *Endocrinology* 2013; **154**: 2687-2701.
  62. Zhu Y, Tchkonja T, Stout MB, Giorgadze N, Wang L, Li PW, Hoppelmann CJ, Bouloumie A, Jensen MD, Bergen HR, 3rd, Kirkland JL. Inflammation and the Depot-Specific Secretome of Human Preadipocytes. *Obesity (Silver Spring)* 2015; **23**: 989-999.
  63. Nielsen NB, Hojbjerg L, Sonne MP, Alibegovic AC, Vaag A, Dela F, Stallknecht B. Interstitial Concentrations of Adipokines in Subcutaneous Abdominal and Femoral Adipose Tissue. *Regulatory Peptides* 2009; **155**: 39-45.
  64. Yamada T, Kawakami S, Nakanishi N. Fat Depot-Specific Differences in Angiogenic Growth Factor Gene Expression and Its Relation to Adipocyte Size in Cattle. *Journal of Veterinary Medical Science* 2010; **72**: 991-997.
  65. Hube F, Birgel M, Lee YM, Hauner H. Expression Pattern of Tumour Necrosis Factor Receptors in Subcutaneous and Omental Human Adipose Tissue: Role of Obesity and Non-Insulin-Dependent Diabetes Mellitus. *European Journal of Clinical Investigation* 1999; **29**: 672-678.
  66. Winkler G, Kiss S, Keszthelyi L, Sapi Z, Ory I, Salamon F, Kovacs M, Vargha P, Szekeres O, Speer G, Karadi I, Sikter M, Kaszas E, Dworak O, Gero G, Cseh K. Expression of Tumor Necrosis Factor (Tnf)-Alpha Protein in the Subcutaneous and Visceral Adipose Tissue in Correlation with Adipocyte Cell Volume, Serum Tnf-Alpha, Soluble Serum Tnf-Receptor-2 Concentrations and C-Peptide Level. *European Journal of Endocrinology of the European Federation of Endocrine Societies* 2003; **149**: 129-135.
  67. von Eyben FE, Kroustrup JP, Larsen JF, Celis J. Abdominal Obesity Study G. Comparison of Gene Expression in Intra-Abdominal and Subcutaneous Fat: A Study of Men with Morbid Obesity and Nonobese Men Using Microarray and Proteomics. *Annals of the New York Academy of Sciences* 2004; **1030**: 508-536.
  68. Ishikawa K, Takahashi K, Bujo H, Hashimoto N, Yagui K, Saito Y. Subcutaneous Fat Modulates Insulin Sensitivity in Mice by Regulating Tnf-Alpha Expression in Visceral Fat. *Hormone and Metabolic Research* 2006; **38**: 631-638.
  69. van Beek EA, Bakker AH, Kruyt PM, Hofker MH, Saris WH, Keijer J. Intra- and Interindividual Variation in Gene Expression in Human Adipose Tissue. *Pflügers Archiv European Journal of Physiology* 2007; **453**: 851-861.
  70. Lee MJ, Gong DW, Burke BF, Fried SK. Pathways Regulated by Glucocorticoids in Omental and Subcutaneous Human Adipose Tissues: A Microarray Study. *American Journal of Physiology: Endocrinology and Metabolism* 2011; **300**: E571-580.
  71. Engela AU, Baan CC, Peeters AM, Weimar W, Hoogduijn MJ. Interaction between Adipose Tissue-Derived Mesenchymal Stem Cells and Regulatory T-Cells. *Cell Transplantation* 2013; **22**: 41-54.
  72. Blazquez R, Sanchez-Margallo FM, de la Rosa O, Dalemans W, Alvarez V, Tarazona R, Casado JG. Immunomodulatory Potential of Human Adipose Mesenchymal Stem Cells Derived Exosomes

- on in Vitro Stimulated T Cells. *Frontiers in Immunology* 2014; **5**: 556.
73. DelaRosa O, Lombardo E, Beraza A, Mancheno-Corvo P, Ramirez C, Menta R, Rico L, Camarillo E, Garcia L, Abad JL, Trigueros C, Delgado M, Buscher D. Requirement of Ifn-Gamma-Mediated Indoleamine 2,3-Dioxygenase Expression in the Modulation of Lymphocyte Proliferation by Human Adipose-Derived Stem Cells. *Tissue Eng Part A* 2009; **15**: 2795-2806.
