Annals of Medicine and Surgery 20 (2017) 41–48

Contents lists available at ScienceDirect

Annals of Medicine and Surgery

journal homepage: www.annalsjournal.com

Review

The biological and clinical basis for the use of adipose-derived stem cells in the field of wound healing

Nicolò Bertozzi [a, b, *], Francesco Simonacci [a, b], Michele Pio Grieco [a, b], Eugenio Grignaffini [a, b], Edoardo Raposio [a, b]

[a] Department of Medicine and Surgery, Plastic Surgery Division, University of Parma, Via Gramsci, 14, 43126, Parma, Italy
[b] Cutaneous, Mininvasive, Regenerative and Plastic Surgery Unit, Parma University Hospital, Via Gramsci, 14, 43126, Parma, Italy

HIGHLIGHTS

- Worldwide, hard-to-heal wounds are a matter of economic and public concern.
- The emerging fields of regenerative medicine and stem cell-based therapies hold great promise for wound healing.
- ASCs can potentially give the support necessary for recovery of hard-to-heal wounds.
- ASCs can be easily harvested from adipose tissue by means of standard wet liposuction technique.
- ASCs have been widely studied in vitro and in vivo to demonstrate their potential and safety.

ARTICLE INFO

Article history:
Received 12 February 2017
Received in revised form
21 June 2017
Accepted 21 June 2017

Keywords:
Regenerative medicine
Tissue engineering
Adipose-derived stem cells
Wound healing

ABSTRACT

Worldwide, hard-to-heal lower limb wounds are estimated to affect 1.5–3% of the adult population with a treatment-related annual cost of $10 billion. Thus, chronic skin ulcers of the lower limb are a matter of economic and public concern. Over the years, multiple medical and surgical approaches have been proposed but they are still inadequate, and no effective therapy yet exists. Regenerative medicine and stem cell-based therapies hold great promise for wound healing. Recently, many plastic surgeons have studied the potential clinical application of adipose-derived stem cells (ASCs), which are a readily available adult stem cell population that can undergo multilineage differentiation and secrete growth factors that can enhance wound-healing processes by promoting angiogenesis, and hence increase local blood supply. ASCs have been widely studied *in vitro* and *in vivo* in animal models. However, there are few randomized clinical trials on humans, and these are still ongoing or recruiting patients. Moreover, there is no consensus on a common isolation protocol that is clinically feasible and which would ensure reproducible results. The authors aim to provide readers with an overview of the biological properties of ASCs as well as their clinical application, to help better understanding of present and future strategies for the treatment of hard-to-heal wounds by means of stem cell-based therapies.

Contents

* Corresponding author. Department of Medicine and Surgery, Plastic Surgery Division, University of Parma, Via Gramsci, 14, 43126, Parma, Italy; Cutaneous, Mininvasive, Regenerative and Plastic Surgery Unit, Parma University Hospital, Via Gramsci, 14, 43126, Parma, Italy.
E-mail address: nicolo.bertozzi@yahoo.com (N. Bertozzi).

http://dx.doi.org/10.1016/j.amsu.2017.06.058

1. Introduction

A chronic wound can be defined as any wound that fails to heal within 30 days, even though there is no clear-cut definition regarding the chronicity of the wound.

As hard-to-heal wounds of the lower limb are estimated to affect 1.5–3% of the adult population and up to 5% of people aged over 65 years, they are a matter of economic and public concern [1,2]. Worldwide, the annual costs related to chronic wound treatment are about $10 billion, and they are expected to exceed $22 billion per year by 2020 [3,4]. Multiple medical and surgical approaches have been proposed for the treatment of chronic cutaneous ulcers. However, currently available treatments are still inadequate and are often mainly supportive, as truly effective therapies do not yet exist. The physiologically impaired healing response of the chronic wound is still poorly understood and thus a matter of debate. The emerging fields of regenerative medicine and stem cell-based therapies hold great promise for wound healing. Recently, many plastic surgeons have studied the potential clinical application of adipose-derived stem cells (ASCs), which represent a readily available adult stem cell population that has gathered a lot of attention in the field of regenerative medicine [5].

ASCs can undergo multilineage differentiation and to secrete growth factors that can enhance wound-healing processes by promoting angiogenesis, and hence increase local blood supply [6]. As a result, hard-to-heal wounds can potentially receive the support necessary for recovery. Although ASCs have been widely studied *in vitro* and *in vivo* in animal models, which have demonstrated their potential and safety, randomized clinical trials on humans are either ongoing or recruiting patients, and are still very few [6]. Moreover, there is no consensus on a common isolation protocol feasible for clinical application that could ensure reproducibility of results. In this review, the authors aim to provide readers with an overview of the biological properties of ASCs as well as their clinical application, to help better understanding of present and future strategies for the treatment of hard-to-heal wounds by means of stem cell-based therapies.

2. Regenerative medicine and cell-based therapy

Tissue engineering and regenerative medicine are multidisciplinary sciences, involving physicians, engineers, and scientists, which have evolved in parallel with recent biotechnological advances and may provide novel tools for reconstructive surgery. Tissue engineering combines the use of biomaterials, growth factors, and stem cells to repair failing organs. In particular, stem cell therapies hold high therapeutic promise based on the possibility of *ex vivo/in vivo* stimulation of stem cell expansion and differentiation into functional progeny that may repair and even replace damaged tissues or organs [7,8].

Ideally, a stem cell for regenerative medical applications should meet the following criteria:

1. Can be found in large quantities (millions to billions of cells).
2. Can be harvested using a minimally invasive procedure.
3. Can be differentiated along multiple cell lineage pathways in a controllable and reproducible manner.
4. Can be safely and effectively transplanted to either an autologous or allogeneic host.
5. Can be manufactured in accordance with current Good Manufacturing Practice guidelines [9,10].

