Human Adipose-Derived Stem Cells for Tissue Engineering Approaches: Current Challenges and Perspectives

Sorina Dinescu, Anca Hermenean and Marieta Costache

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Abstract

Human adipose-derived stem cells (hASCs) currently represent a viable source of mesenchymal-*like* stem cells, with similar properties and differentiation potential to bone-marrow-derived mesenchymal stem cells (BM-MSCs) but with a different and more accessible source—the adipose tissue. hASCs are able to produce almost all of the factors that contribute to normal wound healing, and therefore, they are preferred for all types of tissue engineering (TE) and regenerative medical applications. This chapter will review hASCs regeneration potential and the most modern approaches in TE for bone, cartilage and adipose tissue regeneration using hASCs. Furthermore, an overview of novel and original hASCs-scaffold constructs studied in our group completes an up-to-date presentation of hASCs multiple uses. Additionally, this chapter will highlight the relevance of ultimate advances in regenerative medicine and the need for this evolution to increase the quality of life in patients with tissue defects.

Keywords: human adipose-derived stem cells, tissue engineering, regenerative medicine, stem cell differentiation

1. Introduction

Current tissue engineering (TE) approaches registered an evolution over the past few years in the area of regenerative medicine. If the last decade was dedicated to repair and reconstructive procedures involving the implantation of inert materials to solve tissue defects, the modern trend uses regenerative strategies frequently based on advanced biomaterials and the differentiation potential of stem cells.

The basic principle underlying this modern tissue engineering concept is that an equilibrium should be established simultaneously between the elements required to obtain a functional engineered tissue—suitable cells, appropriate scaffolds and the adequate signalling molecules. The goal of tissue engineering is to recreate a microenvironment as similar as possible to the *in vivo* natural tissue.

Human adipose-derived stem cells (hASCs) represent a viable source of mesenchymal-*like* stem cells, with similar properties to bone-marrow-derived mesenchymal stem cells (BM-MSCs) but with a different and more accessible source—the adipose tissue. A particular population of stem cells with self-renewal properties and multilineage differentiation potential, isolated from the adipose stromal-vascular compartment, was first reported by Zuk et al. [1]. In further studies, it has been proved that these cells display (i) a hypoimmunogenic profile [2], (ii) can be easily harvested from subcutaneous adipose tissue during liposuction procedures [3] and (iii) since they can be isolated from autologous fat, there is no ethical issue involved in case of transplantation for regenerative purposes. All these properties that hASCs display make them a more viable solution for regenerative medicine approaches then MSCs or other adult stem cells.

Apart from the fact they can be more easily harvested than MSCs, hASCs are able to produce almost all of the factors that contribute to normal wound healing [4]. Consequently, at the injury site, implanted cells that undergo differentiation generate not only an inert filling tissue but also are able to stimulate cell recruitment from stem cell niches in order to aesthetically restore the site of injury in a paracrine manner (by secretion of growth factors and cytokines) [5]. These observations suggest that hASCs could be better candidates for TE applications than other traditional cell sources.

hASCs' clinical and TE applications: Modern regenerative therapies use hASCs, based on their abundance, distribution and multilineage differentiation ability. There are certain studies that put, under a question mark, the safety issues related to hASCs use for regeneration purposes, since several genetic abnormalities after their *in vitro* expansion or differentiation have been reported by several groups [6–8]. However, no interdiction of hASCs in clinical practice for TE has been yet announced.

Regarding the scaffolds appropriate for TE applications, there has been an evolution in the composition and properties of biomaterials in the last decade. Nowadays, biomaterials tend to be made of natural and biodegradable compounds, thus favouring their biocompatibility. In addition, a 3D scaffold displays a significantly increase in capacity to closely mimic *in vivo* cellular microenvironments [9, 10].

Different biomaterials have been used in studies to favour hASCs growth in 3D scaffolds. hASCs displayed potential to attach, proliferate and differentiate in contact with favourable biomaterial compositions. Ideally, biofabricated scaffolds should offer hASCs proper environments to facilitate their proliferation and maintain their differentiation potentials [3]. Besides the biocompatibility condition, these materials should also be synthesized to have highly porous structures with interconnected architecture to mimic the native tissue niche [3]. Science in the field of scaffold engineering has evolved towards biofabrication using modern approaches

such as bioprinting, patterning, self-assembling and organ-on-a-chip [10]. According to Dai et al., most of these approaches have been employed to encapsulate hASCs in 3D structures (resulting in 3D culture systems) specifically designed for a TE application.

Related to *in vivo* experiments using hASCs, it was both experimentally and clinically shown that the topical administration of hASCs to full-thickness radiated wounds increases the healing rate of the wound [4]. It was also shown that hASCs stimulate fibroblast proliferation and migration and type I collagen secretion in an *in vivo* wound model. These findings suggest that hASCs may promote *in vivo* wound healing.

hASCs are currently recognized as an attractive and efficient adult stem cell type for regenerative medicine. Still, there are problems that need to be clarified including the mechanisms of the interactions among hASCs and their long-term safety. Only a small number of clinical trials have been performed by now [3].

