

ADIPOSE-DERIVED STEM CELLS IN TISSUE ENGINEERING: LABORATORY TO BEDSIDE

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ABSTRACT

Human adipose tissue has been recognized as an alternative source of adult stem cells. The abundance and ease of harvest of adipose tissue has made it suitable for use in regenerative medicine and tissue engineering. Adipose-derived stem cells isolated from human adipose tissue are able to differentiate into several mesenchymal lineages and secrete growth factors that exhibit therapeutic potential. Protein profiles have been established using various isolation methods, which has expanded researchers' understanding of adipose-derived stem cells in clinical applications. This review highlights the properties, isolation methods, immunophenotype and clinical applications of adipose-derived stem cells.

Keywords: *adipose-derived stem cells, immunophenotyping, isolation methods, clinical applications*

Introduction

Advances in the fields of biomedical engineering, biochemistry, genetics, material science, cell biology and molecular biology have given rise to the remarkable new cross-disciplinary field of tissue engineering. Large-scale human and animal cell cultures that include skin, cartilage, muscle, bone, endothelial and stem cells have been studied and clinically applied to replace damaged tissues or organs in humans. This new branch of modern medicine has been termed regenerative medicine. Regenerative medicine combines several factors, including cells, growth factors and biomaterials. Strategies include cell therapy, biomaterials alone and combinations of cells and biomaterials (1). When implanted in the body as temporary structures, naturally derived or synthetic biomaterials can provide templates that guide tissue formation while the scaffold is gradually biodegraded. Stem cells for regenerative medicine should have the following criteria: they (a) are abundantly present, (b) can be harvested using minimally invasive procedures, (c) are able to reproducibly differentiate into multiple cell lineages, (d) can be safely and effectively transplanted to either an autologous or allogeneic host, and (e) can be manufactured in accordance with current Good Manufacturing Practice (GMP) guidelines (2).

Generally, stem cells are unspecialized cells that are able to multiply themselves or become specialized types of cells.

Stem cells can be divided into several classes according to potency; namely, totipotent stem cells can build an entire organism, and pluripotent stem cells can become all tissue types except for extraembryonic tissues such as placenta and amnion. Multipotent stem cells are able to develop into more than one cell type in the body. Cells that can differentiate into only one cell type are known as precursor cells or progenitor cells (3). Bone marrow is a recognized source of mesenchymal stem cells for regenerative medicine. Mesenchymal stem cells (MSC) from bone marrow are aspirated from patients using painful procedures and with relatively lower yields of cells than MSCs from adipose tissue (4).

Embryonic stem cells (ESCs) are derived from the inner cell mass of the blastocyst during embryogenesis. ESCs were first isolated from mouse embryos in 1980 and then later from humans in 1998 (5). Despite their pluripotency, humanity and ethics concerns relating to the destruction of embryos to obtain ESCs have been raised by various religious and research institutions worldwide. Differentiated cells reprogrammed to stem cells, called induced pluripotent stem (iPS) cells, are another interesting source of stem cells. Typically, iPS cells are produced by transfecting differentiated or adult cells with stem cell-associated genes. Thus, their properties are remarkably similar to ESCs (6).

Adult stem cells can be isolated from a mature organ such as bone marrow, adipose tissue, dermis, trabecular bone, periosteum, pericytes, blood and synovial membrane (7). These stem cells usually replicate to replace the dead cells of their origin organ. Thus, their multi-potency to differentiate into other lineages can be employed in stem cell-based therapy. In 2006, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy provided several guidelines to define human mesenchymal stromal cells, such as adherence to plastic culture flasks; expression of specific antigens/markers such as CD105, CD73 and CD90; and multipotent *in vitro* multi-lineage differentiation into osteoblasts, adipocytes and chondroblasts (8).

