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Article *in* Stem cell reviews · June 2011

DOI: 10.1007/s12015-010-9193-7 · Source: PubMed

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Stem Cell Rev and Rep (2011) 7:269–291
DOI 10.1007/s12015-010-9193-7

The Potential of Adipose Stem Cells in Regenerative Medicine

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Published online: 18 September 2010

Abstract Adipose stem cells (ASCs) are an attractive and abundant stem cell source with therapeutic applicability in diverse fields for the repair and regeneration of acute and chronically damaged tissues. Importantly, unlike the human bone marrow stromal/stem stem cells (BMSCs) that are present at low frequency in the bone marrow, ASCs can be retrieved in high number from either liposuction aspirates or subcutaneous adipose tissue fragments and can easily be expanded in vitro. ASCs display properties similar to that observed in BMSCs and, upon induction, undergo at least osteogenic, chondrogenic, adipogenic and neurogenic, differentiation in vitro. Furthermore, ASCs have been shown to be immunoprivileged, prevent severe graft-versus-host disease in vitro and in vivo and to be genetically stable in long-term culture. They have also proven applicability in other functions, such as providing hematopoietic support and gene transfer. Due to these characteristics, ASCs have rapidly advanced into clinical trials for treatment of a broad range of conditions. As cell therapies are becoming more frequent, clinical laboratories following good manufacturing practices are needed. At the same time as laboratory processes become more extensive, the need for control in the processing laboratory grows consequently involving a greater risk of complications and possibly adverse events for the recipient. Therefore, the safety, reproducibility and quality of the stem cells must thoroughly be examined prior to extensive use in clinical applications. In this review, some of the aspects of examination on ASCs in vitro and the utilization of ASCs in clinical studies are discussed.

Keywords Adipose stem cells · Stem cell therapy · Good manufacturing practice · Defined serum-free culturing conditions · Flow cytometry

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Introduction

In order to use stem cells for clinical treatments, there has to be a means to isolate cells in large enough quantities. While adult stem cells have a lower capacity of differentiating [1, 2] than embryonic stem cells (ESCs) [3, 4] and induced pluripotent stem (iPS) cells [5–7], the use of stem cells from adult tissues circumvent ethical issues associated with the application of embryonic stem cells and the production cost issues associated with iPS cells. Since the identification of pluripotent stem cells in the bone marrow stroma 40 years ago [8], bone marrow stromal/stem cells (BMSCs) have become a standard in the field of adult stem cell biology and in regenerative medicine due to their high differentiation potentials and low morbidity during harvesting [1, 9, 10]. Nevertheless, harvesting of BMSCs by bone marrow aspiration is a painful procedure and the number of cells acquired is usually low. Adipose tissue can therefore be considered an attractive alternative source [11–14] since it can be collected in large quantities from adipose tissue fragments. Human adipose stem cells (ASCs) are an abundant cell source with

therapeutic applicability in pre-clinical studies in diverse fields, due to their ability to readily be expanded and their capacity to undergo adipogenic, osteogenic, chondrogenic, neurogenic and myogenic differentiation in vitro [2, 12–19]. Furthermore, ASCs have been shown to be immunoprivileged [20, 21] and appear to be more genetically stable in long-term culture [22] compared to BMSCs [23].

The safety and efficacy of ASCs for tissue regeneration or reconstruction is currently under assessment in clinical trials. The number of trials have risen rapidly from a total of nine in December 2009 to 18 by May 2010 investigating the efficacy in treating conditions such as type I and II diabetes, liver cirrhosis and regeneration, fistulas, cardiovascular disease, limb ischemia, amyotrophic lateral sclerosis and lipodystrophy, but only two studies are yet completed (http://clinicaltrials.gov) (Table 1). Furthermore, ASCs are also under examination in clinical case studies for graft-versus-host disease [24–27], immunosuppression (rheumatoid arthritis, Crohn's disease and ulcerous colitis) [20, 21, 28], multiple sclerosis [29], soft tissue augmentation [30, 31] and bone tissue repair [32, 33]. Clinical bone tissue reconstruction studies using autologous ASCs are also ongoing at our institute Regea, University of Tampere, with 23 patients treated so far [33], Numminen et al., submitted, Thesleff et al., submitted]. With clinical trials and case studies under way, thorough characterization of adipose stem cells is critical for safe and reproducible utilization in cell therapy applications. In this review, aspects of the in vitro characterization of ASCs are discussed with an emphasis on requirements and challenges related to clinical cell therapy.

Stem Cells in Adult Tissues

While ESCs and iPS cells exhibit nearly unlimited potential to differentiate in vitro and in vivo, the applications of these cells are limited by ethical, legal, and political concerns, as well as by scientific and clinical issues of safety and efficacy. Therefore, tissue-specific stem cells derived from adults offer alternative approaches that circumvent many of these concerns.

The first stem cells to be described were the hematopoietic stem cells (HSCs) by Becker and co-workers in 1963 [34]. These cells reside in the bone marrow and are arranged in a hierarchy of progenitors that have the ability to self-renew and differentiate [35], furthermore, reports claim that these cells have the capacity to transdifferentiate into e.g. hepatocytes [36], which gives them broader potential in regenerative medicine than expected. However, the focus of this review will be on mesenchymal stem cells and adipose stem cells in particular.

In 1968, Friedenstein and co-workers were the first to report the observation of a small number of adherent cells from rat whole bone marrow that were spindle-shaped in appearance and demonstrated ability to differentiate into colonies that resembled small deposits of bone or cartilage [8]. The potential of these marrow stromal cells were further investigated in the 1980s, particularly by Piersma and co-workers [37] and by Owen and co-workers [38]. In the early 1990s, Caplan popularized the term mesenchymal stem cell (MSC) [39], but some investigators still preferred not to refer to these cells as stem cells when publishing pre-clinical [40, 41] or clinical [42–45] studies of MSC. Since the 'stem cell' label has scientific implications, i.e. the cell type must possess self-renewal and multilineage differentiation potential, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy (ISCT) proposed a uniform nomenclature for these important cells in two position statement papers published in the mid 2000s [46, 47]. They proposed that the plastic-adherent cells currently described as mesenchymal stem cells should be termed multipotent mesenchymal stromal cells, while the term mesenchymal stem cell should be reserved for a subset of these cells that demonstrate stem cell activity by clearly stated criteria. The criteria state that the acronym MSC may be used for both populations, as long as the acronym is defined in the presentation of the work. To define MSCs, some minimal criteria were suggested by the ISCT for in vitro demonstrations of long-term survival with self-renewal capacity and tissue repopulation with multilineage differentiation. Firstly, the MSCs must be plastic-adherent when maintained in standard culture conditions. Secondly, since monospecific and unique molecular probes do not exist to unequivocally identify these cells in situ, several markers are needed, therefore the cells must express surface markers CD105, CD73 and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA-DR surface molecules. And thirdly, they must differentiate to osteoblasts, adipocytes and chondroblasts in vitro. Apart from being able to differentiate osteoblasts, adipocytes and chondroblasts MSCs have been shown differentiate into a large variety of specialized mesenchymal cell types including myocytes, tendocytes and ligament cells [1, 48, 49]. Furthermore, MSCs reside in various locations throughout the body, e.g. in bone marrow, around blood vessels (as pericytes), in fat, skin, muscle, teeth and other locations [1, 48, 50]. In addition to their potential to differentiate, MSCs have also been shown to possess some level of plasticity [51, 52], transdifferentiating in vitro across germinal boundaries. Until recently, it was believed that tissue-specific stem cells only differentiated into mature phenotypes within their restricted lineages. This novel notion of stem cell plasticity is perhaps not surprising, since within the mesenchymal cell lineages, plasticity of mature cells was proposed several decades ago by showing that chondrocytes could transdifferentiate into osteoblasts [53], and that adipocytes could

Table 1 Clinical trials using ASCs reported at clinicaltrials.gov as of May, 2010