The majority of clinical trials involving hASCs or hASCs-enriched fat grafts are incipient phase clinical trials (phase I or II), while only one trial reached phase IV in human subjects (NCT00616135). Several directions were approached by clinicians in order to test the efficiency of hASCs in tissue regeneration. Breast reconstruction is one of the major applications approached by clinicians, not only for breast augmentation purposes (NCT01771913) but also for breast reconstruction after tumour resection (NCT00616135). Special care should be given for implantation of hASCs in a former tumour microenvironment, since little is known about hASCs stability at genomic level. Both clinical trials resulted in a favourable primary outcome. However, this direction is considered to have limitations since the implanted fat graft suffered resorption over time and thus the implant shape and dimension altered after 6–12 months. Additional successful applications where hASCs were involved are liver tissue reconstruction (NCT01062750), cardiovascular disease (NCT01449032; NCT00442806; NCT00426868; NCT01216995), osteoarthritis (NCT01585857) or brain injury (NCT01649700). All results showed hASCs display high therapeutic potential and generally display safety for *in vivo* implantation.

Considering all the abovementioned, the chapter will further present a few biomaterial compositions that we used in conjunction with hASCs and stimulating factors. In particular, we have studied the potential of hASCs differentiation towards the adipose, cartilage and bone lineages during *in vitro* studies in different 3D original scaffolds. For adipose tissue engineering (ATE), we have identified novel biomaterials based on gelatin-alginate-polyacrylamide (GAPAA) [11] and collagen-sericin (CollSS) [12] which proved to be efficient for soft tissue reconstruction. In parallel, the addition of hyaluronic acid and chondroitin sulphate proved to increase the quality of the CollSS hydrogel and to transform it in a scaffold designed for cartilage tissue engineering (CTE) [13]. We have also tested and validated different scaffolds based on chitosan, polyvinyl alcohol, polysulphone, etc. each improved by the addition of graphene oxide (GO), giving stability to the structure. An appropriate concentration of GO in scaffolds composition resulted in significantly better cell differentiation towards bone tissue.

Both chitosan/GO biomaterials and improved collagen scaffolds for cartilage repair were tested for response during *in vivo* studies on mouse models. Results supported the conclusions

obtained *in vitro* and confirmed the efficiency of these differentiation-specific cell-scaffold systems. However, only a small number of studies have addressed *in vivo* applications involving ASCs. More studies need to be developed in this direction in order to evaluate the good and the bad potential of hASCs.

2. Human adipose-derived stem cells: source, properties and differentiation potential

2.1. hASCs isolation and characterization

hASCs can be reproducibly isolated from liposuction aspirates through a procedure involving collagenase digestion, differential centrifugation and expansion in culture [5, 14]. Undifferentiated hASCs express a distinct immunophenotype (hASCs express the MSC markers CD10, CD13, CD29, CD34, CD44, CD54, CD71, CD90, CD105, CD106, CD117 and STRO-1 and are negative for hematopoietic lineage and endothelial cell markers) detectable by flow cytometry and produce additional adipocyte-specific proteins upon induction [15, 16]. The hASCs immunophenotype was also assessed by flow cytometry in our team and the results were successfully reported [14].

The protocol for the isolation of hASCs involves the removal of subcutaneous fat by liposuction and treatment with collagenase, a hydrolytic enzyme [1]. hASCs are found in a homogeneous mixture called stromal vascular fraction (SVF), together with endothelial cells, stromal and hematopoietic cells; due to their tendency to adhere to the substrate in the culture medium, hASCs can be isolated easily [17].

Mainly distributed at subcutaneous and visceral fat, adipose tissue is an excellent source of stem cells, providing approximately 300,000 cells/ml, 5 times more than in the bone marrow [1]. Similar to BM-MSCs, hASCs may differentiate towards osteogenic, adipogenic, myogenic, neurogenic and chondrogenic pathways, depending on the experimental conditions [16]. Similarities between hASCs and BM-MSCs are found in the secretory profile and CD surface markers [16, 18].

Adipose tissue has an endocrine auxiliary function, secreting cytokines and growth factors. Thus, it was found that hASCs produce increased amounts of epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), the keratinocytes growth factor (KGF), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), hepatocyte growth factor (HGF), transforming growth factor-β (TGF-β), insulin-*like* growth factor (IGF) and brain-derived neurotrophic factor (BDNF) [2, 19]. There are also products and cytokines such as Flt-3-ligand, granulocyte colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), macrophage colony stimulating factor (M-CSF), bone morphogenetic proteins (BMP), interleukins-6,-7,-8,-11,-12, leukaemia inhibitory factor (LIF) and tumour necrosis factor alpha (TNF-α) [2, 19]. Based on hASCs properties to secrete molecules that modulate all stages of healing and to differentiate into multiple cell types, stem cells are actively involved in wound healing when administered in the vicinity of the affected

tissues [20]. In many experiments, hASCs administered at a lesion site have stimulated the development of granulation tissue and increased cell density and traumatic wound re-epithelialization [20, 21]. Recent research developments have shown that hASCs produce a volume of cytokines and growth factors superior to BM-MSCs and to human dermal fibroblasts, thus being ideal tools in tissue regeneration [22].