Adipose tissue and adipose-derived stem cells

Adipose tissue modulates energy homeostasis and secretes lipids and proteins, as well as peptides such as hormones, adipokines and cytokines (9). The majority of adipose tissue in mammals can be found in the subcutaneous and intra-abdominal areas (10). Subcutaneous fat is the fat present below the skin, and intra-abdominal fat is omental, mesenteric and perirenal. There are two types of adipose tissue: white adipose tissue (WAT) and brown adipose tissue (BAT). White adipose tissue is predominantly present in adults, while brown adipose tissue is present in newborns. The largest deposits of WAT are in the subcutaneous and visceral areas (11). Based on structural and ultra-structural features, three types of subcutaneous WAT have emerged: deposit WAT (dWAT), structural WAT (sWAT) and fibrous WAT (fWAT) (12).

Adipose tissue originates from the mesodermal layer during embryogenesis. Adipose tissue is vascularized by a capillary network of blood vessels during development. Mature adipocytes are filled with a single lipid droplet interlarded with stroma, which is composed of fibroblasts, blood vessels, leukocytes, macrophages and preadipocytes (lacking the lipid droplet) (13). Approximately 10% of fat cells are renewed each year, irrespective of body mass index and at all adult ages (14). As adipocytes are a terminally differentiated cell, their replacement with new adipocytes to compensate for lost adipocytes originates from a population of progenitor cells (pre-adipocytes) that are present in the stromal fraction of the adipose tissue (15, 16).

Adipose tissue progenitor cells were initially thought to only differentiate into adipocytes. However, these cells have gained greater attention through recent research work showing that cells isolated from lipoaspirate have multipotential ability *in vitro* to differentiate outside of the adipogenic lineage, including differentiation to chondrogenic, myogenic, and osteogenic cells via the use of specific growth medium induction (17). Therefore, adipose tissue harvested with minimal pain and great availability from surgical operations presents an alternative and promising source of stem cells for use in regenerative medicine. Many terms have been used in the literature to

refer to cells isolated from adipose tissue, including adipose derived stem/stromal cells (ASCs), adipose-derived adult stem (ADAS) cells, adipose-derived adult stromal cells, adipose-derived stromal cells (ADSC), adipose stromal cells (ASC), adipose mesenchymal stem cells (AdMSCs), lipoblasts, pericytes, pre-adipocytes, and processed lipoaspirate (PLA) cells (18). To avoid confusion, the International Federation for Adipose Therapeutics and Science (IFATS) agreed to adopt the term “adipose-derived stem cells” (ASCs) to identify the isolated, plastic-adherent and multipotent cell population (18).

ASCs from adipose tissue have similar multipotency to that of MSCs from bone marrow (4). The population of ASCs may partly originate from pericytes and fibroblasts from surrounding adipose tissue environment (19). Pericytes are cells that line endothelial cells in capillaries and microvessels. Pericytic markers are expressed in ASCs cultures, implying that ASCs cultures may originate from pericytes resident in proximity to adipose tissue (20, 21). However, native ASCs do not express *in situ* pericytic markers, and ASCs can be found scattered in adipose tissue stromal (16).

The stromal vascular fraction (SVF) is the pellet obtained from collagenase digestion of adipose tissue. It can be composed of a heterogeneous cell population that includes pre-adipocytes, endothelial cells, fibroblasts, smooth muscle cells, pericytes, leukocytes and mast cells (22). However, cultured SVF displayed a fibroblast-like morphology and was maintained *in vitro* long term with a low level of senescence (17, 23).

There is increasing evidence that ASCs can be differentiated into mesodermal lineages, including adipogenic (24, 25), osteogenic (24, 26), chondrogenic (27, 28) and myogenic (29, 30) lineages in their respective growth medium. Moreover, ASCs can also be differentiated into non-mesodermal lineages, such as endothelial cells (24, 31), neuronal cells (32), epithelial cells (33, 34), pancreatic cells (35) and hepatocytes (36). Despite their multipotency, ASCs secrete several angiogenic and antiapoptotic factors such as vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF), which may act synergistically for cardiovascular protection (37).