Trial	Study phase	Condition	Locations
Study of Autologous Fat Enhanced w/ Regenerative Cells Transplanted to Reconstruct Breast Deformities After Lumpectomy (RESTORE-2)	IV, Active, not recruiting	Breast Neoplasms Carcinoma; Ductal; Breast Mammoplasty Mastectomy; Segmental, Lumpectomy; Breast Reconstruction	Brussels, Belgium; Florence, Italy; Madrid, Spain; Valencia, Spain; Glasgow, United Kingdom
Efficacy and Safety of Adipose Stem Cells to Treat Complex Perianal Fistulas Not Associated to Crohn's Disease (FATT1)	III, Completed	Anal Fistula	Mannheim, Germany; Madrid, Spain; Zaragoza, Spain; Pamplona, Spain; Tarrasa, Spain; Oxford, United Kingdom
Safety and Efficacy of Autologous Cultured Adipocytes in Patient With Depressed Scar	II/III, Completed	Depressed Scar	Seoul, Republic of Korea
Autologous Stem Cells Derived From Lipoaspirates for the Non-Surgical Treatment of Complex Perianal Fistula	II, Active, not recruiting	Perianal Fistula	Madrid, Spain
Safety and Efficacy of Autologous Adipose-Derived Stem Cell Transplantation in Type 2 Diabetics	I/II, Active, not recruiting	Type 2 Diabetes Mellitus	Makati City, Philippines; Quezon City, Philippines
Safety and Efficacy of Autologous Adipose-Derived Stem Cell Transplantation in Patients With Type 1 Diabetes	I/II, Recruiting	Type 1 Diabetes Mellitus	Makati City, Philippines; Quezon City, Philippines
Autologous Mesenchymal Stem Cells From Adipose Tissue in Patients With Secondary Progressive Multiple Sclerosis (CMM/EM/2008)	I/II, Recruiting	Secondary Progressive Multiple Sclerosis	Sevilla, Spain
Allogenic Stem Cells Derived From Lipoaspirates for the Treatment of Recto-vaginal Fistulas Associated to Crohn's Disease (ALOREVA)	I/II, Recruiting	Rectovaginal Fistula; Crohn Disease	Madrid, Spain
Intraarterial Infusion of Autologous Mesenchymal Stem Cells From Adipose Tissue in Diabetic Patients With Chronic Critical Limb Ischemia (CeTMAd/ICPD200)	I/II, Suspended	Chronic Critical Limb Ischemia	Sevilla, Spain
A Randomized Clinical Trial of AdiPOse-Derived Stem ceLLs in the Treatment of Patients With ST-Elevation myOcardial Infarction - The APOLLO Trial	I, Active, not recruiting	Myocardial Infarction; Coronary Arteriosclerosis; Cardiovascular Disease; Coronary Disease	Rotterdam, Netherlands; Madrid, Spain
A Randomized Clinical Trial of adiPose-deRived stEm & Regenerative Cells In the Treatment of Patients With Non revaScularizable ischEmic Myocardium - The PRECISE Trial	I, Active, not recruiting	Ischemic Heart Disease; Coronary Arteriosclerosis; Cardiovascular Disease; Coronary Disease; Coronary Artery Disease	Copenhagen, Denmark; Rotterdam, Netherlands; Utrecht, Netherlands; Madrid, Spain
Safety and Efficacy Study of Autologous Cultured Adipose -Derived Stem Cells for the Crohn's Fistula	I, Active, not recruiting	Crohn's Fistula	Seoul, Republic of Korea
Autologous Adipose-Derived Stem Cell Transplantation in Patients With Lipodystrophy (AADSCTPL)	I, Recruiting	Lipodystrophy	Porto Alegre, Brazil
Liver Regeneration Therapy by Intrahepatic Arterial Administration of Autologous	I, Recruiting	Liver Regeneration Therapy	Kanazawa, Japan

Table 1 (continued)

Trial	Study phase	Condition	Locations
Adipose Tissue Derived Stromal Cells			
Safety Study of Autologous Cultured Adipose -Derived Stem Cells for the Fecal Incontinence	I, Recruiting	Fecal Incontinence	Seoul, Republic of Korea
Long-term Safety and Efficacy of Adipose-derived Stem Cells to Treat Complex Perianal Fistulas in Patients Participating in the FATT-1 Randomized Controlled Trial (LTE)	Recruiting	Perianal Fistulas	Terrasa, Spain; Cantabria, Spain; Madrid, Spain; Valencia, Spain
Liver Regeneration Therapy Using Autologous Adipose Tissue Derived Stromal Cells	Recruiting	Liver Cirrhosis	Kanazawa, Japan
Mesenchymal Stromal Cells Secreting Neurotrophic Factors (MSC-NTF), in Patients With Amyotrophic Lateral Sclerosis (ALS)	Not yet recruiting	Amyotrophic Lateral Sclerosis	Jerusalem, Israel

switch their phenotype to that of osteoblasts [54]. In summary, the performance of MSCs have an impact on the overall health status of individuals by controlling the body's capacity to naturally remodel, repair, and upon demand, rejuvenate various tissues.

Adipose Stem Cells

Although the BMSCs continue to be a viable option for a stem cell population for cell therapy applications, there are drawbacks to utilizing the source. A bone marrow harvest is a painful procedure with possible donor site morbidity as a result [11]. Secondly, although MSCs grow well under standard tissue culture conditions, ex vivo expansion is necessary due to relatively low numbers of MSCs present in the harvested marrow [11]. In light of this, adipose tissue has become an attractive option and represents an alternative source of stem cells. Subcutaneous adipose depots are accessible and abundant, thereby providing a potential adult stem cell reservoir for each individual.

Historically, adipose tissue has been considered a metabolic reservoir for packaging, storing, and releasing high-energy substrates in the forms of triglycerides and cholesterol as well as lipid-soluble vitamins. In the mid 1980s, the conception was altered when the tissue was found to be involved in the metabolism of sex steroids such as estrogens [55]. Today, adipose tissue is a compartment known for its abundant population of stem cells.

Adipose tissue is comprised of adipocytes and a heterogeneous set of cell populations that surround and support them, which upon isolation are termed the stromal vascular fraction (SVF) (Fig. 1). The SVF includes the stromal cells, ASCs, that have the ability to differentiate into cells of several lineages such as adipocytes osteoblasts, chondrocytes, myocytes, endothelial cells, hematopoietic cells, hepatocytes and neuronal cells

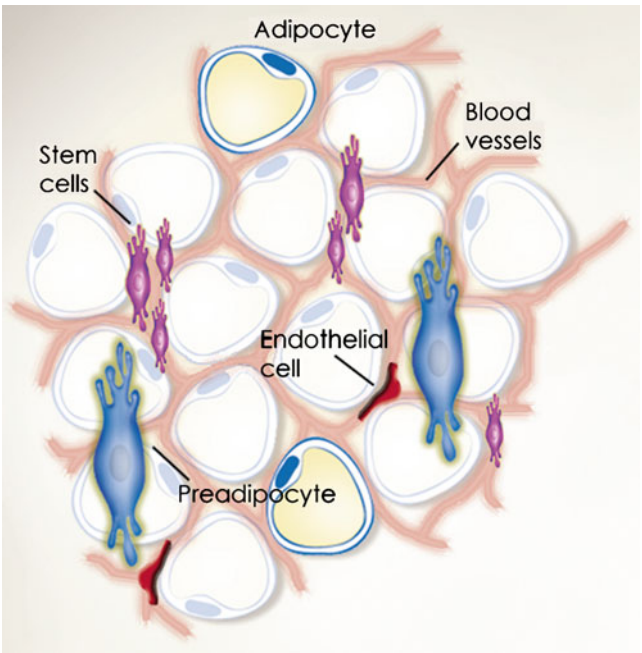

Fig. 1 A proposed schematic view of components of adipose tissue modified from [217]

[2, 12–19, 56]. Furthermore, the SVF contains cells from the microvasculature, such as vascular endothelial cells and their progenitors, vascular smooth muscle cells and also cells with hematopoietic progenitor activity [57, 58]. Also present in the SVF are leukocytes that may be resident in the parenchyma of adipose tissue [57]. Despite the fact that the SVF is a heterogeneous cell population, subsequent expansion of human adipose-derived cells selects for a relatively homogeneous cell population, enriching for cells expressing a stromal immunophenotype, compared with the heterogeneity of the crude SVF. For reviews, see [14, 55, 58, 59].

As in many rapidly developing fields, a range of names have been used to describe the plastic adherent cell population isolated from collagenase digests of adipose tissue, e.g. lipoblast, pericyte, preadipocyte, processed lipoaspirate (PLA) cells, adipose derived stem/stromal cells (ASCs), adipose-derived adult stem (ADAS) cells, adipose-derived adult stromal cells, adipose-derived stromal cells (ADSCs), adipose stromal cells (ASCs), multipotent adipose-derived stem cells (hMADS) and adipose mesenchymal stem cells (AdMSCs). To address the problem, the International Fat Applied Technology Society (IFATS) proposed a standardized nomenclature during the 2004 IFATS meeting in Pittsburgh by adopting the term adipose-derived stem cells (ASCs) to identify the isolated, plastic-adherent, multipotent cell population [57]. As for MSCs, the use of the term stem cell may be questioned and thus it is widely accepted that investigators may use the acronym ASC to mean adipose-derived stromal cells [14].

Isolation of Adipose Stem Cells

In humans, ASCs can be isolated from fat tissue wastes resulting from plastic surgery, i.e. liposuction aspirates and from reconstructive surgeries, through resection of a large tissue fragment [60]. When the starting material is obtained from liposuction procedures, the isolation method is simplified, as the procedure generates finely minced tissue fragments that are more homogeneous, allowing a more efficient enzymatic digestion. When working with whole tissue pieces as starting material, the tissue is minced manually, requiring more time and effort for thorough enzymatic digestion [55].

Furthermore, the isolation procedure may be sped up by commercial bench top closed systems for isolating unexpanded ASCs directly for cell therapy, such as Cytori's Celution™ system [61–64] and Tissue Genesis' TGI 1000™ platform [65]. The Cytori's Celution™ system is a CE-marked medical technology and has also been approved by the U.S. FDA as a medical device [66], that allows the clinician to generate a high yield, single cell suspension of stem and regenerative cells at the bedside for immediate application, which can be directly appended within the same procedural setting. The Celution system has been reported used in a clinical study for the treatment of stress urinary incontinence [63] with promising results. The Tissue Genesis' TGI 1000™ platform has so far not received approval by the FDA.