Since fat tissue actively intervenes in the endocrine processes, paracrine action of transplanted hASCs can negatively influence peripheral tissues. For example, the secretion of active hormones such as leptin, certain cytokines such as IL-6 and TNF-α or of certain growth factors can lead to a disturbance of the peripheral metabolic pathways [23].

2.2. hASCs regenerative potential

Most organs display "reservoirs" of adult stem cells that are activated in case of trauma, infection or disease [24]. In many of these cases, the endogenous stem cell populations are insufficient to cope with compromised tissue regeneration process, and therefore, modern strategies in the field of regenerative medicine involve the use of exogenous stem cells. Restoration of damaged structures, as well as the resumption of the restored tissue functionality, was associated with stem cells ability to adopt a specific phenotype through differentiation and to paracrine actions of stem cells [25].

Numerous studies showed that hASCs have the potential to differentiate into bone, cartilage and muscle, as well as adipose and neural tissue [15, 26, 27]. This ability to differentiate towards different mesenchymal lineages has stimulated interest in their clinical use. hASCs have also been used for breast augmentation and to treat congenital deformities and other defects as well as for reconstruction after mastectomy [28]. Immunomodulatory molecules, growth factors, angiogenic and antifibrotic factors released by hASCs, matrix metalloproteinases and collagen stimulate the regeneration and remodelling of altered structures [29], whereas secreted anti-apoptotic molecules and antioxidants protect cells in proximity [30].

The most important differentiation pathways reported for hASCs will be further described:

2.2.1. Adipogenic differentiation and adipose tissue engineering involving hASCs

Adipogenic differentiation is induced *in vitro* by treatment with a cocktail based on dexamethasone, indomethacin, isobutyl-methyl-xanthine [31] and insulin [32]. The mixture stimulates expression of the receptor peroxisome proliferation-activated receptor ɣ2 (PPAR-ɣ2), key inducer of adipogenic differentiation, which, once activated, triggers the transcription of a set of genes involved in differentiation of terminal adipocytes [33]. These include genes encoding the synthase fatty acid (FAS), the protein binding of fatty acids P2 (aP2), perilipin marker of adipogenic differentiation, lipoprotein lipase (LPL), the carrier protein fatty acid-1 (FATP-1) and adipocytokines (adiponectin, leptin and resistin). All of these events stimulate the lipid metabolism, leading in the end to the formation of intracellular lipid vesicles [34].

Paracrine action of MSCs plays a key role in the modulation of adipogenic differentiation. Thus, BMP, with cytokine function, stimulates *in vivo* adipogenesis. Bone morphogenetic

proteins receptor type 1A (BMPR-1A) can direct the differentiation of mesenchymal stem cells either to adipogenic lineage or osteogenic direction [35]. Insulin, glucocorticoids and FGF factors promote adipogenic differentiation, while molecules such as TNF-α and Wnt stop this process [36].

Eljaafari et al. [37] have recently found that hASCs isolated from obese individuals are able to induce a pro-inflammatory response by monocyte activation and stimulation of T helper 17 cells (Th17), which inhibit adipogenesis and response of adipocytes to insulin. Vascularization of *de novo* generated tissue is promoted by molecules such as bFGF, VEGF, TGF-β, PDGF, angiopoietin-1, monocyte chemoattractant protein-1 (MCP-1) and even extracellular vesicles (exosomes) that transfer genetic material and pro-angiogenic molecules from stem cells to cells in proximity [38].

In many experiments, hASCs were combined with biodegradable polymer-based scaffolds in order to validate efficient systems for adipogenesis. Adipogenic differentiation is exploited most often for breast reconstruction, either in normal conditions or after a breast tumour removal [17]. In contrast to the classical inert materials used as breast implants, the tissue generated *de novo* after differentiation from hASCs exhibits an optimal biocompatibility, is well vascularized, does not shrink, is not absorbed over time and does not trigger allergic reactions. In conclusion, autologous fat implants with active biomolecules and synthetic substitutes appear to be more efficient for adipose tissue regeneration than other methods in the field and ensure better quality of life in patients with fat tissue defects.