In vitro study has shown that ASCs promote dermal wound healing by increasing collagen synthesis, thus promoting fibroblast proliferation via the paracrine activity of ASCs secretory factors (38). *In vivo* study has shown that ASCs significantly reduce wound size in mice (38). Furthermore, factors secreted by ASCs produce a whitening effect by inhibiting melanin synthesis (39). ASC-conditioned medium has antioxidant properties because they protect human dermal fibroblasts from oxidative injury (40). Hair growth is stimulated upon treatment with the growth factors secreted by ASCs (41). In addition to wound healing, ASCs also have anti-ageing properties that act by stimulating collagen synthesis and angiogenesis (42).

In reconstructive surgery, skin flaps are used to repair defects caused by injuries and other causes. A lack

of blood supply results in necrosis of the flap tissue. New blood vessel formation and the elongation of existing blood vessels are two mechanisms that assist revascularization. Newly formed blood vessels originating from the differentiation of ASCs into endothelial cells and the secretion of angiogenic factors improve the viability of the skin flap (43, 44).

***In vitro* isolation and propagation technology for ASCs**

Primary cultures are created when cells grow out from an explants or attach to substrate after enzymatic or mechanical disaggregation (45). Primary cultures can be defined as a mixture of cells or highly purified cells isolated directly from organism. The function of cells from a primary culture is expected to resemble that of *in vivo* cells (46). The cell isolation procedure is performed in a class II biosafety cabinet to minimize contamination. There is no standard protocol for the isolation of human ASCs. Generally, adipose tissue is enzymatically digested, centrifuged and filtered (see Figure 1). Factors including heterogeneity between patients, tissue depot, concentration and collagenase type, centrifugation and media formulations may result in variability in the yield and *in vitro* characteristics of the cells. These factors may affect marker expression, differentiation capability and therapeutic potential.

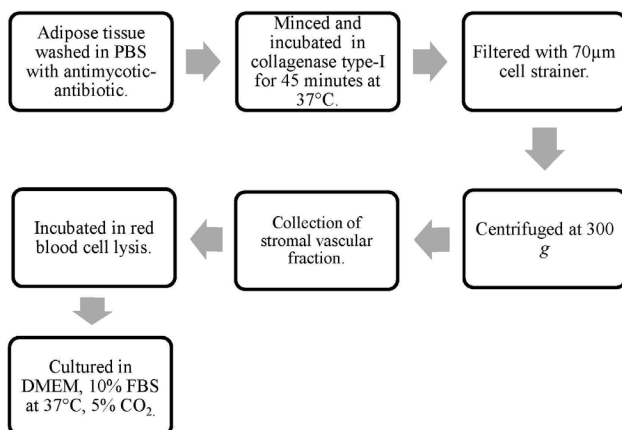


Figure 1: Isolation of ADSCs from human adipose tissue. Abbreviations: PBS: phosphate buffered saline, DMEM: Dulbecco's modified eagle medium, FBS: fetal bovine serum.

One of the ways to isolate cells from tissue is by enzymatic disaggregation. Tissue is washed with phosphate-buffered saline to remove debris and blood. It is then minced and incubated in collagenase with continuous rotation. Collagenase is widely used as an enzyme to help disperse the collagenase bundles that are present in the extracellular matrix and thus dissociate cells from tissue. Many types of digestion buffer have been used to digest the tissue, including collagenase type I (18) and collagenase with trypsin (23). The optimal collagenase concentration and

digestion time are 0.2% and 1 hour, respectively (47). The combination of collagenase with trypsin can shorten the time of incubation from 3 hours to 20 minutes (23). Trypsin is used to dissociate the cells. After incubation, the activity of collagenase and trypsin can be neutralized with growth medium containing serum. Collagenase is less aggressive than trypsin (48). The presence of erythrocytes can reduce ASCs adherence and proliferation (49). Therefore, erythrocyte cross-contamination can be overcome by lysing the red blood cells in lysis buffer. However, erythrocytes are also non-adherent to plastic, allowing primarily ASCs to attach.