Also, the effects on yield and cell proliferation using different harvesting techniques and harvesting sites have also been investigated, and contradictory reports have been published. In a report by Fraser and co-workers [67] the results showed that neither the site of harvest nor the harvesting technique (liposuction, syringe-based and pump-assisted) affected the number of ASCs obtained. Nevertheless, the number of clonogenic cells varied with the harvesting site. Oedayrajsingh-Varma and co-workers studied three harvesting techniques (resection, tumescent liposuction and ultrasound-assisted liposuction) and the results suggested that the harvesting technique affected the recovery of ASCs, with ultrasound-assisted liposuction yielding the lowest number of proliferative ASCs [68]. Later, the same group also concluded that the site of harvest also affected the yield of ASCs [69]. Furthermore, von Heimburg and co-workers reported that resection yielded lower numbers of viable progenitors as compared to liposuction aspirates [70]. However, due to the small number of reports published and the variations in the protocols used, it is difficult to conclude the optimal harvesting technique, site of harvest and optimal isolation procedure.

In 1964, Martin Rodbell was the first to present a method for in vitro isolation of mature adipocytes and adipogenic progenitors from rat fat tissue [71]. In his protocol, the tissue was minced into small fragments, digested at 37°C with type I collagenase, and the cellular components were separated by centrifugation. Following centrifugation, the supernatant contained the mature adipocytes, which floated due to their high lipid content, and the pellet contained the SVF components, including the presumptive adipocyte progenitor cells in addition to cells of the hematopoietic lineages. Katz, Zuk and co-workers were first to show that the SVF fraction isolated from human lipoaspirates in fact contained cells with multilineage potential and termed these cells processed lipoaspirate (PLA) cells [12, 13, 72]. They isolated the adipose stem cells from liposuction aspirates using the collagenase digestion method, and subsequently allowing the ASCs to adhere to the plastic surface of tissue culture flasks, which is still the basis of most methods used to date. Since then, several groups working independently have developed and refined procedures of isolating and characterizing adipose stem cells [2, 73–76]. For an overview of tissue sources, enzyme and applications used, see [55].

Characterization of Adipose Stem Cells

Adipose stem cells are commonly characterized by the same methods used for characterizing BMSCs: by their immunophenotype in the undifferentiated state and by their differentiation potential towards the adipogenic, osteogenic, and chondrogenic lineages in the presence of lineage-specific induction factors [1, 2].

ASC Immunophenotype

Unlike embryonic stem cells [4], undifferentiated ASCs cannot be identified by a single surface marker or a few surface markers but rather, a panel of markers are needed for identification of the population. Still, a number of reports have been published suggesting markers for identifying the mesenchymal stem cell population, such as STRO-1 [77], CD271 [78], STRO-3 [79] and MSCA-1 +CD56+ [80].

BMSCs and ASCs show very similar surface marker expression patterns [59, 81–83]. Furthermore, both cell sources express the surface markers characteristic for MSCs, meeting the criteria set by ISCT [46, 84, 85]. However, minor differences exist: BMSCs lack expression of CD49d, which is strongly expressed on ASCs, while ASCs lack expression of CD106, which is expressed on BMSCs [59]. This reciprocal expression pattern is interesting because CD106 is the cognate receptor of CD49d and both molecules are involved in hematopoietic stem and progenitor cell homing to and mobilization from the bone marrow [83, 86, 87].

Markers CD13, CD29, CD73, CD90, CD133, MHC I and MHC II have been detected with highly consistent patterns of expression on the surface of ASCs (for references, see Table 2). Markers that are uniformly reported to have strong positive expression are CD13, CD29, CD44, CD73, CD90, CD105, CD166 and MHC I, while markers of the hematopoietic and angiogenic lineages, such as CD31, CD45 and CD133, have been reported to show low or lack of expression on ASCs. Moreover, MHC II has also been found to be absent on ASCs. Moderate expression has been reported for markers CD9, CD34, CD49d, CD106, CD146 and STRO-1, i.e. surface marker expression levels of lower than 50%. The presence or absence of STRO-1 is particularly controversial because while Gronthos and co-workers reported absence of this marker in ASC cultures [75] Zuk and co-workers reported its presence [13]. Similar controversies are seen for CD34 and CD106, where Gronthos et al. [75] reported detection of these markers in ASCs, while Zuk et al. [13] and Katz et al. [73] reported their absence or expression on a small population of cells. Also MHC I shows great variation, varying from strongly positive to low or no expression. Moreover, the expressions of some surface markers change during cell culturing and passaging. For instance, the expression level of CD29, CD44, CD73, CD90 and CD166 increase from the SVF to passage 2 (p2), whereupon they stabilize at a high expression level [85]. On the contrary, hematopoietic cell markers, such as CD11, CD14, CD34 and CD45, expressed on cells in the SVF decrease or are lost with increasing passage number, suggesting that adherence to plastic and subsequent expansion will select for a relatively homogeneous cell population compared with the SVF [81, 84]. Most of the results are produced on ASCs cultured in medium supplemented with FBS, but the results for ASCs cultured in medium supplemented with human serum derivatives in or in serum-free (SF) conditions show a similar pattern, with slight variations particularly in markers CD14 and CD49.

Despite several reports being published to establish the markers of the ASC phenotype, there is still a lack of consensus in the results. Also, the results on ASCs cultured in medium supplemented with human serum and in SF conditions still have been reported by very few. Nevertheless, the expression profiles of surface proteins reported on ASCs are by and large consistent. To note, these inconsistencies in the results reported by different teams is by no means unique for ASCs; similar differences in expression profiles have been detected for BMSCs [75]. The inconsistencies may in part be explained by the differences in marker antibodies sources and sensitivity differences between detection methods used in the referred studies, the proliferative stage of the cells in culture or donor heterogeneity. It may prove impossible to unify the protocols of surface marker characterization due to the reasons mentioned above; however, some minimal criteria for characterization of ASCs by surface markers may prove useful.

ASC Differentiation Potential

In order to utilize adipose stem cells for clinical cell therapy applications, the multipotentiality of ASCs must be established. In the following sections, some in vitro characterization methods will be described. The differentiation protocols for BMSCs/ASCs from different species may vary, however in this review only protocols related to the determination of human ASC differentiation potential are discussed.

Adipogenesis The cells of the adipose lineage differentiate from a multipotent stem cell population residing in the vascular stroma of adipose tissue and undergo a multi-step process by an initial commitment step, in which cells become restricted to the adipocyte lineage, but do not yet express markers of terminal differentiation. Subsequent

Table 2 Surface markers expressed on adipose stem cells

Antigen	Surface marker expression					
	FBS		HS		SF/XF	
CD9	+	[75, 98, 219]	+	[98, 219]	+	[98]
CD10	++	[75, 98, 219]	++	[98, 219]	++	[98]
CD13	+++	[75, 85, 98, 150, 219]	+++	[98, 219]	+++	[98]
CD14	±	[75, 84, 147, 220, 221], *	++	[147], *		
CD19	±	*	±	*		
CD29	+++	[73, 75, 85, 98, 147, 219–221]	+++	[98, 147, 219]	+++	[98]
CD31	+/±	[75, 85, 147, 172, 219, 221, 222]	±	[98, 147, 172, 219]	±	[98, 172]
CD34	+	[75, 85, 147, 150, 172, 219–221]	+	[98, 99, 172, 219]	+	[98, 99, 172]
CD44	+++/++	[75, 85, 147, 219–222]	++	[98, 147, 219]	+++	[98]
CD45	+/±	[75, 147, 150, 219–222]	+	[98, 99, 147, 219]	±	[98, 99]
CD49d	++/+	[73, 75, 219]	+	[98, 219]	±	[98]
CD73	+++	[85, 147, 220, 221]	+++	[147]		
CD90	+++	[85, 98, 150, 219–222]	+++	[98, 99, 147, 219]	+++	[98, 99]
CD105	++	[75, 85, 98, 150, 219–222]	++	[98, 99, 219]	++	[98, 99]
CD106	+/±	[73, 98, 219, 220, 222]	±	[98, 219]	±	[98]
CD117	+++/±	[73, 221]	±	*		
CD133	±	[73, 147, 172, 220]	±	[147, 172]	±	[172]
CD146	+	[85, 98, 222]	±	[98]	±	[98]
CD166	++/+	[75, 85, 98, 219, 221, 222]	+	[98, 219]	+	[98]
MHC I	+++/+	[73, 75, 98, 147, 172, 220]	+++/±	[98, 99, 147, 172, 219]	+++/+/±	[98, 99, 172]
MHC II	+/±	[73, 75, 98, 147, 220], *	±	[98, 99, 147], *	±	[98, 99]
STRO-1	+	[219, 222]	+	[219]		

FBS = fetal bovine serum, HS = human serum, SF = serum-free, XF = xeno-free

+++ = strong expression >90%, ++ = positive expression <90% >50%, + = moderate expression <50% >2%, ± = low or no expression ≤2%

* = Lindroos et al, unpublished results

differentiation occurs by activation of several transcription factors resulting in the adipocyte phenotype. Factors that lead to the commitment of mesenchymal stem cells to the adipose lineage ex vivo have been identified, but the molecular mechanisms by which these pathways are regulated have not been determined [88].