Several different types of stem cells have been considered for clinical applications. Embryonic stem cells (ESCs), pluripotent-amniotic epithelial cells, umbilical cord mesenchymal stem cells, and induced-pluripotent stem cells (iPSCs) are very promising since all show nearly unlimited potential to differentiate *in vitro* and *in vivo* into specific progenitor cells or mature and specialized cell lineages of all three embryonic germ layers [11–17]. However, the clinical use of these cells is limited by ethical, legal, and political considerations, as well as by scientific and clinical issues of safety and efficacy. One of the main issues that hampers successful and safe clinical use of ESCs is the possibility of immune rejection, and *in vivo* formation of teratoma or teratocarcinoma [12,13,15,18,19]. iPSCs have a low reprogramming efficiency and thus require the introduction of exogenous transcription factors using viral vectors, or require other significant *ex vivo* manipulations, which mean that iPSCs are not currently feasible for practical clinical use [20–22].

Tissue-specific stem cells derived from adults offer an alternative approach that circumvents many of these concerns [23].

3. Mesenchymal stem cells

Mesenchymal stem cells (MSCs) are a well-characterized population of tissue-resident adult stem cells identified in most tissues/organs within specific cell niches, where they colocalize with

supporting cells [12]. MSCs fulfill a critical role in homeostatic maintenance by replenishing the mature cell types within the tissues in which they reside over a lifetime [12,24]. MSCs were first identified in the whole bone marrow of rats in 1968 by Friedenstein et al. [25,26], who in 1976 described a method for their isolation based on differential adhesion properties. MSCs were immediately shown to be adherent, clonogenic, non-phagocytic and fibroblastic, with the ability to give rise to fibroblast colony forming units [25,27]. The potential of these marrow stromal cells was further investigated in the 1980s, particularly by Piersma et al. [28] and by Owen et al. [29]. Given the high interest generated by MSCs, in the mid-2000s, the Tissue Stem Cell Committee of the International Society for Cellular Therapy identified three minimal criteria to define a MSC: plastic-adherence in standard culture; expression of CD105 (SH2), CD73 (SH3/4), and CD90 surface markers and lack of expression of CD45, CD34, CD14 or CD11b, CD79a or CD19, and HLA-DR surface molecules; and multilineage differentiation into osteoblasts, adipocytes and chondroblasts [4,30]. Subsequently, MSCs have also been shown to possess some level of plasticity, transdifferentiating *in vitro* across germinal boundaries [31].

MSCs were found to reside not only in the bone marrow but also throughout the body, around blood vessels (as pericytes) and in the fat, skin, muscle, teeth and other locations [30,31,32]. Bone marrow stem cells (BMSCs) were the first to be studied in the field of regenerative medicine because of their high differentiation potential [33]. MSCs have rapidly moved from *in vitro* and animal studies into human trials as a therapeutic modality for a diverse group of clinical applications. However, the clinical use of BMSCs has two main drawbacks: the harvesting procedure from bone marrow is painful with possible donor site morbidity, and MSCs need *ex vivo* expansion before their application because of the relatively low yield upon isolation [34].

4. Adipose-derived stem cells (ASCs)

The evidence that MSCs could be isolated from adipose tissue has resulted in the shared idea that subcutaneous adipose tissue can be regarded as the ideal source of MSCs and as a viable alternative to bone marrow [34]. Indeed, subcutaneous adipose deposits are accessible, abundant, and can be collected in large quantities, thus providing a potential adult stem cell reservoir for each individual. Adipocytes constitute almost 90% of adipose tissue volume and nearly 65% of the total cell number [6]. When enzymatically digested, adipose tissue yields a heterogeneous population of many cell types (preadipocytes, fibroblasts, vascular smooth muscle cells, endothelial cells, resident monocytes/macrophages, and lymphocytes), which upon isolation are termed the stromal vascular fraction (SVF) [7]. The SVF includes ASCs, which account for 30% of the total SVF cells [6]. ASCs are a plastic-adherent, multipotent stem cell population, which display a similar differentiation potential to other MSCs, and the ability to differentiate into cells of several lineages from all three germinal layers [35]. Moreover, ASCs have been shown to be immunoprivileged and more genetically stable in long-term culture, with a greater proliferative rate, than BMSCs [36–39]. The discovery that ASCs can readily be expanded and have the capacity to undergo adipogenic, osteogenic, chondrogenic, neurogenic and myogenic differentiation *in vitro* was a significant milestone in ASC therapeutic applicability [40–42].

4.1. ASC characterization

ASCs are commonly characterized 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 [23]. Like other MSCs, undifferentiated ASCs must be identified using a panel of surface markers on which no clear consensus has been reached. However, based on current literature, markers that are uniformly reported to have strong positive expression are CD13, CD29, CD34 (a well-established stem cell marker), CD44, CD73, CD90, CD105 (a mesenchymal stem cell-associated marker), CD166 and MHC I. CD14, CD31, CD45, and CD133 (all markers of the hematopoietic and angiogenic lineages) have been reported to show low or no expression in ASCs [23,43,44].

4.2. Differentiation potential

When cultured in suitable medium *in vitro*, ASCs show multipotency and plasticity [45,46]. Since ASCs are of mesodermal origin, they can differentiate into the adipogenic, osteogenic, and chondrogenic lineages, and even the myogenic lineage, leading to skeletal muscle, smooth muscle, and cardiomyocytes [41,42,47,48]. Interestingly, however, ASCs have also been shown to possess the potential to differentiate into cells of the ectodermal and endodermal lineages, such as neuron-like cells, endothelial cells, epithelial cells, hepatocytes, pancreatic cells, and hematopoietic supporting cells [49–57].