  74. Yanez R, Lamana ML, Garcia-Castro J, Colmenero I, Ramirez M, Bueren JA. Adipose Tissue-Derived Mesenchymal Stem Cells Have in Vivo Immunosuppressive Properties Applicable for the Control of the Graft-Versus-Host Disease. *Stem Cells* 2006; **24**: 2582-2591.
  75. McIntosh K, Zvonic S, Garrett S, Mitchell JB, Floyd ZE, Hammill L, Kloster A, Di Halvorsen Y, Ting JP, Storms RW, Goh B, Kilroy G, Wu X, Gimble JM. The Immunogenicity of Human Adipose-Derived Cells: Temporal Changes in Vitro. *Stem Cells* 2006; **24**: 1246-1253.
  76. DelaRosa O, Sanchez-Correa B, Morgado S, Ramirez C, del Rio B, Menta R, Lombardo E, Tarazona R, Casado JG. Human Adipose-Derived Stem Cells Impair Natural Killer Cell Function and Exhibit Low Susceptibility to Natural Killer-Mediated Lysis. *Stem Cells Dev* 2012; **21**: 1333-1343.
  77. Cohen CA, Shea AA, Heffron CL, Schmelz EM, Roberts PC. Intra-Abdominal Fat Depots Represent Distinct Immunomodulatory Microenvironments: A Murine Model. *PLoS One* 2013; **8**: e66477.
  78. Choudhery MS, Badowski M, Muise A, Pierce J, Harris DT. Subcutaneous Adipose Tissue-Derived Stem Cell Utility Is Independent of Anatomical Harvest Site. *Biores Open Access* 2015; **4**: 131-145.
  79. Sadie-Van Gijzen H, Smith W, du Toit EF, Michie J, Hough FS, Ferris WF. Depot-Specific and Hypercaloric Diet-Induced Effects on the Osteoblast and Adipocyte Differentiation Potential of Adipose-Derived Stromal Cells. *Molecular and Cellular Endocrinology* 2012; **348**: 55-66.
  80. Watson JE, Patel NA, Carter G, Moor A, Patel R, Ghansah T, Mathur A, Murr MM, Bickford P, Gould LJ, Cooper DR. Comparison of Markers and Functional Attributes of Human Adipose-Derived Stem Cells and Dedifferentiated Adipocyte Cells from Subcutaneous Fat of an Obese Diabetic Donor. *Adv Wound Care (New Rochelle)* 2014; **3**: 219-228.
  81. De Girolamo L, Stanco D, Salvatori L, Coroniti G, Arrigoni E, Silecchia G, Russo MA, Niada S, Petrangeli E, Brini AT. Stemness and Osteogenic and Adipogenic Potential Are Differently Impaired in Subcutaneous and Visceral Adipose Derived Stem Cells (Ascs) Isolated from Obese Donors. *International Journal of Immunopathology and Pharmacology* 2013; **26**: 11-21.
  82. Frazier TP, Gimble JM, Devay JW, Tucker HA, Chiu ES, Rowan BG. Body Mass Index Affects Proliferation and Osteogenic Differentiation of Human Subcutaneous Adipose Tissue-Derived Stem Cells. *BMC Cell Biology* 2013; **14**: 34.
  83. Lopa S, Colombini A, Stanco D, de Girolamo L, Sansone V, Moretti M. Donor-Matched Mesenchymal Stem Cells from Knee Infrapatellar and Subcutaneous Adipose Tissue of Osteoarthritic Donors Display Differential Chondrogenic and Osteogenic Commitment. *Eur Cell Mater* 2014; **27**: 298-311.
  84. Schipper BM, Marra KG, Zhang W, Donnenberg AD, Rubin JP. Regional Anatomic and Age Effects on Cell Function of Human Adipose-Derived Stem Cells. *Annals of Plastic Surgery* 2008; **60**: 538-544.
  85. Ding DC, Chou HL, Hung WT, Liu HW, Chu TY. Human Adipose-Derived Stem Cells Cultured in Keratinocyte Serum Free Medium: Donor's Age Does Not Affect the Proliferation and Differentiation Capacities. *Journal of Biomedical Science* 2013; **20**: 59.
  86. Wu W, Niklason L, Steinbacher DM. The Effect of Age on Human Adipose-Derived Stem Cells. *Plastic and Reconstructive Surgery* 2013; **131**: 27-37.
  87. Bielli A, Scioli MG, Gentile P, Agostinelli S, Tarquini C, Cervelli V, Orlandi A. Adult Adipose-Derived Stem Cells and Breast Cancer: A Controversial Relationship. *Springerplus* 2014; **3**: 345.