2.2.2. Osteogenic differentiation potential and bone tissue engineering involving hASCs

hASCs secretory profile includes many molecules that promote bone regeneration. Cytokines IGF-1, VEGF, HGF, BMP-1, IL-6, IL-3, MCP-1 and MCP-3 modulate the most important step in the process of bone formation: angiogenesis, cell migration and proliferation and differentiation of osteoblastic precursors [39]. IGF-1 is the main factor that stimulates cell proliferation and migration, VEGF promotes angiogenesis, while the other factors promote bone formation, favouring the recruitment of osteoprogenitor cells, their proliferation and differentiation into osteocytes [40]. Secretory profile and osteogenic differentiation capacity of hASCs sites are ideal resources for bone regeneration.

It was observed that hASCs exposed to a cocktail of pro-osteogenic inducers are able to initiate and develop osteogenic differentiation process. In a study, Halvorsen et al. [41] used a medium containing ascorbic acid, BMP-2, dexamethasone and 1, 25 dihydroxyvitamin D3 to induce osteogenesis. Results indicated that osteoblast-*like* cells were obtained.

The first attempts of bone regeneration were carried out on animal experimental models in order to determine the ideal conditions for mineralization of the new tissue. Then, in a study involving mice infected with severe combined immunodeficiency (SCID), the formation of osteoid in 80% of mice under investigation due to an implant of hydroxyapatite-tricalcium phosphate scaffold seeded with hASCs was revealed [42].

Microenvironment conditions affect the ability of hASCs to commit to osteogenesis *in vitro*. This was shown, for example, when comparing hASCs cultured in a medium supplemented

with human serum (better differentiation) with cells exposed to a serum-free medium [43]. Gender differences between donors can also affect the ability of hASCs to differentiate, probably because of differences in steroid hormones [44]. Osteogenic potential decreases with increasing age [45].

Using hASCs in bone tissue engineering (BTE) applications is an alternative strategy to replace or restore bone function, where the tissue was traumatized, damaged or lost. Typically, when hASCs differentiate towards bone-*like* cells, three phases can be observed: cell proliferation, extracellular matrix synthesis and mineralization of the matrix [16]. After 2–3 weeks of differentiation, the induction of alkaline phosphatase activity is observed, an enzyme involved in calcification of the matrix. In parallel, synthesis of calcium phosphate in the extracellular matrix is activated. Extracellular matrix mineralization can be detected by Alizarin red staining or von Kossa staining [16, 41].

Mesimäki et al. [46] reported a method to reconstruct a defect in the jaw in an adult patient using hASCs exposed to BMP-2 treatment. Another study developed on 23 patients with craniofacial defects [47] revealed that bone-*like* tissue could be obtained by a synergistic effect of hASCs and a tricalcium phosphate scaffold (β-TCP) in the presence of growth factors (BMP-2). Similarly, skull defects were successfully reconstructed or their healing was accelerated by using hASCs and pro-osteogenic conditions [46, 47].

A more recent study revealed that hASCs were able to differentiate towards the osteogenic lineage also in contact with synthetic polymers, such as polyethylene glycol diacrylate co-N-acryloyl-6-aminocaproic acid [48]. In this case, cells were allowed to infiltrate in this gel and then to differentiate, leading to the increase in the osteoblast cell differentiation. Differentiation was confirmed by analysis of calcium deposits, by quantification of alkaline phosphatase and by specific determination of molecular markers of bone formation, such as osteocalcin, osteopontin and the transcription factor correlated with Runt-2 (Runx2).

Another experiment involved osteogenesis of MSCs encapsulated in microspheres made of chitosan and collagen in a media supplemented with dexamethasone, β-glycerophosphate and ascorbic acid 2-phosphate. During differentiation, the level of expression found in genes encoding for collagen I, bone sialoprotein and osterix osteogenic markers demonstrated hASCs capacity to differentiate the osteogenic lineage [49].

2.2.3. Chondrogenic differentiation potential and cartilage tissue engineering involving hASCs

A crucial condition for chondrogenic differentiation is that hASCs need to be cultured in a pellet system to form spheroids. These systems are suitable because they allow cell condensation that occurs during embryonic development, increasing the interactions between cells and eventually forming a cartilaginous matrix [50]. Thus, 3D biomaterials with certain architectural and mechanical properties would be very suitable microenvironments to allow hASCs condensation and differentiation.

It was generally shown that there are several growth factors which stimulate cell proliferation and differentiation towards the chondrogenic lineage. Adding these growth factors to the scaffold structure or in the culture media greatly favours chondrogenesis. They maintain

homeostasis, integrity and influence the degree of development of hyaline cartilage [51]. The most used factors are TGF-β1-which stimulates collagen II and aggrecan overexpression—molecules present in the extracellular cartilage matrix [52], transforming growth factor-β3 (TGF-β3) induces the production of glycosaminoglycans in the extracellular matrix [53], bone morphogenetic protein-7 (BMP-7) is synthesized *in vivo* where the cartilage is damaged [54] and *in vitro* decreases the rate of proliferation of MSC and increases the rate of differentiation [55], growth and differentiation factor-5 (GDF-5) increases the expression of Sox9, the main inducer of chondrogenesis [56], IGF stimulates the proliferation of chondrocytes and induces the expression of specific genes [57] and fibroblast growth factor (FGF-2) is present in the extracellular matrix and plays a role in the prevention of native cartilage degradation [58].