4.3. Proliferative capacity

Based on telomere length and β-galactosidase activity, the senescence of ASCs appears similar to that of BMSCs, while their proliferative capacity appears to be greater [7]. The doubling times of ASCs range from 40 to 120 h during the logarithmic phase of growth, and this may be influenced by donor age, type and location of the adipose tissue, and the harvesting procedure. Interestingly, Wu et al. [58] reported that infant-derived cells exhibit enhanced angiogenic and osteogenic capabilities compared with adult and senescent cells. However, the same group theorized that the effectiveness of cells from adult donors was still conserved and thus their clinical use was not precluded. ASCs are generally considered to be stable throughout long-term culture, as it has been reported that even ASCs that had passed through more than 100 population doublings had a normal diploid karyotype [59]. The proliferation of ASCs can be stimulated by various growth factors [fibroblast growth factor-2 (FGF-2), epithelial growth factor (EGF), insulin-like growth factor-1 (IGF-1), platelet-derived growth factor (PDGF), and tumor necrosis factor-α (TNF-α)] via different signaling pathways such as the extracellular signal-related kinase pathway, the Jun amino-terminal kinase pathway, and the JAK3/STAT1 pathway, and by Oncostatin M [7].

4.4. Potential for in vivo tissue regeneration

A variety of tissues and organs engineered using ASCs have been described [5]. *In vitro* studies rapidly progressed to *in vivo* experiments, where ASCs were tested with or without appropriate scaffolds to assess their capability to effectively regenerate and repair tissues or organs. ASCs were initially assessed for their commitment into cell lineages of mesodermal origin. ASCs have been successfully induced to differentiate into adipose tissue after being preinduced in adipogenic differentiation medium, seeded onto a scaffold of poly(lactic-co-glycolic acid), type I collagen sponge, and fibrin glue. This result was confirmed using macroscopic morphology observations, histology, and immunohistochemistry [60,61]. Similar findings were reported by other groups that tested the *in vivo* commitment of ASCs into cell lineages of mesodermal, ectodermal and endodermal origins [6,7]. ASC therapeutic effects in cranial bone, articular chondrocytes, cardiac wall regeneration, functional repair after myocardial infarction, and functional

improvement after stroke have all been investigated [62–66].

4.5. Isolation process

In 1964, Martin Rodbell [67] was the first to present a method for the *in vitro* isolation of mature adipocytes and adipogenic progenitors from rat fat tissue. In 2001, Zuk et al. [41,68] were the first to isolate ASCs from adipose tissue after a liposuction procedure by means of existing enzymatic strategies. Since then, interest in ASCs has grown dramatically and several groups working independently have developed procedures to isolate and characterize them. However, it has emerged that, depending on the isolation process, 1 g of adipose tissue can yield an inconsistent number of ASCs. Mizuno et al. [69] reported a yield of approximately 5×10^3 stem cells for each gram of adipose tissue, which is 500-fold greater than the number of BMSCs in 1 g of bone marrow; Bouquest et al. [70] reported a yield of 1×10^7 ASCs from 300 mL of lipoaspirate. Francis et al. [71] described a rapid collagenase-free isolation protocol with a yield of 2.5×10^5 ASCs starting from 250 mL of lipoaspirate. Other studies have demonstrated that 1 g of adipose tissue can yield 2×10^6 SVF cells, with 10% of these cells thought to be ASCs [72–74].

The isolation process described by Zuk et al. [75] has come to be the most commonly published method for ASC isolation. Briefly, the freshly harvested lipoaspirate is washed with sterile phosphate buffered solution to remove blood cells, saline and local anesthetics. Then, it is enzymatically digested using 0.075% collagenase type I, which subsequently must be inactivated by means of addition of an equal volume of Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. Finally, red blood cell lysis is performed and the high-density ASC pellet is separated from the infranatant by centrifugation. Although the procedure has proven effective, it can be complex, expensive and time-consuming for clinical application, since the entire process takes an average of 2 h.

In 2016, Raposio et al. [76–78] described a method that was specifically designed for clinical application, which appeared easy, safe and fast (80 min), allowing collection of a ready-to-use ASC pellet. Just like previous isolation processes, first the adipose tissue was harvested by a standard liposuction procedure, and then the isolation process was carried out through both mechanical (centrifugation) and enzymatic (collagenase) means [78]. The entire process was performed in a closed-circuit system, which guaranteed sterility and the safety. Once an ASC pellet was obtained in this manner, Raposio and colleagues were able to inject it into the skin at the wound edges, as well as at the bottom of chronic skin ulcers, to promote wound-healing processes [79]. Based on a flow cytometric assay, 9.06×10^5 (SD $\pm$ 6.6×10^5) ASCs were isolated from 100 mL of adipose tissue. ASCs accounted for 25.9% of the pelleted cells, which is a considerably higher yield compared with those reported in the literature [7].

ASCs have come to be regarded as the ideal stem cell for regenerative clinical applications since their yield upon isolation is so high that they do not require extensive manipulation before delivery. Thus, there is no need for compliance with “cell manufacturing” in accordance with current Good Manufacturing Practice Guidelines [80]. These restrictions do not apply in the case of minimal manipulation [Regulation (EC) No 1394/2007 of the European Parliament and of the Council]. The isolation of ASCs by means of collagenase digestion in a clinical setting is not regarded as extensive manipulation and hence it is not prohibited in Europe where ASCs are considered as Advanced Therapy Medicinal Products [75]. Conversely, in the United States, enzymatically isolated ASCs are regarded as more than just minimally manipulated and are therefore classified as a drug. Consequently, their clinical application following enzymatic digestion is regulated by the Food and Drug Administration [81]. This implies the need for an Investigational New Drug application to be submitted by every surgeon who wishes to use enzymatically isolated ASCs in clinical settings. However, the clinical application of ASCs isolated using mechanical means during the same operative session falls under the jurisdiction of medical practice and is thus allowed. Therefore, the development of high-yield isolation processes for ASCs with minimal handling would be highly desirable for clinical applications [76].