When applying the appropriate induction factors in vitro, human ASCs are capable of differentiating into their original differentiation pathway, adipogenesis. The first adipogenic induction media reported was a chemically defined SF media containing insulin or IGF-1, triiodothyronine and transferrin, with serum only used briefly for cell attachment [89]. Further refinements to the induction media supplement composition have been made with the addition of isobutylmethylxanthine (IBMX), hydrocortisone or dexamethasone, indomethacin or thiazolidinedione, pantothenate, biotin and serum [12, 13, 90, 91]. After a week of induction, neutral lipid containing vacuoles accumulate in ASCs and the production of adipogenic mRNAs, such as lipoprotein lipase (LPL), proliferator-activated receptor γ (PPARγ), CCAAT/enhancer binding protein α/β/δ (C/EBPα/β/δ), adipocyte fatty-acid binding protein (FABP4/aP2) and leptin can be detected [12, 13, 90–95]. Expression of LPL has often been cited as an early sign of adipocyte differentiation; however, LPL expression occurs spontaneously at confluence and is independent of the addition of reagents used for induction of adipogenesis [95]. Lipid vacuoles accumulated in the ASCs can be detected by Oil Red O or Nile red staining [13, 96].

The culture media formulation also appear to affect the adipogenic differentiation in vitro, with a few studies reporting more robust adipogenic differentiation seen in ASCs cultured in medium supplemented with human serum derivatives in or in SF conditions than in FBS supplemented media (Figs. 2 and 3) [97–99].

Osteogenesis The mechanisms that drive the ASCs into the osteoblast lineage are still not clear, but the process has been more extensively studied in BMSCs [100]. Irrespective of the tissue source, it is now commonly believed that osteogenic cells arise from multipotential mesenchymal

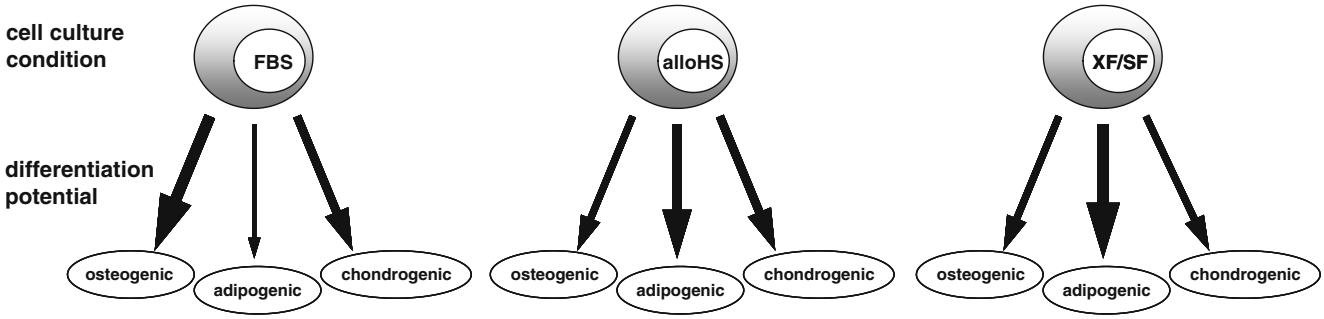

Fig. 2 Schematic outline of the differentiation potential of the adipose stem cells in different culture conditions. The thickness of the arrow demonstrates the differentiation potential towards each lineage. Image modified from [218]

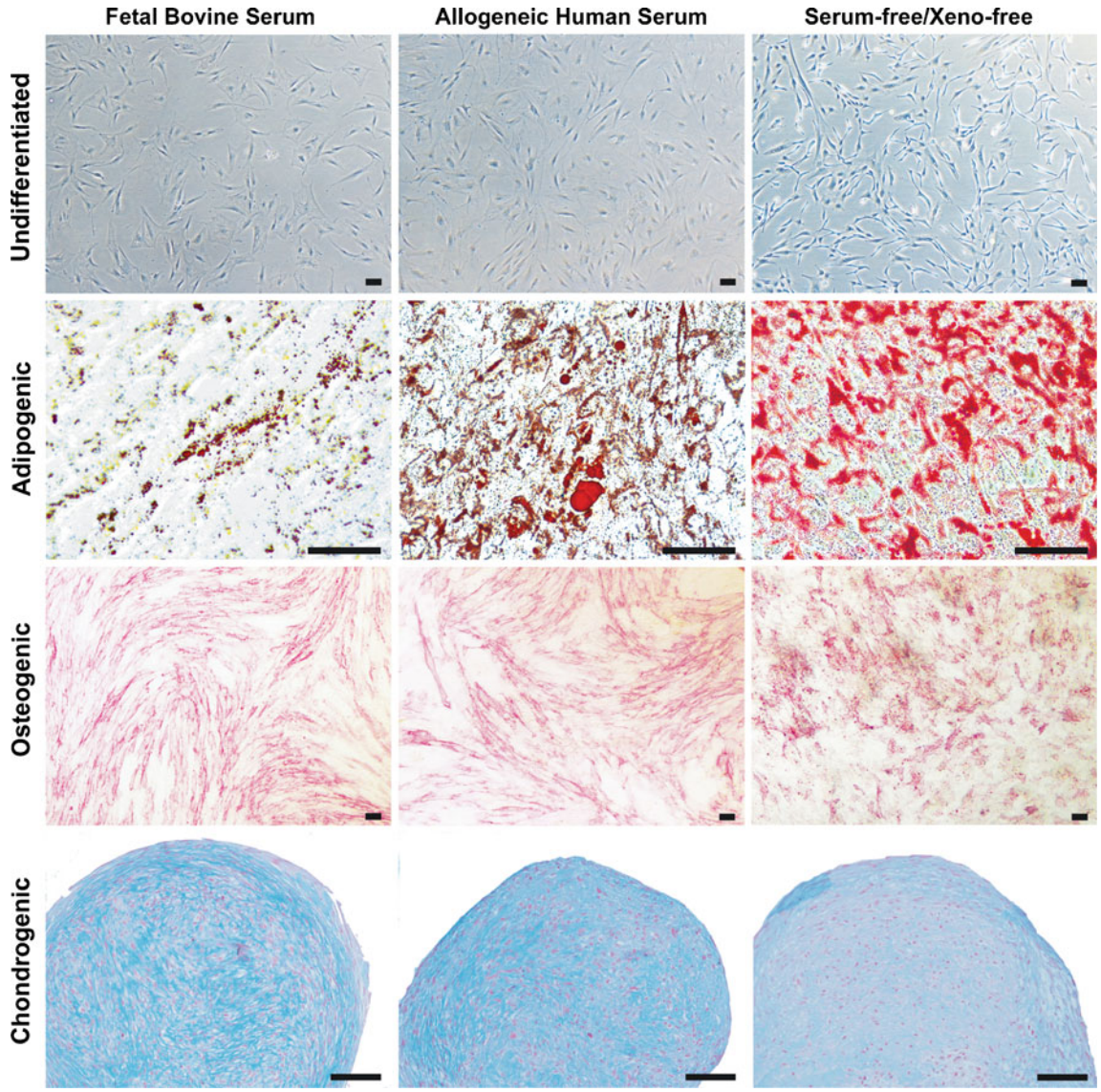

Fig. 3 Multipotentiality of ASCs cultured in fetal bovine serum, allogeneic human serum and in xeno-free/serum-free conditions. Alkaline phosphatase staining confirming osteogenesis, Alcian blue staining verifying chondrogenesis and Oil red O staining substantiating adipogenesis. Scale bar 100 μm. Images by Lindroos et al

cells found in bone marrow or adipose tissue that have the capacity to undergo a number of commitment or restriction steps to give rise to progeny with more limited capacities [101–104]. Many kinds of inducers mediate these commitment steps, such as glucocorticoids and molecules of the transforming growth factor-β (TGF-β) superfamily, including bone morphogenetic proteins (BMPs) [100]. As the stem cells or progenitor cells differentiate, expression of osteoblast-associated genes, e.g. type I collagen (COLL I), alkaline phosphatase (ALP), osteopontin (OPN), bone sialoprotein (BSP), osteocalcin (OCN), parathyroid hormone/parathyroid hormone-related protein (PTH/PTHrP) and receptor (PTH1R) are asynchronously active and/or lost, as the bone matrix matures and mineralizes [105].

In the presence of ascorbate, β-glycerophosphate, dexamethasone and/or 1,25 vitamin D_3, ASCs differentiate into osteoblast-like cells in vitro [2, 106, 107]. Moreover, it has been demonstrated that ASCs cultured in the presence of these factors express ALP, RUNX2, BMP-2, BMP-4, BMP receptors I and II, and PTH receptor genes characteristic of osteoblast-like cells [13, 55, 59, 81, 106]. When induced for 2 to 4 weeks in vitro in the appropriate induction conditions, ASCs start to produce calcium phosphate mineral within their extracellular matrix that can be detected by Alizarin Red or von Kossa staining, and begin to express osteogenic genes and proteins [12, 13, 106].

Also in the case of osteogenic differentiation, the culture conditions appear to affect the differentiation capacity in vitro [97–99], with more robust osteogenic differentiation seen in ASCs cultured in medium supplemented with human serum derivatives in or in SF conditions compared with FBS supplemented media (Figs. 2 and 3). Furthermore, gender differences affect the osteogenic capacity of ASCs, with male ASCs differentiating more rapidly and more effectively than female ASCs in vitro [108], moreover, Zhu and co-workers showed that while the adipogenic potential is unchanged irrespective of age, the osteogenic potential appears to decrease with increasing age [109]. While these differences are likely due to the different steroid functions in males and females with hormone levels varying at different phases of life, they must be taken into account when designing clinical treatments for patients.