Numerous studies in CTE concluded that collagen-based materials display the highest biocompatibility among the tested materials and an increased rate of biodegradation [59, 60]. Moreover, it was found that the addition of active biomolecules (e.g. sericin) in the structure of scaffolds seeded with stem cells can actually improve their performance, stimulating adhesion and proliferation and even synthesis of extracellular matrix [61, 62].

Surprisingly, it was observed that most studies aiming to regenerate elastic or hyaline cartilage using hASCs failed. Consequently, it was concluded that hASCs can only regenerate fibrocartilage [63].

In 2004, Awad et al. [64] conducted an experiment that involved seeding hASCs in a scaffold made of alginate, agarose and gelatin in a medium and differentiation towards the chondrogenic pathway. Biomaterials favoured adhesion, cell proliferation and differentiation, and at the end of the experiment, the ability of hASC to differentiate into chondrocytes was proved.

Im et al. [65] used a synthetic scaffold based on poly-lactic-co-glycolic acid seeded with hASCs transfected with a plasmid vector expressing Sox5, Sox6 and Sox9 genes. A culture medium supplemented with insulin-transferrin-selenium (ITS), dexamethasone, ascorbate-2-phosphate, proline and sodium pyruvate was used. Results indicated the presence of DNA coding for chondrogenesis inducers of Sox9, Sox5 and Sox6, showing a positive influence on the rate of cell differentiation.

Mardani et al. [66] has shown that hASCs cultivated in the presence of an inducing chondrogenic cocktail (culture medium supplemented with sodium pyruvate, transforming growth factor-β (TGF-β1), dexamethasone, insulin-transferrin-selenium (ITS), proline, ascorbic acid 2-phosphate) are able to differentiate into chondrocyte-*like* cells. Platelet-rich plasma (PRP) contains many growth factors, such as TGF-β, insulin growth factor (IGF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and vascular endothelial growth factor (VEGF). An experiment investigated hASCs chondrogenic differentiation when the culture medium was enriched with 10% of TGF-β1 from PRP; in this case, the cells differentiated and expressed Sox9 and ColIIα1 in similar levels to the control, represented by cells cultured in a classic medium [51].

Chondrogenic differentiation was also studied *in vivo* [67]; hASCs cultured at high cell density on surfaces coated with fibrin and maintained in a culture media supplemented with TGF-β and FGF-2 generated a chondrogenic matrix; these structures were implanted in areas of joint damage in rabbits, and the cells adopted a chondrocyte phenotype [67].

2.2.4. Neural regeneration using mesenchymal-like stem cells

Nervous tissue reconstruction involves injecting mesenchymal-*like* stem cells by the intrathecal, intracerebral or by the intranasal route, in the form of infusion [68]. Stem cells migrate to the lesion site, secrete neurotrophic factors and thus the survival and functioning of the affected nerve tissue can be supported [69].

However, there are opinions arguing that the generated neuronal extensions are non-authentic and without signalling capacity [70]. These assumptions are due to the fact that MSCs have the ability to carry membrane proteins by exosome transport, trans-endocytosis or even as a result of cell-cell contact. Despite this theory, neuroregenerative and neuroprotective capabilities of stem cells cannot be questioned.

Current research directions in the field of neurodegenerative diseases (degenerative myopathies, Parkinson's disease, amyotrophic lateral sclerosis, Huntington disease, etc.) target to exploit the neuroprotective effects of stem cells by investigating the mechanisms of production and action of neurotrophic factors. Analysis of the secretory profiles of human MSCs showed that all types of stem cells have the ability to secrete compounds that exert a protective action in the central nervous system (CNS), of which the most important are tissue glial-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factors (BDNF [71]. Experiments conducted on animal models revealed that genetically modified hMSCs overexpressing GDNF were able to induce novel neuromuscular junctions and stop motor neuron degeneration, thus offering new perspectives in the treatment of amyotrophic lateral sclerosis [72].

2.2.5. The regeneration of the optic nerve and retinal cells

hASCs also display the ability to regenerate optic nerves responsible for visual function. Optical nerve damage occurs frequently in the population; it is associated with local inflammation, infection or brain trauma.

For this application, murine experimental models received therapy with BM-MSCs; based on their paracrine action, these mesenchymal stem cells were actively involved in the repair and replacement of the epithelial cells of the retina (RPE), glial cells of the retina (RGC) and neurons of the optic nerve [73]. Another experiment revealed an increase in survival of RGC-treated retina and increased production of ciliary neurotrophic factor after injection of hASCs at lesion site [74]. With these results, it becomes increasingly clear that the paracrine secretion of MSCs enhances their therapeutic effect.