Several alternative isolation methods have been proposed which avoid enzymatic digestion completely, in keeping with the findings of Yoshimura et al. [82–86]. Yoshimura and co-workers found that a significant number of ASCs could be isolated from what they called the liposuction aspirate fluid, and although cell numbers were less than those obtained with enzymatic digestion, they were still enough to be used clinically without cell expansion. In the light of these findings, Francis et al. [71] isolated cells capable of trilineage differentiation by mechanically separating the SVF directly from the lipoaspirate. Soon after, Bianchi et al. [86] described a non-expanded, ready-to-use fat product obtained by pushing aspirated fat through size reduction filters while allowing waste products to exit in a closed system.

Raposio et al. [76] also described an effective alternative procedure in which the isolation process was performed by mechanical means at a laminar airflow bench. Once the adipose tissue was harvested during a conventional tumescent liposuction, it was placed in a vibrating shaker at 600 vibrations per minute for 6 min and then centrifuged at 1600 rpm for 6 min so that the previously detached ASCs were collected into the resulting pellet at the bottom of each 10-mL test tube containing the lipoaspirate. The SVF was then collected using a pipette and added to a 10-mL Luer Lock syringe, ready to be injected. The entire isolation process lasted approximately 15 min and it yielded a mean of 5×10^5 (SD: $\pm$ 1×10^5) ASCs with 97% cell viability.

4.6. ASCs in wound healing

Currently, ASCs are being investigated as a therapeutic strategy for a diverse group of pathological conditions, including hard-to-heal wounds. Wound healing is not a series of individual and independent progressive steps, but a complex process involving inflammation, epithelialization, neoangiogenesis, proliferation, and collagen matrix formation [87]. This complex process is carried out and regulated by numerous growth factors, cytokines, and chemokines, such as transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), granulocyte macrophage colony-stimulating factor, the interleukin family, EGF, FGF and TNF-α [88,89]. A reduction in the cytokines released by local inflammatory cells and decreased neovascularization are the main obstacles to healing processes. BMSCs and ASCs have been studied as potential solutions for these major issues. Both types of MSC have been shown to be effective in enhancing wound healing by modulating the immune response, secreting paracrine factors, and promoting therapeutic angiogenesis, thereby providing the building blocks for wound regeneration [90]. Given their higher isolation yield, ease of harvest and abundance, ASCs are more clinically attractive and have generated interest as the most favored cell type for wound repair and regeneration [91]. It has been demonstrated that ASCs not only have potential for stimulating angiogenesis, but may also function *in situ* as pericytes providing vascular stability, and can communicate with endothelial cells in response to environmental stimuli [92]. As a consequence of a newly re-established blood supply, hard-to-heal wounds can obtain the necessary support for wound recovery.

In a rat ischemic hind-limb model, the intravenous or

intramuscular administration of CD31-negative ASCs dramatically improved the vascular supply [93,94]. This mechanism is accounted for by both the direct differentiation of ASCs into endothelial cells and the indirect paracrine effects of ASCs, which secrete angiogenic growth factors. Consistent with the results of the ischemic hind-limb study, the survival area of an ischemic skin flap can also be increased by local injection of autologous ASCs into the skin flap [95]. Further studies performed by the same research group showed that skin ulcers in diabetic mice were repaired using autologous ASCs, indicating that ASCs can be used to treat skin ulcers, even in patients with diabetes [96]. Marino et al. [97] employed ASC therapy in the chronic skin ulcers of patients with peripheral artery disease, gaining encouraging results. In fact, skin ulcers may accompany critical limb ischemia, but even after successful revascularization, they still may not heal and often require special treatment. Several studies have adopted an approach based on the concept of improving the vascularization of ischemic limbs so that perfusion increases sufficiently to promote wound healing, reduce the pain at rest, and allow limb salvation [98]. In these studies, ASCs were delivered mainly using multiple intramuscular injections and intra-arterial or intravenous administration [99]. It emerged that after cell implantation, perfusion was increased in the treated limbs, as confirmed by improvements in ankle-brachial index, transcutaneous oximetry ($TcpO_2$), rest pain and pain-free walking distance, with a reduction in amputation rates. In a still-unpublished pilot study, Raposio and co-workers successfully treated seven patients presenting with ischemic ulcers of the lower limb with topical ASC administration. All patients were affected either by diabetes, peripheral artery disease, or both and were candidates for amputation. All patients were completely healed, with or without undergoing percutaneous transluminal angioplasty, and hence avoided limb amputation. Moreover, both Laser Doppler Flowmetry and $TcpO_2$ values showed perilesional improvements in oxygenation and perfusion during the follow-up, validating the effectiveness of ASCs in inducing angiogenesis. The same group also reported successful treatment of 21 chronic wounds of the lower limb using a combination of ASCs and platelet-rich-plasma (PRP) [79]. Compared with the 31 chronic wounds of the control group that were treated using standard wound care, the experimental group showed a significantly higher wound closure rate, resulting in faster recovery. Indeed, PRP combined with ASCs might act synergistically because of the degranulation of the platelet α-granules, which contain various growth factors such as PDGF-BB, TGF-β1, VEGF and EGF. Moreover, when the PRP is activated by adding calcium gluconate and thrombin, a fibrin matrix is generated, providing a scaffold for the ASCs [100]. Numerous studies have demonstrated that ASCs may benefit from an appropriate scaffold, which can provide a 3D architecture to enhance cell behavior. In fact, a cell-seeded matrix can deliver stem cells directly where needed in the ischemic tissue and can also facilitate the infiltration of native cellular agents including fibroblasts and vascular tissue [92]. Several biomaterials have been tested in this regard, and particular attention has been given to materials derived from the extracellular matrix [101]. Skin substitutes appear to provide an optimal 3D scaffold for the delivery of ASCs to the wound site. In this regard, human acellular dermal matrix (ADM), silk fibroin-chitosan scaffolds, and atelocollagen matrix have been studied for their potential to assist the integration between local tissue and the graft [102]. Unlike synthetic materials, ADM possesses favorable handling properties and resistance to infection, as well as facilitating local cell infiltration and revascularization through integration at the wound site [103,104]. Altman et al. [105] applied an ADM scaffold seeded with ASCs at a wound site and observed a significant improvement in wound healing and an increased local retention of ASCs, which enhanced the microvascular network, leading to tissue regeneration.