The cells are centrifuged at 180 *g* for 5 minutes and filtered through a 70- μ m cell strainer to obtain a single cell suspension and remove the undigested tissue. Centrifugation is not a precise cell separation technique; however, it concentrates the cells and removes fractions of cells based on density. Two measurements of centrifugal force are used: rpm (revolutions per minute) and *g* (relative centrifugal force). The same centrifugal force may correspond to different rpm values among researchers if they have different radii of centrifugation. Therefore, specific gravity is used for standardization. Centrifugation of adipose tissue at more than 3000 *g* significantly damaged the adipose-derived stem cells, and the number of ASCs remained unchanged at 1200 *g* (50). Another finding suggested 1126 *g* as the optimal centrifugal force in comparison to 12,519 *g* and 4507 *g*, which resulted in more viable ASCs (51). To date, there is no extensive research on the relationship of various centrifugal forces on the yield of ASCs.

ASCs in the culture appear spindle-shaped or fibroblast-like (17, 23, 52). When 70-80% confluence is reached, the cultured cells need to be further propagated in additional vessels, a procedure called sub-culture. Cells can be detached from the culture flask by incubating the cells in dissociating agents, such as trypsin, for 3-5 minutes at 37°C. Culture flasks can be tapped to assist with cell detachment. Detached cells usually float and exhibit a round shape in the culture medium.

A simple and fast method to quantify viable cells utilizes the trypan blue exclusion assay. Viable cells with intact membranes do not take up the blue dye; thus, dead cells with blue color can be distinguished from viable cells. The expansion of cells *in vitro* requires growth medium. Growth medium provides necessary nutrients, growth factors and hormones, and it regulates the culture pH and osmotic pressure. This medium is a combination of basal media and 10-20% of serum. Basal media is a media that contains nutrients such as amino acids, vitamins, inorganic salts and a carbon source, such as glucose. The most frequently used basal media for ASC culture are Dulbecco's modified Eagle medium (DMEM) (35, 53-57), DMEM/F12 (58-62) and alpha-modified Eagle medium (α -MEM) (63). The expansion of ASCs in α -MEM is significantly better than the expansion in DMEM, DMEM/F12, F12 and LPO2 (commercial media free from animal product) when all are supplemented with 10% serum (64). This parameter

is evaluated based on the differentiation capacity of ASCs rather than the immunophenotype of the ASCs (64). Laboratory-made media composed of basal media, several growth factors such as basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF), and serum have preserved the stemness of ASCs, as measured by their proliferation and expression of stemness transcriptional markers (49). However, human MSC stemness from bone marrow is better preserved in DMEM than in Iscove's modified Dulbecco's medium (IMDM) (65). High calcium medium can cause cell differentiation. Low calcium medium can accelerate proliferation and prolong their lifespan (66).

ASC stemness should be preserved in both *in vitro* and *in vivo* situations for optimal effects in regenerative medicine. The most common serum used is animal-derived, such as fetal bovine serum and fetal calf serum. Sera from animals may cause contamination and are expensive. Therefore, researchers are looking into new alternative formulations of media, such as serum-free media or animal-free sera (67-69). In addition, we can avoid transmission of diseases derived from animals if ASCs are employed clinically in humans. Similar results are obtained when porcine-derived trypsin is compared with animal protein-free products based on yield, viability and immunophenotype of the human ASCs (70). There is also a similar result for *in vitro* and *in vivo* proliferation and differentiation of human skeletal-derived MSCs when cultured in human serum and fetal bovine serum (71). Another study states that ASCs cultured in human allogeneic serum and fetal bovine serum have a similar surface marker phenotype but differ in gene expression after differentiation (72). Artificial serum substitute induces more profound cellular physiological changes in comparison to ASCs grown in fetal calf serum, pretested fetal calf serum or human allogeneic serum (73). Despite the fact that animal-free serum such as human serum may be a great alternative, mass production of serum from healthy humans may be needed for long term ASC culture.