Johnson and his collaborators [75] investigated which biomolecules secreted by MSCs are essential to protect nerve structures of the eye. For this purpose, a co-culture system using retinal cells and BM-MSCs was established. Retinal cells expressed an increased survival rate in the presence of BM-MSCs possibly because of the paracrine action of MSCs.

2.2.6. Cardiac regeneration and cardioprotective effect of hASCs

Stem cell-based therapies have proven effective in heart regeneration, in particular in the post-myocardial infarction. Heart attack triggers irreversible alteration of the cardiac function

by the permanent loss of cardiomyocytes and myocardial tissue scarring [76]. Even if traditional medications containing aspirin, beta blockers, and statins and angiotensin-converting enzyme (ACE) inhibitors of angiotensin prevent possible damage to the affected tissue and reduce the risk of heart attacks, it is necessary to replace altered cardiomyocytes; mesenchymal stem cells were successfully used in this case. Furthermore, recent *in vivo* studies demonstrated that injected BM-MSCs survive and differentiate to form the complex junction structure of the damaged cardiac myocytes [77].

Chen et al. conducted a clinical trial involving 69 patients who suffered heart attacks. Following transplantation of BM-MSCs at the lesion sites, the cells infiltrated within the damaged myocardial areas and improved cardiac activity without risks of morbidity, mortality, arrhythmias or malignant processes [78]. Subsequent studies have shown that hASCs have the ability to form functional cardiomyocyte-*like* cells and exert protective effects on the heart. One such experiment, conducted by Bai et al. [79], showed that hASCs moved to the altered regions in close proximity to cardiomyocytes and adopted similar phenotypes, regenerating the damaged structure and stimulating its functioning.

Both *in vitro* and *in vivo* studies have shown that BM-MSCs and hASCs secrete molecules such as VEGF, PDGF, IL-1β, IL-10, stem cell-derived factor (SDF-1), IGF-1 and HGF proteins angiopoietin-1 and-2 (Ang-1 and-2) that act synergistically to regenerate and protect cardiac tissue [80].

2.2.7. Liver regeneration using hASCs

Mesenchymal-*like* stem cells, including hASCs, can regenerate the liver tissue. This tissue is composed mostly of hepatocytes, polarized epithelial cells representing approximately 80% of liver mass [81].

In 2005, Seo and co-workers reported, for the first time, to obtain a population of cells structurally and functionally similar to hepatocytes by inducing hASCs differentiation using a culture medium supplemented with growth factors, cytokines and dimethyl sulfoxide (DMSO) [82]. Continuing to investigate the regenerative potential of hASCs, the researchers transplanted cells in nude mice with acute liver lesions and found repopulation of the damaged tissue with newly generated hepatocyte-*like* cells embedded in liver parenchyma [82]. Microarray analysis showed that there were considerable similarities between sets of genes expressed in normal hepatocytes and the cells generated from hASCs. Thus, mesenchymal differentiation-specific genes (vimentin and N-cadherin-2) are downregulated, while genes that are overexpressed are direct epithelial differentiation [83]. These results suggest that hASCs are able to differentiate into hepatocyte-*like* cells through a mesenchymal-epithelial transition process [84]. However, it is obvious that liver regeneration would not be possible in the absence of stem-cell paracrine action.

In a more recent study [85], revealed that the factors released by MSCs and hASCs have immunosuppressive properties (IL-6, IL-8, IL-1RA and VEGF), can inhibit fibrosis and apoptosis of liver cells, can promote angiogenesis or they can stimulate progenitor cells to divide and differentiate to regenerate damaged tissue (G-CSF, TNF-α and IL-6). Currently, very few

details are known about the mechanisms by which these biomolecules modulate an integrated dynamic response to mitigate damage and scarring of the liver tissue *in vivo*. New clinical strategies in the field are oriented towards the use of MSCs Akt-modified Frizzled protein 2, which can produce large amounts of VEGF, HGF, FGF2 and insulin-*like* growth factors [86]. Increased production of these molecules ensures a viable regeneration; for example, an increased amount of HGF ensures suppression of the immune response of the graft (transplanted tissue) versus host which has disastrous consequences for the host organism, whereas the other molecules maintain angiogenesis, cell growth and proliferation [86].

3. Original biocompatible scaffolds validated for hASCs differentiation in the context of tissue engineering applications

Advanced research in tissue engineering promotes the use of bioconstructs as an effective solution to perform regeneration of damaged tissues. Using 3D microenvironments brings benefits for tissue engineering applications, since 3D microenvironment recreates better *in vivo* conditions and mimics closely the natural tissue. A number of cell-scaffold bioconstructs with structure and properties adapted to the nature of the tissue in need of reconstruction have been shown to be useful in the production of functional *de novo* tissue. These bioconstructs can function as 3D (i) transport systems ("shuttle") to deliver the cells to the injured site, encouraging the self-healing ability of the tissue and (ii) biocompatible and biodegradable bioconstructs, supporting the cellular component during tissue reconstruction.