4.7. Mechanism of action

Although ASCs have been shown to be effective in the treatment of acute and chronic wounds in preclinical and clinical settings, their exact mechanism of action is still under investigation. ASCs have been found to initiate or enhance tissue regeneration through two main mechanisms, either by differentiating into skin cells, or by secretion of paracrine factors which downregulate the inflammatory response [91]. The primary mechanism is thought to be paracrine secretion, which leads to subsequent differentiation of the stem cells into endothelial cells, fibroblasts, or keratinocytes. Moreover, ASCs may modulate the “stem cell niche” of the host by stimulating the recruitment of endogenous stem cells and promoting their differentiation along the required lineage pathway. ASCs have also been shown to possess antioxidant effects during wound healing [106].

4.8. Differentiation or transdifferentiation

Topical administration of ASCs on ulcers is effective in enhancing wound closure, mainly through neovascularization and epithelialization, as demonstrated through the *in vivo* application of ASCs in rat, pig, and mouse wounds [91]. Tamarat et al. [107] showed enhanced healing in physiological and pathological wounds in mice, where green fluorescent protein-positive ASCs were demonstrated to differentiate *in situ* into endothelial cells and keratinocytes. Huang et al. [108] also reported similar findings. They created a rat model of radiation-induced acute wounds that they treated with ASCs cultured *in vitro*. Wound healing was enhanced, but, more importantly, a histological examination of the wound edge and an immunoblot analysis of the re-epithelialization region suggested that ASCs promoted angiogenesis, either by acting directly as angiogenesis promoters or by differentiating into endothelial cells. Moreover, Nie et al. [109] reported an *in vivo* animal model in which ASCs were shown to differentiate *in situ* into keratinocytes, aiding an epithelialization process. The same group also demonstrated spontaneous ASC differentiation into a vascular endothelial phenotype that led to newly formed capillaries. Traktuev et al. [110] reported that ASCs could be found as pericytes around newly formed blood vessels, providing vascular stability. Additional studies have shown that ASCs seeded onto a biomaterial, such as a silk fibroin-chitosan scaffold, transformed into fibrovascular, endothelial, and epithelial elements of repaired tissue [91]. These studies have indicated that ASCs not only secrete pro-healing factors but also directly take part in the healing process and act on the local environment.

Transdifferentiation of ASCs into skin cells has not been extensively investigated; however, Trottier et al. [111] recently reported to replace skin substitutes that commonly use dermal fibroblasts, with ASCs. This cell replacement strategy led to a trilayered skin that consisted of the epidermis, dermis, and hypodermis. Coculture experiments performed by Kim et al. [112] showed that the proliferation of fibroblasts was boosted by direct cell-cell contact between ASCs and human dermal fibroblasts. As a consequence, there was both up- and downregulation of certain factors with the expression of collagen type I and III and fibronectin being increased 1000-fold in the cocultures, whereas MMP-1 was downregulated.

4.9. Paracrine action

A wide range of cytokines and factors are known to be involved in the beneficial interaction between ASCs and other cells. It is likely because of their paracrine secretion during wound repair that

ASCs can stimulate recruitment, migration and proliferation of endogenous cells in the wound environment. Therefore, ASCs can stimulate angiogenesis, epithelialization, and wound remodeling.

The angiogenic support provided by ASCs can be regarded as a predominant effect since the reestablishment of blood supply is mandatory for the recovery of damaged tissues in non-healing wounds [15,113].

It is known that ASCs express and secrete cytokines which are important for angiogenesis, such as stromal cell-derived factor 1 (SDF-1), VEGF, PDGF-BB, basic FGF, Ang-1, IGF-1, matrix metalloproteinases, IL-6, and IL-8 [15]. VEGF is the most effective and specific growth factor that regulates angiogenic processes, and it can stimulate the mobilization, recruitment, and migration of progenitor endothelial cells, accelerating the onset of angiogenesis [114]. Nie et al. [109] demonstrated that ASCs secrete angiogenic cytokines *in vitro* and *in vivo*, including VEGF, HGF, and FGF-2. SDF-1 activity is essential for endothelial cell survival, vascular branching, and pericyte recruitment. SDF-1a is not only a mobilization signal capable of recruiting CXC chemokine receptor type 4-positive progenitor cells into hypoxic tissues, but it also recruits pericytes and smooth muscle cells that stabilize and mature newly formed blood vessels [115]. Ang-1 inhibits pericyte apoptosis and interacts with the Tie-2 receptor, recruiting pericytes for the maturation of new blood vessels, while FGF may also play a key role in maintaining vascular integrity. The levels of VEGF and/or HGF secreted by ASCs can be induced by exposure of the cells to hypoxia, growth and differentiation factors, TNF-α, FGF, EGF, and ascorbate [91]. Moreover, Zografou et al. [116] reported that the transplantation of ASCs can enhance skin graft survival through the secretion of growth factors such as VEGF and TGF-β3, which inhibits scarring and promotes better collagen organization *in vivo*, regulating wound re-epithelialization. The same group demonstrated increased collagen density as a result of the ASC paracrine effect on collagen production and secretion.