The site of tissue harvesting may influence the concentration and function of human ASCs. Lipoaspirates from the inner thigh and lower abdomen may have a higher concentration of MSCs than lipoaspirates harvested from the upper abdomen, trochanteric region, knee, and flank (74). Different locations on the same individual may yield different amounts of stem cells. Subcutaneous adipose tissue from the hip has more stem cells than the subcutaneous space of the abdomen (75). This finding contradicts the results of a study, which found that subcutaneous adipose tissue abdomen yields more ASCs than adipose tissue from the hip or thigh region (22). In terms of gender, the abdomen has the highest cell yield in males compared to the knees and back region, whereas there is no significant relationship between the collection site and cell yield in females (47). The osteogenic differentiation capability of ASCs from superficial and deep fat and the dependence on gender and age have been examined. ASCs from the superficial layer of fat from males differentiate into osteocytes faster than those from the deep layer, while

there is no significant differences of osteogenic properties of both depot in female (76). In humans, there are no data supporting which type of adipose tissue has greater plasticity. Nevertheless, subcutaneous structural WAT sites that have weak collagenic bundles and are rich in stem cells are preferable to sources of deposit WAT and fibrous WAT (12). However, in mice, inguinal WAT has shown greater differentiation potential than BAT (77).

There is inconsistency among researchers regarding the influence of age and body mass index (BMI) on the proliferation of ASCs. ASCs from younger donors have a higher proliferation rate than those from older donors (78). However, a study has found no significant relationship between age and the proliferation rate of ASCs (79). There is no significant correlation between the frequency of adipose-derived stem cells and BMI (22), and there are no statistical correlations between age and BMI and the proliferation and yield of ASCs (47, 80). Different isolation methods, culture conditions and sites from which adipose tissue is taken may cause variation between studies.

In addition to the manual isolation of cells, there is an automated device called the Celution™ system that is designed to wash, digest and concentrate cells from adipose tissue. These isolated cells comprise a heterogeneous population that contains ASCs, endothelial cells and vascular smooth muscle cells. The cells isolated by a Celution™ system possess equivalent expression results for certain markers and equivalent differentiation ability to cells isolated by the manual method (81). In comparison to enzymatic isolation, the mechanical isolation of cells from lipoaspirates can reduce the cost of collagenase and time of digestion while maintaining their antigen expression and differentiation capacity (82). ASCs can also be derived from mature adipocytes by dedifferentiating them into lipid-free fibroblast-like cells (83, 84).

There are heterogeneous populations of cells that may adhere to the plastic surfaces of a culture flask after the enzymatic aggregation of tissue. The isolation of ASCs by immuno-magnetic beads prior to culturing can reduce cross-contamination with unwanted cells. The cells are incubated with beads that are conjugated with MSC-associated antibodies, such as CD 105, CD 90 and CD73. The magnetically labeled cells are retained within the magnetic field in the column and collected by flushing out the cells after removing the column from the magnetic field. The isolated cells can be cultured, and the attached beads are biodegradable. The immuno-magnetic bead separation of cells after collagenase digestion of adipose tissue results in several subpopulations that share typical marker expression (85). Three different isolation methods have been studied to reduce the heterogeneity of cultures; these methods are based on adherence on plastic, washing after one hour of cell seeding and immuno-magnetic isolation by specific markers. Washing after one hour of seeding significantly reduces the heterogeneity of the cell population and increases stem cell marker expression. A more homogenous population is achieved when loosely attached cells are washed away after one hour of seeding instead of changing the medium after 24 hours or more (86).

Many protocols have been developed for the cryopreservation of ASCs. Cryopreservation requires a medium and a cryoprotectant. Fetal bovine serum prevents the viability loss of ASCs, and cryoprotective agents such as dimethylsulphoxide prevent cell death due to extreme temperature. ASCs can be cryopreserved for at least 6 months while maintaining their proliferation capability and surface marker profiles (87). Storage of liposuction samples for 24 hours at 4°C before processing the samples could increase the yield of stem cells (88).