Chondrogenesis Similarly to adipocytes and osteoblasts, chondrocytes likely develop from multipotent mesenchymal cells that give rise to progeny with more limited capacities [102]. Nevertheless, there is little evidence distinguishing whether bone and cartilage forming cells arise from a common bipotential progenitor, or whether the two cell types arise from two separate monopotential precursors [102]. Furthermore, a common set of genes has been elucidated to be necessary for early adipogenic, osteogenic and chondrogenic differentiation in both BMSCs and ASCs, although osteogenesis and adipogenesis appear to be linked in a differentiation branch separate from chondrogenesis [103]. Yet, there is a particularly intriguing connection between the osteogenic and chondrogenic lineages, not only due to the possibility of a common bipotential progenitor but also due to the fact that hypertrophic chondrocytes can transdifferentiate into osteoblast-like cells [10, 102].

For chondrogenic differentiation, ASCs are routinely cultured in micro mass culture or pellet culture systems [10, 110]. The micro mass or pellet culture model mimics pre-cartilage condensation during embryonic development, which increases the cell-to-cell interaction and leads to the production of a cartilage-like matrix [111]. The suspension of cells in hydrogel scaffolds [112] has been done in the attempt to mimic the composition of native cartilage. However, the cell configuration is not adequate for induction of chondrogenesis. Chondrogenic differentiation requires the use of a defined medium supplemented with ascorbate-2-phosphate, dexamethasone, L-proline and TGF-β1. Other factors of the BMP family have also been studied for chondrogenic induction of ASCs. For example, while BMP-6 promotes chondrogenic differentiation, BMP-7 induces chondrogenic differentiation only when present in high doses [113–115]. With the addition of chondrogenic induction factors and when maintained in an appropriate 3D environment in vitro, ASCs will start to secrete the extracellular matrix proteins of cartilage, including COLL II, COLL VI and aggrecan [116, 117].

The expression profiles of human ASCs and BMSCs under chondrogenic conditions has been compared in vitro [19]. The two cell types displayed a similar gene expression profile of adipogenic, chondrogenic and osteogenic markers in monolayer, nonetheless, when both cell sources are cultured as chondrogenic micromass pellets, the BMSCs exhibited greater chondrogenic differentiation capacity than ASCs [19, 56, 103, 118, 119]. Although ASCs have may have lower intrinsic chondrogenic potential than BMSCs, results have shown, that chondrogenesis comparable to BMSCs can be induced from ASCs using a greater dose combination of growth factors such as TGF-β2 and IGF-I [120]. Furthermore, the chondrogenic differentiation capacity appears similar in ASCs expanded in either FBS or HS supplemented media prior to switching to serum-free chondrogenic conditions, while completely SF/XF medium formulations appear to support cell proliferation, and perhaps extracellular matrix production to a lesser extent in vitro (Figs. 2 and 3) [97–99]. However, further work is needed to establish culture conditions to maximize ASCs chondrogenic potential in vitro.

Other Lineages and Functions Apart from osteogenesis, adipogenesis and chondrogenesis, ASCs have shown

function in various other differentiation processes with potential clinical applicability. Human ASCs also have potential to differentiate along the cardiomyocyte pathway [121]. Nevertheless, in vitro studies on human ASCs are limited, and in most studies only low percentages (<2%) of differentiation have been described [122–124]. Only one team has reported success in producing higher percentages (61%) of differentiated cells by treatment of cells with 2-deoxy-5-azacytidin [121]. In vitro expanded ASCs have also been shown to have applicability in vivo when administered intracoronarily in a porcine myocardial infarction model, demonstrating improved cardiac function and perfusion via angiogenesis [124]. These preliminary reports point towards ASCs having potential in regenerating cardiac tissue damaged through infarctions or ischemic injury [2]. Under appropriate induction conditions ASCs also, demonstrate in vitro evidence for differentiation along the skeletal myocyte pathway by expressing myoD and myogenin and transcription factors regulating skeletal muscle differentiation [12, 13, 125]. The cells fuse, form multi-nucleated myotubes, and express protein markers of the skeletal myocyte lineage, such as myosin light chain kinase.

In vitro expanded ASCs contain progenitor cells that have the ability to differentiate into mature endothelial cells and participate in blood vessel formation [15, 126–128] although the capacity may be limited [129]. ASC induced vessel formation and growth may be related to the secretion of proangiogenic factors [130] or through perivascular functions of the ASCs [126] or perhaps both. Apart from the endothelial cells, ASCs have also been shown to differentiate towards other cell types of endodermal origin [131]. In a study by Timper and co-workers [18], human ASCs differentiated into cells with a pancreatic endocrine phenotype expressing insulin, glucagon, and somatostatin. Using regenerative pancreas extract from pancreatectomized rats, Lee and co-workers [132] reported induction of C-peptide-positive cells from ASCs by observing expression of genes related to early pancreatic differentiation. Furthermore, Kang and co-workers [133] showed that differentiated eyelid ASCs transplanted into diabetic mice regulated blood glucose levels in the mouse by releasing human insulin. In a similar in vivo study using subcutaneously derived ASCs, Lin and co-workers [134] reported the derivation of insulin-producing cells from ASCs by transduction with the pancreatic duodenal homeobox 1 (Pdx1) gene.

There is some evidence to suggest that human ASCs can differentiate into cells of ectodermal origin, such as hepatocytes and neurons [16, 17, 135]. When hepatogenically induced, ASCs differentiate into hepatocyte-like cells, although the mechanisms are not yet clear, however, the cells expressed albumin and α-fetoprotein, and showed LDL uptake and production of urea. Additionally, when transplanted into a SCID mouse model, the transplanted cells expressed albumin in vivo. When proper induction cues are applied in vitro, ASCs display neuronal and/or oligodendrocytic markers [16, 136–138]. ASCs take on a bipolar morphology, similar to that of neuronal cells, while expressing neuronal associated proteins such as nestin, intermediate filament M, Neu N, as well as glial fibrillary acidic protein (GFAP), a protein associated with oligodendrocyte differentiation. Physiologic and/or chemical signal transduction properties of ASC in neurogenesis need to be characterized further, still looking forward, this work will hopefully lead to in vivo analyses assessing the applicability of ASCs in the regeneration of the central or peripheral nervous system following traumatic injury.

Good Manufacturing Practice (GMP) Requirements

As cellular and gene therapies are becoming more common, clinical laboratories following good manufacturing practices (GMPs) are needed. The need for control in the processing laboratory grows as laboratory processes become more extensive, consequently involving a greater risk of complications and possibly adverse events for the recipient [139].

Current regulations concerning stem cell therapies are developing slowly and therefore leaves room for interpretation. New regulations by the FDA and EU divide procedures according to the degree of manipulation involved and the risk of adverse processing-related events [140–144]. Minimal manipulation, such as cryopreservation of autologous peripheral blood progenitor cells (PBPC), may be performed using good tissue practices (GTPs), a level of control similar to that already practiced by most clinical laboratories. With regards to more-than-minimal manipulation, an elevated degree of process control and laboratory complexity is required, i.e. current good manufacturing practices (GMPs). More-than-minimal manipulation includes transduction, ex vivo expansion, activation, combination with non-tissue components, use for other than the tissue's normal function, and transplantation of unrelated allogeneic cells and tissues [140].

In the case of GMPs, much can be learned from the biopharmaceutical experience, but advanced clinical cell engineering applications set different demands on laboratory design and operation. Perhaps the most fundamentally different characteristic of GMP cell engineering is that most applications are custom-made; unlike biopharmaceutical processing, in which mass production is the aim, an advanced cell-engineering laboratory often produces a widely processed cell product for the individual patient.

In Europe, MSCs (including BMSCs and ASCs) are considered advanced therapy medicinal products, as defined by the European Regulation [141, 145]. Furthermore, MSCs are considered somatic-cell therapy products or tissue-engineered products depending on the source, manufacturing process and proposed indications.

The transformation of protocols into GMP compliant procedures for production of clinical-grade ASCs requires careful assessment of the risks and benefits to identify and control all critical aspects [146]. Quality controls must be carried out at all phases of production. Process controls, qualifying the technique of producing the cells including functional tests, and controls of the production e.g. bacteriological tests, phenotypic controls, a visual follow-up of the cultures are necessary. Controls must also integrate analyses that allow one to ensure that the culture protocol does not lead to cell transformation (karyotype, FISH, quantitative expression of telomerase, c-myc etc.). The final quality controls must include viability and phenotype tests, which are compatible with a rapid release of the graft [146]. Furthermore, parameters such as proliferation rate and differentiation capacity of the cells must be maintained. Thus, for large-scale production of MSCs, in vitro expansion is needed to obtain a large enough number of cells. In order to maintain phenotypic and genotypic stability of MSCs during multiple passages, optimization of culture conditions is one of the most crucial aims of MSC GMP production. Even though consensus is yet lacking on the optimal method of culturing MSCs, basically DMEM or α-MEM is commonly used, with the supplementation of FBS, human serum or plasma and growth factors. For the use of FBS in GMP production, a certificate should be obtained to control the risk of infectious disease transmission. In the following chapter, some aspects of culture media and supplements used for GMP production will be discussed.