  88. Walter M, Liang S, Ghosh S, Hornsby PJ, Li R. Interleukin 6 Secreted from Adipose Stromal Cells Promotes Migration and Invasion of Breast Cancer Cells. *Oncogene* 2009; **28**: 2745-2755.
  89. Bellotti C, Stanco D, Ragazzini S, Romagnoli L, Martella E, Lazzati S, Marchetti C, Donati D, Lucarelli E. Analysis of the Karyotype of Expanded Human Adipose-Derived Stem Cells for Bone Reconstruction of the Maxillo-Facial Region. *International Journal of Immunopathology and Pharmacology* 2013; **26**: 3-9.
  90. Dahl JA, Duggal S, Coulston N, Millar D, Melki J, Shahdadfar A, Brinchmann JE, Collas P. Genetic and Epigenetic Instability of Human Bone Marrow Mesenchymal Stem Cells Expanded in Autologous Serum or Fetal Bovine Serum. *International Journal of Developmental Biology* 2008; **52**: 1033-1042.
  91. Kang BJ, Ryu HH, Park SS, Koyama Y, Kikuchi M, Woo HM, Kim WH, Kweon OK. Comparing the Osteogenic Potential of Canine Mesenchymal Stem Cells Derived from Adipose Tissues, Bone Marrow, Umbilical Cord Blood, and Wharton's Jelly for Treating Bone Defects. *Journal of Veterinary Science* 2012; **13**: 299-310.
  92. Kang BJ, Ryu HH, Park SS, Kim Y, Woo HM, Kim WH, Kweon OK. Effect of Matrigel on the Osteogenic Potential of Canine Adipose Tissue-Derived Mesenchymal Stem Cells. *Journal of Veterinary Medical Science* 2012; **74**: 827-836.
  93. de la Garza-Rodea AS, van der Velde-van Dijke I, Boersma H, Goncalves MA, van Bekkum DW, de Vries AA, Knaan-Shanzer S. Myogenic Properties of Human Mesenchymal Stem Cells Derived from Three Different Sources. *Cell Transplantation* 2012; **21**: 153-173.
  94. Jung SN, Rhie JW, Kwon H, Jun YJ, Seo JW, Yoo G, Oh DY, Ahn ST, Woo J, Oh J. In Vivo Cartilage Formation Using Chondrogenic-Differentiated Human Adipose-Derived Mesenchymal Stem Cells Mixed with Fibrin Glue. *Journal of Craniofacial Surgery* 2010; **21**: 468-472.
  95. Feisst V, Meidinger S, Locke MB. From Bench to Bedside: Use of Human Adipose-Derived Stem Cells. *Stem Cells Cloning* 2015; **8**: 149-162.
  96. Jeong JO, Han JW, Kim JM, Cho HJ, Park C, Lee N, Kim DW, Yoon YS. Malignant Tumor Formation after Transplantation of Short-Term Cultured Bone Marrow Mesenchymal Stem Cells in Experimental Myocardial Infarction and Diabetic Neuropathy. *Circulation Research* 2011; **108**: 1340-1347.
  97. Im GI. Regeneration of Articular Cartilage Using Adipose Stem Cells. *J Biomed Mater Res A* 2016:
  98. Suzuki E, Fujita D, Takahashi M, Oba S, Nishimatsu H. Adipose Tissue-Derived Stem Cells as a Therapeutic Tool for Cardiovascular Disease. *World Journal of Cardiology* 2015; **7**: 454-465.
  99. Jin SE, Sung JH. Hair Regeneration Using Adipose-Derived Stem Cells. *Histology and Histopathology* 2016; **31**: 249-256.
  100. He Y, Lu F. Development of Synthetic and Natural Materials for Tissue Engineering Applications Using Adipose Stem Cells. *Stem Cells Int* 2016; **2016**: 5786257.
- Peer reviewers:** Gaël Rochefort, PhD HDR, EA2496, Faculté de chirurgie dentaire, Université Paris Descartes, 1, rue Maurice Arnoux, 92120 Montrouge France; Rubén Daniel Arellano, PhD, Full time Professor Researcher Medicine School, Torre, México; Andrea Del Fattore, PhD, Regenerative Medicine Area, Children's Hospital Bambino Gesù, Padiglione Giovanni Paolo II, Lab Ricerca, piazza Sant'Onofrio 4, 00165, Rome, Italy.