4.10. Immunomodulation

Most chronic wounds fail to heal since they remain in a chronic inflammatory state. Thus, reducing the inflammatory response may improve wound healing. ASCs have been shown to modulate monocytes/macrophages, dendritic cells, T-cells, and B-cells in terms of adaptive immunity. However, the mechanisms underlying the immunosuppressive effects of ASCs are still unclear [91].

ASCs can interact with monocytes, granulocytes, and melanoma cells through expression of Thy-1, an inflammation-dependent adhesion molecule. *In vitro*, ASC cell-cell binding and paracrine signaling can suppress immunity by inhibiting the proliferation of activated lymphocytes [117]. *In vivo*, expanded ASCs have demonstrated immunosuppressive properties in mice, alleviating graft-versus-host disease, colitis, and arthritis [6]. ASCs exert their immunoregulatory effects mainly through the secretion of paracrine factors. Indeed, oxidative stress induces ASCs to secrete TGF-β, which promotes premature T helper (Th) differentiation toward regulatory T cells. Consequently, the Th1 inflammatory response is downregulated, promoting immune tolerance. ASCs also secrete galectin-1 and -3, which are essential in T-cell suppression, and metabolize L-arginine, limiting its bioavailability, thereby reducing T-cell proliferation and function [6].

Moreover, once ASCs are activated by monocytes and leukocytes at sites of injury, nitric oxide, prostaglandin E2, indolamine 2,3-dioxygenase and IL-6 can all be secreted. These soluble molecules can regulate many components of the immune system: cytotoxic T-cell proliferation and development and B-lymphocyte differentiation into plasma cells are prevented, and dendritic cell differentiation, maturation and function are hindered [118].

5. Conclusions

Since ASCs were first isolated in 2001 by Zuk et al. [41], stem cell therapy has gained a prominent and promising role in the future of wound healing and much has been done to thoroughly understand ASC biology [119]. To date, ASCs have mainly been studied *in vitro* and *in vivo* in animal models, and only a few clinical trials have been reported in the literature. The preliminary results reported so far are promising, and from a practical standpoint the clinical application of ASCs in wound healing seems effective and safe, even if it is currently in its early stages. However, further research is needed: the mechanism of action of ASCs is still not completely understood, and the isolation protocol and delivery system must be improved. The combination of ASCs with PRP and 3D scaffolds seeded with stem cells seems to be a step forward in the field of regenerative medicine since these strategies may have the potential to enhance the proangiogenic effects of the stem cells. Stem cell therapy is a new and noteworthy chapter in the history of plastic and regenerative surgery and it is a shared hope that ASCs may soon become a readily available tool for the treatment of hard-to-heal wounds. However, to turn that hope into a reality, further research and evaluation of the literature are mandatory, so that evidence of safety and effectiveness may inform clinical practice.

Ethical approval

Nothing to declare.

Sources of funding

Nothing to declare.

Author contribution

Dr. Bertozzi Nicolo', writing.
Dr. Simonacci Francesco, data collections.
Dr. Grieco Michele Pio, data collections.
Prof. Eugenio Grignaffini, data collections.
Prof. Raposio Edoardo, study design.

Conflicts of interest

The authors declares that there is no conflict of interest regarding the publication of this paper.

Guarantor

Dr. Bertozzi Nicolo'.
Dr. Simonacci Francesco.
Dr. Grieco Michele Pio.
Prof. Grignaffini Eugenio.
Prof. Raposio Edoardo.

Consent

Nothing to declare.

Registration of research studies

Not needed.

References

[1] D.J. Margolis, W. Bilker, J. Santanna, et al., Venous leg ulcer: incidence and prevalence in the elderly, J. Am. Acad. Dermatol. 46 (2002) 381–386.