Immunophenotyping of ASCs

In addition to adhering to plastic, stem cells must express specific surface antigens or proteins. The expression of specific antigen is crucial for clinical applications. Immunophenotyping of ASCs can be performed at various stages of culture (freshly isolated cells, early passages and late passages) and using various experimental methods (western blot, flow cytometry, immunofluorescence and immunocytochemistry). These methods offer either quantitative or qualitative expression of antigen. The depot of adipose tissue and type of sample (liposuction or excised adipose tissue) may influence the percentage of expression.

The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy has proposed that more than 95% of the MSC population must express CD105, CD73 and CD90, as measured by flow cytometry. Additionally, these cells must lack expression (less than 2% positive) of CD45, CD34, CD 14 or CD11b, CD79a or CD19 and HLA class II (8). Positive and negative markers of ASCs are summarized according to the publication in which they appear (see Table 1).

Table 1: Positive and negative markers of ASCs, as reviewed:

Positive	Negative/ Very Low
CD29 (23, 89, 90, 109, 110)	CD31 (22, 89, 90, 109, 111)
CD44 (23, 90, 109-111)	CD45 (22, 23, 89, 90, 109, 111, 112)
CD49d (89, 111)	CD34 (23, 90, 91, 109, 111)
CD73 (89, 90, 109, 110, 112)	CD106 (22, 109, 111)
CD90(22, 89, 109-112)	HLA-II (109)
CD105 (22, 23, 89, 90, 109-113)	CD14 (109)
CD13 (23, 111, 113)	CD44 (110)
CD166 (22, 23)	HLA-DR (22, 23, 90, 91)
CD34 (22)	CD146 (22)
HLA-ABC (22)	CD117 (91)
CD71 (111)	

ASCs express surface markers such as integrins (CD29, CD49d), surface enzymes (CD73), extracellular matrix proteins (CD90 and CD105) and hyaluronate (CD44). In contrast, ASCs do not express hematopoietic progenitor cell marker (CD34), vascular cell adhesion molecule (CD106),

monocyte differentiation antigen (CD14), endothelial cell adhesion molecule (CD31) and leukocyte common antigen (CD45). Katz et al. demonstrated that ASCs strongly express proteins related to cell adhesion, matrix proteins, growth factors and receptors, and proteases (58).

Most papers report that cultured ASCs exhibit significantly increased expression of positive markers in the subsequent cultures (subcultures) (89-91). High expression of CD105, which is an MSC marker, has indicated that these cells can be applied to stem cells therapy (89). In contrast, decreased expression of CD105 is related to the differentiation of MSC into osteogenic, adipogenic, chondrogenic and epithelial pathways (92). CD34, CD31, CD45 are highly expressed in freshly isolated cells, and most reports claim that their expression decreases with successive cultures. This finding implies that the population of ASCs becomes more homogenous with increasing sub-cultures. Positive expression may be correlated with heterogeneous cell population in freshly isolated cells (SVF), which are composed of ASCs, endothelial cells and vascular smooth muscle cells (81). While CD34+ ASCs have greater proliferation potential, CD34- ASCs exhibit greater ability to differentiate into mesenchymal lineages (93).

ASCs share the same profile of antigen expression as MSCs from bone marrow: CD29, CD13, CD44, CD 73, CD90, CD105 and CD166 (59, 94). The differences between ASCs and bone-marrow-derived MSC are the expression of CD106 (only expressed by bone-marrow-derived MSCs) and CD 49d (only expressed by ASCs) (95). Mature fibroblasts in connective tissue also express CD29, CD44, CD90, CD105 and are negative for CD34, CD117 and CD146 (96).

Current clinical applications of ASCs

The multipotential nature and secretion ability of ASCs encourage to their clinical application. Here, we discuss the application of ASCs to soft tissue reconstruction and augmentation, wound management (radiation injury) and orthopedic clinical applications.