Culture Media and Serum

Media used for cell culture have an important impact on growth and differentiation of ASCs. ASCs are often plated and expanded in classical culture media containing balanced salt solutions such as MEM, DMEM, RPMI-1640 and DMEM:F-12 supplemented with FBS [2, 75, 85, 147].

Culture medium is commonly supplemented with serum. From a cell culturing point of view, serum supplementation is practical because it provides the cells with vital nutrients, attachment factors and growth factors [148]. Nevertheless, species of origin and serum concentrations affect the proliferation of ASCs [147, 149, 150]. FBS, for instance, is known to be rich in growth factors and stimulates protein accretion in cell cultures [149, 151, 152]. However, FBS replacement with human serum derivatives, such as allogeneic AB serum, thrombin-activated platelet-rich plasma and human platelet lysate have been reported to support equal or higher proliferation rates and multilineage differentiation capacity of ASCs [147, 150, 153, 154].

Nevertheless, culturing cells aimed for clinical therapy in FBS is an unsuitable option with respect to patient safety [152]. Human cells exposed to xenogeneic (i.e. animal-derived) products originating from cell culture reagents may transfer xenogeneic antibodies, such as Neu5GC, into the human body upon transplantation, raising the risk of triggering a severe immune response in the recipient [155–157]. Severe anaphylaxis and immune reactions [158, 159] have been reported induced in patients transplanted with human cells exposed to xenogeneic reagents, albeit in rare cases. Other possible risks to the recipient include viral or bacterial infections, prions, and as yet unidentified zoonoses [159, 160]. The xenogeneic antigens may, however, be removed up to 99.99% as reported by Spees and co-workers [155]. In the study, xenogeneic internalized antigens were removed from BMSCs expanded in 20% FCS (fetal calf serum) by incubating cell samples in AHS+ (autologous human serum supplemented with 10 ng ml-1 EGF and 10 ng ml-1 basic fibroblast growth factor (bFGF)) for 6 h followed by a medium change with the same medium and incubating for another 42 h. Furthermore, culturing of hMSCs in AHS+ for extended periods (5–10 days) reduced FCS contamination even further.

AutoHS is perhaps the obvious option for clinical applications, since it eliminates the problem of introducing xenogeneic or allogeneic antibodies into the patient. However, there are conflicting results of the superiority of autoHS compared to FBS in terms of proliferation rate and differentiation potential [161]. Utilizing BMSCs, some authors have reported higher proliferation rates using autoHS [23, 162–165], while others showed that autoHS yields similar results to FBS [155, 166, 167]. Oreffo et al. [168] reported improved osteogenic and adipogenic differentiation using autoHS compared to FBS, while Yamamoto et al. [167] showed similar results for osteogenic differentiation using autoHS vs. FBS. So far, no reports to quantitatively verify the effect of autoHS on cell proliferation and differentiation of ASCs have been published. Furthermore, utilizing autoHS for large-scale stem cell production for clinical applications is impeded due to limited availability and high variability in cell growth in autoHS [49, 148, 161, 165]. Serum composition is largely uncharacterized, containing variable amounts of cytokines and growth factors, such as platelet derived growth factor (PDGF), BMPs and epidermal growth factor (EGF), and showing some significant lot-to-lot variability that may affect reproducibility [81, 149, 152, 165, 169–175].

The lot-to-lot variability of serum and risk of disease transmission through serum and supplements added to media has raised concerns, leading to the development of

SF media formulations. Formulations, such as reduced serum media [172] containing nutrients to lower serum requirements and SF media containing added proteins [164, 176, 177], are now available for the expansion of BMSCs and ASCs. However, the development of completely defined, SF/xeno-free (XF) medium compositions for expansion of adult stem cells supporting the proliferation rate while maintaining the multipotential capacity of the cells is in its infancy, with only a few papers published, however, with highly promising results [98, 99, 172]. Importantly, the culturing formula must also be capable of expanding the cells multifold in a minimum number of passages, since long-term in vitro culture may alter the biology of ASCs [178, 179].

Stem Cells for Regenerative Medicine

Regenerative medicine is a multidisciplinary field of research that has evolved in parallel with the advances in the biotechnological field. It involves the use of biomaterials, growth factors, and stem cells to repair, replace, or regenerate tissues and organs damaged by injury or disease [180, 181].

What defines a successful tissue engineered construct will likely differ among tissues. For example, tissues that are designed to prolong life may allow for a lower margin of errors than those that are designed to improve the quality of life. For instance, replacement or regeneration of blood vessels or bone may be expected to last the lifetime of the individual, while replacement of cartilage may be considered successful if it delays total joint replacement for five to ten years [180].

Stem cells are ideal candidates for use in regenerative medicine because of their ability to self-renew and to commit to multiple cell lineages [1, 14]. Stem cells for regenerative medicine applications should meet the following criteria [14]:

- Can be found in abundant numbers (millions to billions of cells)
- Can be harvested by a minimally invasive procedure with minimal morbidity
- Can be differentiated along multiple cell lineage pathways in a controllable and reproducible manner
- Can be safely and effectively transplanted to either an autologous or allogeneic host
- Can be produced in accordance with current Good Manufacturing Practice guidelines

Several sources of stem cells likely fulfill the criteria but adipose stem cells have several advantages [58]; with the increased occurrence of obesity, subcutaneous adipose tissue is accessible and thus ASCs can be harvested in large quantities with minimal risk. In addition, adipose tissue yields manifold greater numbers of MSCs compared to bone marrow [58]. Since 2001, when the existence of multipotent stem cells within this tissue was reported [12], adipose tissue has acquired increased importance as a stem cell source for a wide range of potential applications in regenerative medicine strategies, largely owing to its availability and accessibility.

Aspects on Clinical Applicability of ASC

Immunomodulatory Properties

MSC from various tissues are currently the focus of clinical and scientific research due to their exceptional abilities for multilineage differentiation and their immunomodulatory properties. The immunosuppressive properties of BMSCs has been well reported in vitro and in vivo [182–184]. These characteristics support the clinical significance of utilizing BMSCs in allogeneic transplantation, for instance by reducing the incidence and severity of graft-versus-host disease (GvHD) in the recipient [185, 186]. BMSCs likely go undetected by the immune system because they hold a cell surface phenotype which is poorly recognized by T cells, due to the absence of MHC II or co-stimulatory molecule B7, CD40 or CD40L [184, 186]. In contrast, despite the increasing interest in the use of ASCs cells in cell therapy, their immunosuppressive properties have not been studied extensively so far. ASCs exert profound immunomodulatory properties and protective effects on acute GvHD and experimental arthritis [20]. By functional characterization, ASCs have been shown to be immunoprivileged [20, 21], due to lack of HLA-DR expression (major histocompatibility complex class II; MHC II) [75, 187], and the suppression of proliferation of activated allogeneic lymphocytes [84, 85, 188–190]. Additionally, ASCs have been shown to inhibit the production of inflammatory cytokines by both CD4 T helper cells and CD8 Tc1 cells, stimulate the production of the anti-inflammatory/suppressive cytokine IL-10 by monocytes and T cells, and induce the generation of antigen-specific regulatory T cells [20]. Moreover, ASCs have been shown to promote engraftment and prevent or treat severe GvHD in allogeneic stem cell transplantation in vitro and in vivo [189, 191].

Tumorigenic or tumorsuppressive?

Although ASCs present an intriguing new approach for the control of GvHD, recent studies have shown that the immunosuppressive effects of these cells may favor the growth of tumor cells but contradictory results exist and the topic remains a matter of intense debate [192–195]. Muehlberg and co-workers reported that mouse ASCs in a murine model home to the tumor site and promote tumor growth in

vivo, not only when co-injected locally, but also when injected intravenously [194]. Yu and co-workers showed that hASCs together with tumor cells transplanted subcutaneously or intracranially into BALB/c nude mice promoted tumor growth [193]. Conversely, Kucerova and co-workers showed that cytosine deaminase-expressing ASCs deliver the cytosine deaminase transgene to the site of tumor formation and mediate a strong antitumor effect in vivo [192]. Similarly, Grisendi and co-workers demonstrated that ASCs would be suitable as cellular vectors in TRAIL-based cancer therapy [196]. Cousin and co-workers reported that ASC strongly inhibit proliferation of pancreatic ductal adenocarcinoma cells, both in vitro and in vivo by interfering with the proliferation of tumor cells by altering cell cycle progression [195]. These divergent reports may in part be explained by variations in the protocols used in vivo and in vitro. In some studies, ASC were injected in vivo in combination with the cancer cells, making it difficult to compare the effects of ASC on tumor formation. In addition, some data were obtained using mouse cells, whereas in some experiments human cells were utilized. Moreover, cancer cells from diverse origins were investigated. Although an experimental model is never completely consistent with the complex mechanisms in nature, these contradictory results indicate that the work is far from done, and further studies and consensual protocols are necessary to fully elucidate the true effect of ASCs on tumor formation.