[2] J. Cornwall, C. Doré, J.D. Lewis, Leg ulcers: epidemiology and aetiology, Br. J. Surg. 73 (2005) 693–696.
[3] D.R. Bickers, H.W. Lim, D. Margolis, et al., The burden of skin diseases: 2004 a joint project of the american academy of dermatology association and the society for Investigative dermatology, J. Am. Acad. Dermatol. 55 (2006) 490–500.
[4] M. Dominici, K. Le Blanc, I. Mueller, et al., Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement, Cytother 8 (2006) 315–317.
[5] L. Gould, P. Abadir, H. Brem, et al., Chronic wound repair and healing in older adults: current status and future research, Wound Rep. Reg. 23 (2015) 1–13.
[6] L.E. Kokai, K. Marra, J.P. Rubin, Adipose stem cells: biology and clinical applications for tissue repair and regeneration, Trans. Res. 163 (2014) 399–408.
[7] H. Mizuno, M. Tobita, A.C. Uysal, Concise review: adipose-derived stem cells as a novel tool for future regenerative medicine, Stem Cell 30 (2012) 804–810.
[8] M. Mimeault, R. Hauke, S.K. Batra, Stem cells: a revolution in therapeutics - recent advances in stem cell biology and their therapeutic applications in regenerative medicine and cancer therapies, Clin. Pharmacol. Ther. 82 (2007) 252–264.
[9] J.M. Gimble, A.J. Katz, B.A. Bunnell, Adipose-derived stem cells for regenerative medicine, Circ. Res. 100 (2007) 1249–1260.
[10] J.M. Gimble, Adipose tissue-derived therapeutics, Expert Opin. Biol. Ther. 3 (2003) 705–713.
[11] T. Asahara, A. Kawamoto, Endothelial progenitor cells for postnatal vasculogenesis, Am. J. Physiol. Cell Physiol. 287 (2004) C572–C579.
[12] M. Mimeault, S.K. Batra, Recent advances on the significance of stem cells in tissue regeneration and cancer therapies, Stem Cell 24 (2006) 2319–2345.
[13] A. Trounson, The production and directed differentiation of human embryonic stem cells, Endocr. Rev. 27 (2006) 208–219.
[14] A. Behfar, et al., Cardiopoietic programming of embryonic stem cells for tumor-free heart repair, J. Exp. Med. 204 (2007) 405–420.
[15] D.C. Wu, A.S. Byod, K.J. Wood, Embryonic stem cell transplantation: potential applicability in cell replacement therapy and regenerative medicine, Front. Biosci. 12 (2007) 4525–4535.
[16] N. Lenoir, Europe confronts the embryonic stem cell research challenge, Science 287 (2000) 1425–1427.
[17] U. Ben-David, N. Benvenisty, The tumorigenicity of human embryonic and induced pluripotent stem cells, Nat. Rev. Cancer 11 (2011) 268–277.
[18] W.E. Lowry, L. Richter, R. Yachechko, et al., Generation of human induced pluripotent stem cells from dermal fibroblasts, Proc. Natl. Acad. Sci. 105 (2008) 2883–2888.
[19] C. Takenaka, N. Nishishita, N. Takada, et al., Effective generation of iPS cells from CD34+ cord blood cells by inhibition of p53, Exp. Hematol. 38 (2010) 154–162.
[20] P. Phanthong, H. Raveh-Amit, T. Li, et al., Is aging a barrier to reprogramming? Lessons from induced pluripotent stem cells, Biogerontology 14 (2013) 591–601.
[21] K. Takahashi, S. Yamanaka, Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors, Cell 126 (2006) 663–676.
[22] F. Gonzalez, M. Barragan Monasterio, G. Tiscornia, et al., Generation of mouse-induced pluripotent stem cells by transient expression of a single nonviral polycistronic vector, Proc. Natl. Acad. Sci. 106 (2009) 8918–8922.
[23] B. Lindroos, R. Suuronen, S. Miettinen, The potential of adipose stem cells in Regenerative medicine, Stem Cell Rev. 7 (2011) 269–291.
[24] Kajstura J. LeriA, P. Anversa, Cardiac stem cells and mechanisms of myocardial regeneration, Physiol. Rev. 85 (2005) 1373–1416.
[25] A.J. Friedenstein, K.V. Petrakova, A.I. Kurolesova, et al., Heterotopic of bone marrow. Analysis of precursor cells for osteogenic and hematopoietic tissues, Transplantation 6 (1968) 230–247.
[26] A.J. Friedenstein, Precursor cells of mechanocytes, Int. Rev. Cytol. 47 (1976) 327–359.
[27] D.M. Patel, J. Shah, A.S. Srivastava, Therapeutic potential of mesenchymal stem cells in regenerative medicine, Stem Cell Int. 49 (2013) 21–28.
[28] A.H. Piersma, K.G. Brockbank, R.E. Ploemacher, et al., Characterization of fibroblastic stromal cells from murine bone marrow, Exp. Hematol. 13 (1985) 237–243.
[29] M. Owen, Marrow stromal stem cells, J. Cel. Sci. (Suppl. 10) (1988) 63–76.
[30] J.E. Brinchmann, Expanding autologous multipotent mesenchymal bone marrow stromal cells, J. Neurol. Sci. 265 (2008) 127–130.
[31] G.P. Donald, J.P. Darwin, Concise review: mesenchymal stem/multipotent stromal cells: the state of transdifferentiation and modes of tissue repair - current views, Stem Cell 25 (2007) 2896–2902.
[32] B. Lindroos, K. Mäenpää, T. Ylikomi, et al., Characterisation of human dental stem cells and buccal mucosa fibroblasts, Biochem. Biophys. Res. Commun. 368 (2008) 329–335.
[33] R.K. Jaiswal, N. Jaiswal, S.P. Bruder, et al., Adult human mesenchymal stem cell differentiation to the osteogenic or adipogenic lineage is regulated by mitogen- activated protein kinase, J. Bio. Chem. 275 (2000) 9645–9652.
[34] P.A. Zuk, Tissue engineering craniofacial defects with adult stem cells? Are we ready yet? Pediatr. Res. 63 (2008) 478–486.
[35] S.R. Daher, B.H. Johnstone, D.G. Phinney, et al., Adipose stromal/stem cells: basic and translational advances: the IFATS collection, Stem Cell 26 (2008) 2664–2665.
[36] E. Gonzalez-Rey, M.A. Gonzalez, N. Varela, et al., Human adipose-derived mesenchymal stem cells reduce inflammatory and T cell responses and induce regulatory T cells in vitro in rheumatoid arthritis, Ann. Rheum. Dis. 69 (2010) 241–248.