In this context, a group of researchers from the Department of Biochemistry and Molecular Biology, University of Bucharest, partially in collaboration with the Department of Histology from Western University Vasile Goldis of Arad, investigated hASCs potential to differentiate in contact with several original recipes of materials designed for adipose, cartilage and bone tissue engineering.

For adipose tissue engineering (ATE), the optimal conditions required for successful differentiation of hASCs in case of implantations at a wound healing site were studied. Cells should first proliferate and then receive the optimal amount of pro-adipogenic signals to induce the differentiation process with a certain rate. Therefore, a modulation of the adipogenic conditions would be required, aiming to ensure the long-term proliferation of the precursor cells and to control the kinetics of the differentiation process [87].

One novel scaffold validated for ATE by *in vitro* studies was a spongious 60% collagen and 40% sericin hydrogel preceded with hASCs (CollSS) [12]. In this case, the novel CollSS composition was compared in terms of biocompatibility and ability to support adipogenic differentiation of hASCs to a pure collagen hydrogel (Coll). The addition of the sticky protein sericin in the composition of a classical collagen sponge enhanced the adhesion and also the proliferation rate of hASCs. CollSS proved to be more biocompatible than pure Coll. hASCs-CollSS bioconstruct proved to efficiently support the adipogenic differentiation process, as confirmed by the expression levels of PPARγ2, fatty acid synthase (FAS), adipocyte protein 2 (aP2) and perilipin adipogenic markers [12].

hASCs ability to differentiate towards mature adipocytes in 3D environment was also confirmed when using a gelatin- alginate- polyacril amide (GAPAA) scaffold. This type of material proved to be at least as efficient as the gelatin-alginate biomaterial (GA) for adipogenic differentiation, hASCs generating mature adipocytes in this system in 21 days of induced differentiation [88].

With regard to bone tissue engineering (BTE) direction, original research validated a composite material based on chitosan (CHT) and graphene oxides (GO) (hASCs/CHT/GO 3 wt.%) as most suitable for hASCs osteogenesis [paper under review]. The study of the osteogenic differentiation potential of hASCs in contact with chitosan-based scaffolds enriched with 0.5–3 wt.% GO showed the highest rate of differentiation in cells cultured in the presence of 3 wt.% GO, suggesting that hASCs/CHT/GO 3 wt.% may be a candidate for future bone regeneration applications and BTE. Currently, the potential of graphene and its derivatives is exploited increasingly in tissue engineering because of their positive influence on cellular interaction with the material and on the bioactivity of the material. Graphene oxide (GO) in particular was shown to improve the biological properties of materials and to promote adhesion, proliferation and osteogenic differentiation of mesenchymal stem cells. Experiments demonstrated good biocompatibility of CHT/GO materials, where the degree of biocompatibility depends on the GO content. Similar to previous studies, GO favoured hASCs contact with the materials and influenced the proliferation rate.

For cartilage tissue engineering (CTE), two distinct bioconstructs were studied: (i) a three component inter-polymeric network (IPN) hydrogel based on gelatin, alginate and polyacrylamide (GAPAA) was evaluated for potential to support hASCs chondrogenesis (hASCs/GAPAA) and (ii) a hydrogel based on collagen (Coll), improved with sericin (SS), hyaluronic acid (HA) and chondroitin sulphate (CS) (hASCs/CollSSHACS), was tested for hASCs proliferation, adhesion and chondrogenic differentiation [11, 13].

Chondrogenic differentiation studies conducted in GAPAA IPN have provided important information about the essential characteristics of a 3D scaffold to be used effectively in cartilage reconstruction. In this case, the adequate porosity of the system and the control over scaffold's 3D architecture for hASCs chondrogenic differentiation were achieved by adding the synthetic component polyacrylamide in the composition. Normally, acrylamide (AA) would exert a toxic effect on cells, but the polymerized form of PAA has a lower toxicity when used in very low concentration in the composition of GAPAA to control pore size [11].

Similarly, in hASCs/CollSSHACS bioconstruct exposed to pro-chondrogenic differentiation conditions, the results indicated a more efficient chondrogenesis of hASCs in the collagen hydrogel enriched with hyaluronic acid and chondroitin sulphate (CollSSHACS), two cartilage markers present in the extracellular matrix, as compared to the results obtained for the reference system (hASCs/Coll). The simultaneous presence of both molecules in HA and CS biomaterial composition favoured the initiation and controlled hASC chondrogenesis for 28 days *in vitro* [paper under review]. Therefore, hASCs/CollSSHACS bioconstruct would be a good candidate with high potential for use in CTE applications.

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4. Most recent advances and future perspectives

The optimization of biomaterials composition, structure and properties to mimic as well as possibilities in the *in vivo* architecture has led to the development of the advanced technology of bioprinting. Recently, highly advanced bioprinted 3D constructs comprising of material, stem cells and additives have been reported to successfully support differentiation and regeneration processes.