Clinical Trials Using ASCs

When searching for clinical trials using BMSCs, more than 500 trials can be found that are underway or completed. While searching for clinical trials utilizing either SVF cells or ASCs, a total of 18 trials can be found, of which two are so far completed (see http://clinicaltrials.gov). The only currently ongoing phase III clinical trial investigates autologous ASCs in repairing perianal fistulas not associated with Crohn's Disease in patients [28, 197–200] (Table 1). In phase II of the same study, patients with complex perianal fistulas were randomly assigned to treatment with either fibrin glue or fibrin glue and 20 million ASCs. The results showed that ASCs were more effective than fibrin glue alone, moreover, the quality of life scores were higher in patients who received ASCs than in those who received fibrin glue alone [199].

Clinical Case Studies Using ASCs

Thus far, there are only a few clinical cell therapy case reports published on the use of ASCs. While the treatment modalities and conditions vary dramatically, ASC or SVF administration has, at least, been well tolerated, with no adverse effects reported. Nonetheless, the condition that the cells were intended for was not always improved or cured. Below are brief descriptions of the conditions and the modalities where adipose stem cells have been administered, as well as the outcome of the study. Thus, these reports show that cells from adipose tissue have potential in many areas of clinical cell therapy and regenerative medicine, albeit a lot of work is yet to be done.

ASCs in Immunologic Disorders

Graft-versus-host disease For BMSCs, 20 clinical trials for treatment of GvHD have been reported (see http://clinicaltrials.gov), of which at least ten are already completed. Yet, no clinical trials using ASCs for treating severe and acute GvHD are currently under way. Furthermore, the efficacy of ASCs is currently being studied in GvHD; however, their effect on alloreactivity in solid organ transplant patients is unknown. Fang and co-workers have reported several case studies where ASCs have been used for treating severe and acute GvHD [24–27]. In these studies, severe cases of GvHD of the gut and liver were treated in a 38-year-old woman, a 15-year-old boy and a 12-year-old girl. The administration of ASCs in treating GvHD was successful in all three cases. Still, results from other independent research teams are necessary to impartially verify the effect of ASCs in GvHD.

Immunosuppression by ASCs The use of ASCs for immunologic disorders, for example, in autoimmune-induced rheumatoid arthritis (RA) or inflammatory bowel disease, such as Crohn's disease and ulcerous colitis, is currently under investigation [20, 21, 28, 198]. In the RA case by Gonzales and co-workers, the effects of hASCs on T-cell proliferation and cytokine production, and the production of inflammatory mediators by monocytes and fibroblast-like synoviocytes from RA patients were investigated. The results showed that hASCs are important regulators of immune tolerance, with ability to suppress T-cell and inflammatory responses and inducing the activation of antigen-specific regulatory T cells [20]. In the ulcerous colitis study, acute and chronic colitis as well as sepsis was induced in mice. Colitic and septic mice were treated intraperitoneally with hASCs or murine ASCs, and diverse clinical signs of disease were investigated as well as the levels of various inflammatory cytokines and chemokines, T helper 1-type response and generation of regulatory T cells in affected organs. Systemic infusion of ASCs significantly reduced the severity of colitis by eliminating weight loss, diarrhea and inflammation and increasing survival. ASCs also protected from severe sepsis by reducing the infiltration of inflammatory cells in various target organs and by downregulating the production of various inflammatory mediators [21].

Multiple sclerosis In a recent publication by Riordan and co-workers, autologous SVF cells were administered in a physician-initiated compassionate-use treatment of multiple sclerosis in 3 patients; a 50-year-old man, a 32-year-old man and a patient for whom no age or gender information was submitted [29]. Each patient received two I.V. infusions with 25–75 x 10^6 autologous adipose derived SVF cells and multiple intrathecal and intravenous infusions of allogeneic CD34+ and MSC cells. In the report, no conclusions were drawn in terms of therapeutic efficacy based on the studies, apart from the patient's reporting improved quality of life. Further clinical evaluation of autologous SVF cells is necessary in autoimmune conditions.

Diabetes Mellitus In a report published in 2008 Trivendi and co-workers [201] reported a safe and effective treatment of 5 insulinopenic diabetics using insulin-producing ASCs transfused with unfractionated cultured bone marrow cultured using a completely xeno-free workflow. The study showed that all patients were successfully infused bone marrow and ASC without any adverse effects and showed 30–50% decreased insulin requirements with a 4- to 26-fold increase in serum c-peptide levels during a follow-up period of 2.9 months on average.

Tracheomediastinal Fistulas In a case study, a 67-year-old man with lung cancer with an acquired fistula located in the trachea and upper right lobe was treated with an injection of autologous SVF cells derived from adipose tissue suspended in fibrin glue. Tracheomediastinal fistula is a rare condition and many patients die from hemorrhage resulting from progression of the fistula into the blood vessels. The bronchoscopic postoperative examination revealed closure of the fistula. The results of the treatment were permanent and stable, without any complications during 2 years of follow-up after treatment [202].

Soft-Tissue Augmentation Histioconductive approaches to augment soft-tissue defects using scaffolds seeded with ASCs have been reported [203]. ASCs, or preadipocytes, were seeded on hyaluronic acid-based scaffolds (HYAFF®11), called ADIPOGRAFT® and were implanted subcutaneously in twelve volunteers, aged 20–35 years. Acellular scaffolds (HYAFF®11) were used as controls. After 8 weeks, the ADIPOGRAFT® scaffolds demonstrated matrix deposition and cell infiltration. Nevertheless, despite promising results in rodents and excellent biocompatibility and degradation characteristics in the in vivo human model, the hyaluronic acid-based scaffolds did not support preadipocyte survival and were not inductive towards adipose tissue formation [203].

In two reports, Yoshimura and co-workers used adipose-derived SVF cells for soft tissue augmentation by a novel strategy called cell-assisted lipotransfer (CAL) [30, 31] for treatment of facial lipoatrophy and for breast augmentation. Using this strategy, they isolated the SVF cells from half of an liposuction aspirate and recombined it with the other half of the liposuction aspirate, converting the relatively ASC-poor aspirated fat to ASC-rich fat [30, 31]. In the facial lipoatrophy study, six patients were treated; three patients received conventional lipoinjection (non-CAL), while three patients received CAL. The results showed no adverse side effects or complications, except in one case in the non-CAL group where oral corticosteroids had been used preoperatively. The authors report that lipoinjection is an effective tool for recontouring facial lipoatrophy, and ASC supplementation may presumably have improved its efficacy. Furthermore, the authors suggest larger studies and longer follow-ups to establish the superiority and the durability of the CAL-mediated improvements in facial lipoatrophy patients. In the breast augmentation study, 40 patients with a mean body mass index (BMI) of 19.1±1.9 with healthy thoraxes and breasts underwent CAL for purely cosmetic reasons. In all cases the breast augmentation was successful, with satisfactory clinical results generally achieved without any major complications.

Bone Tissue Repair To date, two clinical case studies have been reported where the capacity of ASCs in bone tissue repair has been investigated [32, 33]. In the first case, the patient was a 7-year-old girl, who had sustained severe head injury after a fall, resulting in a closed multifragment calvarial fracture. Fixation of the calvarial fragments was performed with titanium miniplates, however, due to insufficient fixation, progressive and disseminated calvarial bone resorption occurred over several months, resulting in an unstable scull. Subsequently, the calvarial defect was treated with autologous SVF cells isolated and applied in a single operative procedure in combination with milled autologous bone from the iliac crest. The SVF cells were supported in place using autologous fibrin glue, and mechanical fixation was achieved with two large, resorbable macroporous sheets acting as a soft tissue barrier. The postoperative course was uneventful and new bone formation and near complete calvarial continuity was observed three months after the reconstruction.

The study by Lendeckel and co-workers has some drawbacks that must be considered. Harvesting of bone tissue or a composite microvascular flap is frequently followed by morbidity and a donor site defect despite usually being in an area of lesser importance [204, 205]. Furthermore, a large amount of autologous blood is needed for plasmapheresis, which may in some cases be difficult to

obtain. It is known that the SVF fraction is able to produce angiogenic factors, promote neovascularization and vessel-like structure formation [15, 128] but the capacity has also been reported in expanded ASCs [130, 206]. To note, however, the SVF fraction contains significantly less stem cells with the capacity to differentiate towards mesenchymal lineages, such as osteoblasts.

In the second case [33], that was conducted in collaboration between Tampere University Hospital and Regea—Institute for Regenerative Medicine the problem with harvesting autologous bone was circumvented by using composite graft consisting of autologous ASCs combined with a synthetic bone substitute material, beta tricalcium phosphate, βTCP and rhBMP-2. For this purpose, ASCs were expanded ex vivo and combined with βTCP and rhBMP-2 prior to clinical transplantation procedures. Furthermore, the autologous ASCs were handled and prepared xeno-free in clean rooms according with current GMP regulations. The postoperative healing was uneventful, mature bone structures had developed within the construct, and further rehabilitation with dental implants was carried out. So far, 25 patients with craniomaxillofacial defects have been treated at Regea using similar techniques (Numminen et al., submitted, Thesleff et al., submitted) (Fig. 4). However, further studies are required to assess and verify the safe outcome of the clinical procedure using in vitro expanded stem cells.