Soft tissue reconstruction and augmentation

Adipose tissue has been utilized as an autologous tissue for soft tissue augmentation to repair congenital and acquired tissue damage. To survive, the graft must be vascularized and supplied with adequate nutrients and cell sources. Differentiated adipocytes from autologous ASCs were injected into the depressed scars of thirty-one patients, and long-term follow up in seven patients revealed that the volume of the graft was maintained for up 12 weeks compared to conventional fat transfer (97). The injection of ASCs alone was not sufficient to support adipogenesis; thus, adipocytes differentiated from ASCs enhanced adipose tissue formation (98). Another novel method to enhance survival of graft was achieved by combining autologous fat with fat containing a stromal vascular fraction. This technique is known as cell-assisted lipotransfer (CAL),

which improves the survival rate of the graft and enhances angiogenesis in breast tissue augmentation and facial lipoatrophy (99, 100). ASC differentiation to adipocytes and vascular endothelial cells, secretion of growth factors and replication are important events that contribute to adipose tissue regeneration, angiogenesis and graft survival in CAL treatment (101). ASCs can be employed allogeneically because they possess immunosuppressive properties (lack of HLA-DR expression) (102, 103).

Wound management (Radiation injury)

Autologous lipoaspirate administered into lesions caused by radiation therapy treatment of oncologic patients improved the regeneration of tissue and the restoration of function. ASCs have been reported to actively participate in the regeneration and neovascularization of tissue (104).

In Japan, autologous non-cultured ASCs isolated by the Celution™ System were used as a treatment for local chronic injuries caused by radiation. The treated injuries were caused by nuclear power plant accidents or radiation therapy. In the conventional method of treatment, multiple surgical procedures must be performed before the wound can heal. Autologous non-cultured ASCs applied to the wound area with artificial dermis and sprayed with growth factors resulted in improved tissue regeneration after 1.5 years (105).

Orthopaedic

Calvarial fracture in children was treated with autologous ASCs in autologous fibrin glue. After three months, the fractures showed ossification of the defect (106). Another study also employed autologous ASCs isolated according to GMP guidelines and expanded *in vitro* using autologous serum. These cells were combined with beta-tricalcium phosphate and scaffold construct seeded into the rectus abdominal muscle of patients with hard palate defects. Bone formation and vascularization of tissue occurred after 8 months (107). ASCs were induced to osteocytes and implanted at surgically produced defects in the palatal bones of rats to examine the ability of differentiated ASCs to treat bone defects. The group treated with ASCs and scaffold exhibited significant bone regeneration (108). ASCs offer alternative treatments to traditional bone grafting, and this method is suitable when there is a shortage of autogenous bone, particularly in the cases of children and donor-site morbidity.

Conclusions and future directions

ASCs possess significant potential in regenerative medicine. ASCs have captured researchers' attention because, within 10 years, the investigation of their applications has progressed from bench to bedside. Numerous methods of isolation and culture conditions have been manipulated

to obtain optimum ASCs expansion. Further study and standardization of the isolation protocol and antigen profiles would substantially enhance clinical advancement. ASCs have antioxidant, anti-aging, anti-inflammatory and wound healing properties, as demonstrated *in vitro* and in preclinical models. Cell therapy using ASCs can reduce morbidity and the healthcare cost burden to sustain their development in countries without compromising safety issues.

Basic science knowledge of ASCs can be expanded by further investigation of ASC biology. These findings may facilitate researchers' understanding of the *in vitro* behavior of these cells, which can translate into clinical applications. ASCs production potentially benefitting regenerative medicine should be performed in accordance with the provided code of practice. Further clinical trials are necessary to continue validation of the applications of ASCs and improve their clinical outcome.

*For literature searching, authors key in "adipose-derived stem cells", "mesenchymal stem cells", "adipose tissue", "characterization of adipose-derived stem cells", "isolation of adipose-derived stem cells", "clinical application of adipose-derived stem cells" in PubMed portal and Science Direct.

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