In order to combine as efficiently as possible this modern technology with hASCs properties, several groups have conducted studies to observe hASCs behaviour in these systems and to assess their utility for tissue engineering purposes.

One of these studies explored the behaviour of hASCs when printed in a free-scalable 3D grid pattern by means of laser-assisted bioprinting (LaBP). Cells in this system managed to differentiate towards the adipogenic lineage, thus proving that such 3D bioprints could be used successfully in tissue regeneration or the biofabrication of living grafts [89].

In order to be efficient, the bioprinted constructs should resemble the native tissue microenvironment as close as possible. For example, muscular soft tissue constructs can benefit from bioinks that mimic their nanofibrous matrix constitution [90]. Thus, it is critical to maximize the biocompatibility between cells and the type of fibre materials used to create the constructs. Narayanan et al. have used hASCs with a fibrous bioink composed of alginate hydrogel and polylactic acid nanofibers to obtain bioprinted constructs as replacement for knee meniscus. Results indicated favourable behaviour of hASCs, high levels of cell proliferation as well as positive chondrogenic differentiation.

For bone reconstruction purposes, hASCs were included in a mixture together with nano-hydroxyapatite and bioprinted in a 3D construct, further evaluated for cell proliferation and osteogenesis ability [91]. In this case, it was concluded that nano-hydroxyapatite could increase osteogenic differentiation of the hASCs mixture after bioprinting, in which the cells still have a good proliferation.

Similarly, Wang et al. [92] aimed to investigate osteogenic differentiation of hASCs in 3D bioprinted tissue constructs, both *in vitro* and *in vivo*. After performing tests to assess osteogenic markers expression both at gene and protein levels, researchers reported significantly increased expression levels of RUNX2, OSX and OCN after 7 and 14 days of osteogenesis. *In vivo* studies demonstrated obvious bone matrix formation in the 3D bioprinted constructs. Based on this study, it was concluded that hASCs could be used in 3D bioprinted constructs for the repair of large bone tissue defects.

In cardiac regeneration, a great interest is directed to obtaining 3D cocultures of stem cells and endothelial cells to closely mimic the native tissue conditions. In this context, a team of researchers [93] used laser-induced-forward-transfer (LIFT) cell printing technique to prepare a polyester urethane urea (PEUU) cardiac patch seeded with human umbilical vein endothelial cells (HUVEC) and human MSCs. Results showed an increased production of blood vessels, which reflected an improvement of functionality in infarcted tissue.

4.1. Risks associated with hASCs use for tissue engineering and regenerative medicine

According to good manufacturing practices (GMP) rules, mesenchymal stem cells including hASCs are considered as advanced therapy medical products and are validated safe for medical practice. Thus, these cells are widely used in tissue engineering and regenerative medicine applications based on the fact that they are adult stem cells and that they have a relatively limited potential for proliferation, differentiation and an extremely low risk of transformation.

Most clinical applications involving hASCs, however, require the use of a large number of cells at the implant site for regenerative therapy success and therefore most often, the *in vitro* expansion of these cells is necessary. Risks associated with hASCs use in regenerative medicine are mainly associated with the *in vitro* handling of cells and, thus, most studies are directed towards assessing changes that may occur in the genome of these cells during *ex vivo* cultivation.

Furthermore, a series of contradictory studies discussed the transformation potential of hASCs in different circumstances since the implantation of these cells in the body for regenerative purposes can have long-term consequences if they have tumorigenic potential. Although the accumulation of chromosomal aberrations and mutations was repeatedly reported in the genome of hASCs during *in vitro* culture and expansion, most of these mutations are proven to have a transient nature and can be normally removed by DNA repair systems. Although no case of malignant transformation of hASCs implanted in humans was reported so far, caution is necessary. The interaction of hASCs with the *in vivo* existing microenvironment is specific to each organism and could have risks. In this context, hASCs genomic instability when in contact with tumour microenvironment should be further studied, considering the implications for tissue engineering.

5. Conclusions

Human adipose-derived stem cells are a versatile source of mesenchymal-*like* stem cells, with enormous potential for tissue engineering and regenerative medicine. Regardless of the application and tissue that require restoration, hASCs proved to be efficient in a great number of cases. However, the stem-ness status of these cells and their unexplored risks of transformation should not be forgotten and thus more cautiousness should be taken before recommending therapy with hASCs. If correctly controlled, the potential of these cells could become a very powerful tool to increase the quality of life in patients with tissue defects.

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Authors' contribution

Sorina Dinescu, Anca Hermenean and Marieta Costache contributed to designing the chapter, reviewing the literature and writing the chapter.

Author details

Sorina Dinescu[1], Anca Hermenean[2]* and Marieta Costache[1]

*Address all correspondence to: anca.hermenean@gmail.com

1 Department of Biochemistry and Molecular Biology, University of Bucharest, Romania

2 Department of Histology, Faculty of Medicine, Vasile Goldis Western University of Arad, Romania

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