Future Perspectives

In general, regardless of differences on an individual level, ASCs are shown to survive in a low oxygen environment making them good candidates for cell-based therapies [207] by secreting angiogenic cytokines such as VEGF and HGF, [14, 130, 208], perhaps to a higher degree than for instance BMSCs [206]. Nevertheless, despite having apparently optimal characteristics for clinical applications, showing immunomodulatory properties and protective effects in immunological disorders in addition to the angiogenic properties, the properties may have the opposite effect. Reports have shown that the immunosuppressive capacity of the ASCs may in some cases favor the growth of tumor cells but contradictory results exist and the question is a matter of intense debate [192–195]. These contradictory results indicate that the investigation of the molecular characteristics of ASCs is by far not done, and further studies and consensual protocols are necessary to fully elucidate the true effect of ASCs on tumor formation.

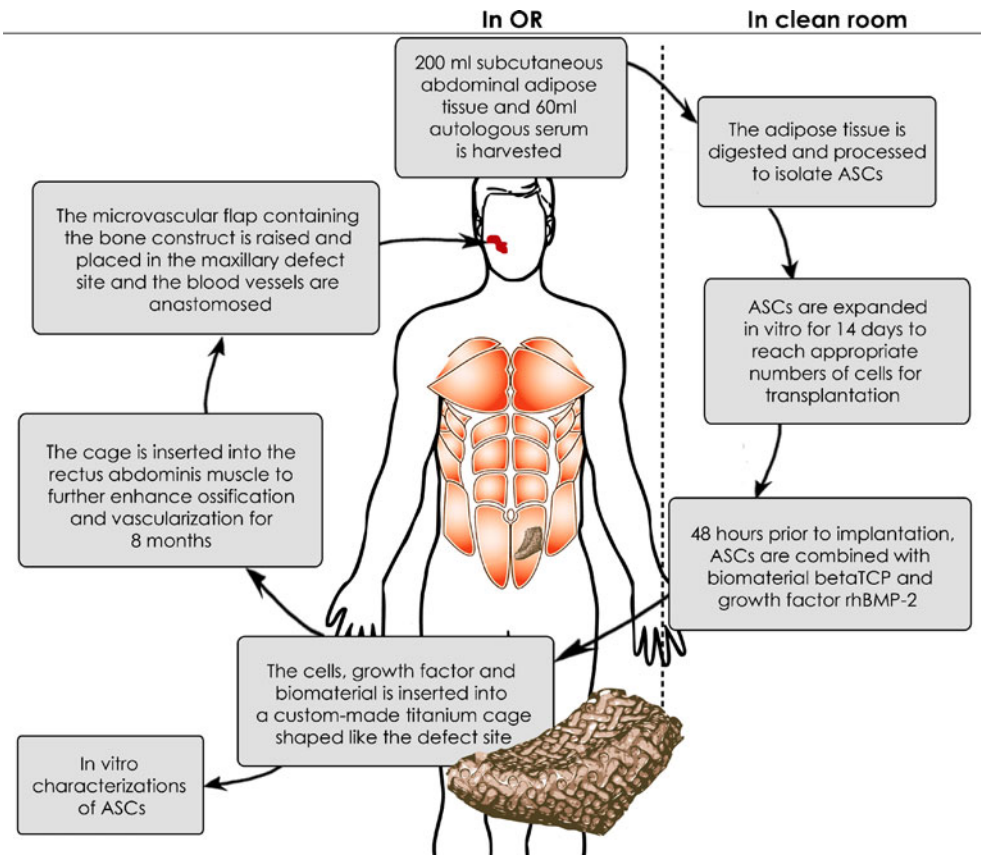

Fig. 4 Schematic outline of the bone reconstruction procedure performed in the clinical case study presented in [33, 218]

From a clinical point of view, since FBS is not recommended for clinical cell therapies for several reasons discussed above, and if autologous serum is not available in large enough quantities for expansion of ASCs for clinical applications, alloHS or SF/XF conditions must be employed. From this point of view, the immunogenicity of ASCs is very important and requires further investigation. Studies have shown [84] that the immunogenic response declined shortly after initiation of culture of ASCs and is essentially lost in higher passages of culture. The alloreactivity is determined by the presence of antigen presenting cells (APCs) within the population, assessed by the expression of MHC class I and class II molecules in addition to co-stimulatory molecules, such as CD80 and CD86 in the cell population, as well as other APC-associated markers (CD45, CD11a, CD14, CD86, and MHC class II antigens) [84]. Early passages of ASCs (SVF or P0) still express APC-associated markers, while late passages do not. This may be due to the early passages of ASCs still containing hematopoietic-derived APCs or alternatively, the adherent ASCs may themselves express the APC-related surface proteins initially during culture and thereby serve as immunogenic stimuli in the mixed lymphocyte reaction (MLR). Since all reports on the immunomodulatory effects of ASCs have been performed on FBS expanded ASCs [84, 188, 189], these markers would need to be assessed on ASCs in alloHS, autoHS and in SF/XF conditions since these more closely resemble the clinical situation. Also, to further consider the immunosuppressive and immunogenic properties of these culture conditions, one-way MLR assay determining the immunogenicity of ASC populations and two-way MLR assay evaluating the suppression by ASC populations should be carried out.

Rare studies have been carried out on the mechanism of immunosuppression by ASCs. Puissant and co-workers established that the immunosuppressive effect of the ASCs was not completely reliant on direct contact with lymphocytes, suggesting that a soluble factor may be involved [189]. BMSCs have been reported to secrete suppressive molecules, including HGF and TGF-β [209] and prostaglandins [210, 211]. ASC have also been studied in the presence of FBS for possible soluble factors, and similarly to BMSCs, elevated levels of prostaglandin E2 (PGE2), were detected but not TGF-β and HGF, when co-cultured in a MLR [188]. Several mechanisms have been suggested to be responsible for BMSC-mediated suppression of lymphocyte proliferation that needs to be determined and possibly confirmed for ASCs. These include assessment of division arrest of activated T cells and B cells by inhibition of cyclin D2 expression [212], induction of regulatory T cells [210] or APCs [213], and interference with dendritic cell [210] and cytotoxic T cell maturation [214].

Although ASCs appear to be fairly stable genetically in long-term culture, some clonal chromosomal aberrations also arise transiently in early passage ASC cultures that may disappear or become negligible later on, at least when cells cultured for approximately one month (35–45 population doublings) [22, 215]. According to a study by Rubio and co-workers, ASCs can be cultured safely during a standard ex vivo expansion period of approximately 6–8 weeks, but spontaneous transformation may occur following long-term in vitro culture of approximately 4–5 months [216]. Due to the relatively slow growth rate of ASCs and their usually normal karyotypes, ASCs may, even at a later stage of their lifespan, maintain a normal cytogenetic profile, which is beneficial considering the expansion times required to reach sufficient cell numbers for clinical therapies, still adhering to the standard ex vivo expansion period maximum of 6–8 weeks. However, in recent studies, minor abnormalities consisting of occasional but minor copy number changes were detected in early passage ASC cultures [22, 215]. Evidently, even a small percentage of chromosomally abnormal cells within a population could pose a risk upon transplantation.

Modification of culture conditions may be an effective way to manage problems of tetraploidy or chromosome instability should they occur in some stem/progenitor cell types but it may also have unwanted or unexpected effects. Therefore, new culture conditions, such as the XF and SF/XF conditions mentioned above, need to be evaluated for their ability to maintain the genomic stability of ASCs. The fact that minor aberrations may occur as a result of in vitro cell expansion, calls for a need for appropriate screening before cell transplantation. Furthermore, in parallel with conventional G-banding, FISH analysis may be necessary to perform since it may detect low level chromosomal changes induced in culture prior to clinical application [215]. Furthermore, in vivo testing of immunodeficient animal tumor with SF/XF conditioned ASCs will be required by a setup similar as to that reported by Rubio and co-workers [216].

Since ASC are gaining popularity in clinical stem cell therapy studies, improved methods to assess safety, reproducibility and quality of the vitro expanded stem cells are urgently called for. The methods must not only verify safety of the cells in vitro, but also in vivo and clinically. The results from this study as well as results reported by others suggest that cells from various adult tissue sources may be utilized for clinical cell therapy. Regardless of the cell source, the choice of culturing supplements as well as the cell expansion time may have considerable effects on the cells on gene level, thus affecting the quality and safety of the cell product. Consequently, assessing the immunogenic properties of the cells in vitro and in vivo is important to assure that anaphylactic reactions in the recipient are avoided. Furthermore, producing cells that are genetically

stable is a step towards ensuring that the cells do not transform, leading to genetically aberrated progeny when transplanted into the recipient. The research adult stem cells are still in its infancy; therefore, extensive pre-clinical safety and efficacy studies will need to be carried out before the promise of the adult stem cells can be fully appreciated.

Acknowledgements The author wishes to thank Prof. Jeffrey Gimble, Pennington Medical Research Center for the valuable comments on the manuscript. The work was supported by TEKES, the Finnish Funding Agency for Technology and Innovation, the competitive research funding of the Pirkanmaa Hospital District, the Finnish Dental Society Apollonia, the City of Tampere, the Maud Kuistila Memorial Foundation and the Finnish Cultural Foundation Pirkanmaa Provincial Foundation.

Conflict of interest The authors declare no potential conflicts of interest.

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