[37] L.A. Meza-Zepeda, A. Noer, J.A. Dahl, et al., High-resolution analysis of genetic stability of human adipose tissue stem cells cultured to senescence, J. Cel. Mol. Med. 12 (2008) 553–563.
[38] A.A. Salibian, A.D. Widgerow, M. Abrouk, et al., Stem cells in plastic surgery: a review of current clinical and translational applications, Arch. Plast. Surg. 40 (2013) 666–675.
[39] J.A. Dahl, S. Duggal, N. Coulston, et al., Genetic and epigenetic instability of human bone marrow mesenchymal stem cells expanded in autologous serum or fetal bovine serum, Int. J. Dev. Biol. 52 (2008) 1033–1042.
[40] J. Gimble, F. Guilak, Adipose-derived adult stem cells: isolation, characterization, and differentiation potential, Cytotherapy 5 (2003) 362–369.
[41] P.A. Zuk, M. Zhu, H. Mizuno, et al., Multilineage cells from human adipose tissue: implications for cell-based therapies, Tissue Eng. 7 (2001) 211–228.
[42] P.A. Zuk, M. Zhu, P. Ashjian, et al., Human adipose tissue is a source of multipotent stem cells, Mol. Biol. Cell 13 (2002) 4279–4295.
[43] K. Yoshimura, T. Shiguera, D. Matsumoto, et al., Characterization of freshly isolated and cultured cells derived from the fatty and fluid portions of liposuction aspirates, J. Cell Physiol. 208 (2006) 64–76.
[44] P.C. Sachs, M.P. Francis, M. Zhao, et al., Defining essential stem cell characteristics in adipose-derived stromal cells extracted from distint anatomical sites, Cell Tissue Res. 349 (2012) 505–515.
[45] E. Raposio, C. Guida, I. Baldelli, et al., Characterization and induction of human pre-adipocytes, Tox. Vitro 21 (2007) 330–334.
[46] E. Raposio, C. Guida, R. Coradeghini, et al., In vitro polydeoxyribonucleotide effects on human pre-adipocytes, Cell Prolif. 41 (2008) 739–754.
[47] L.V. Rodrigues, Z.G. Alfonso, R. Zhang, J. Leung, B. Wu, L.J. Ignarro, Clonogenic multipotent stem cells in human adipose tissue differentiate into functional smooth muscle cells, Proc. Natl. Acad. Sci. 103 (2006) 12167–12172.
[48] J.K. Fraser, R. Schreiber, B. Strem, et al., Plasticity of human adipose stem cells toward endothelial cells and cardiomyocytes, Nat. Clin. Pract. Cardiovasc Med. 3 (2006) S33–S37.
[49] J. Fujimura, R. Ogawa, H. Mizuno, Y. Fukunaga, H. Suzuki, Neural differentiation of adipose-derived stem cells isolated from GFP transgenic mice, Biochem. Biophys. Res. Commun. 333 (2005) 116–121.
[50] V. Planat-Benard, J.S. Silvestre, B. Cousin, et al., Plasticity of human adipose lineage cells toward endothelial cells: physiological and therapeutic perspectives, Circulation 109 (2004) 656–663.
[51] M. Brzoska, H. Geiger, S. Gauer, P. Baer, Epithelial differentiation of human adipose tissue-derived adult stem cells, Biochem. Biophys. Res. Commun. 330 (2005) 142–150.
[52] A. Banas, T. Teratani, Y. Yamamoto, et al., Adipose tissue-derived mesenchymal stem cells as a source of human hepatocytes, Hepatology 46 (2007) 219–228.
[53] K. Timper, D. Seboek, M. Eberhardt, et al., Human adipose tissue-derived mesenchymal stem cells differentiate into insulin, somatostatin, and glucagon expressing cells, Biochem. Biophys. Res. Commun. 341 (2006) 1135–1140.
[54] J. Corre, C. Barreau, B. Cousin, et al., Human subcutaneous adipose cells support complete differentiation but not self-renewal of hematopoietic progenitors, J. Cell Physiol. 208 (2006) 282–288.
[55] C. Scanarotti, A.M. Bassi, M. Catalano, et al., Neurogenic-committed human pre-adipocytes express CYP1A isoforms, Chem. Biol. Interact. 184 (2010) 474–483.
[56] R. Coradeghini, C. Guida, C. Scanarotti, et al., A comparative study of proliferation and hepatic differentiation of human adipose-derived stem cells, Cells Tissues Organs 191 (2010) 466–477.
[57] M.G. Aluigi, R. Coradeghini, C. Guida, et al., Pre-adipocytes commitment to neurogenesis 1: preliminary localisation of cholinergic molecules, Cell Biol. Int. 33 (2009) 594–601.
[58] W. Wei, L. Niklason, D.M. Steinbacher, The effect of age on human adipose-derived stem cells, Plast. Reconstr. Surg. 131 (2013) 27–37.
[59] R. Izadpanah, D. Kaushal, C. Kriedt, et al., Long-term in vitro expansion alters the biology of adult mesenchymal stem cells, Cancer Res. 68 (2008) 4229–4238.
[60] Y.S. Choi, S.M. Cha, Y.Y. Lee, S.W. Kwon, C.J. Park, M. Kim, Adipogenic differentiation of adipose tissue derived adult stem cells in nude mouse, Biochem. Biophys. Res. Commun. 345 (2006) 631–637.
[61] Y. Kimura, M. Ozeki, T. Inamoto, Y. Tabata, Adipose tissue engineering based on human preadipocytes combined with gelatin microspheres containing basic fibroblast growth factor, Biomaterials 24 (2003) 2513–2521.
[62] C.M. Cowan, Y.Y. Shi, O.O. Aalami, et al., Adipose-derived adult stromal cells heal critical-size mouse calvarial defects, Nat. Biotechnol. 22 (2004) 560–567.
[63] K. Masuoka, T. Asazuma, H. Hattori, et al., Tissue engineering of articular cartilage with autologous cultured adipose tissue-derived stromal cells using atelocollagen honeycomb-shaped scaffold with a membrane sealing in rabbits, J. Biomed. Mater Res. B 79 (2006) 25–34.
[64] Y. Miyahara, N. Nagaya, M. Kataoka, et al., Monolayered mesenchymal stem cells repair scarred myocardium after myocardial infarction, Nat. Med. 12 (2006) 459–465.
[65] K. Schenke-Layland, B.M. Strem, M.C. Jordan, et al., Adipose tissue-derived

cells improve cardiac function following myocardial infarction, J. Surg. Res. 153 (2009) 217–223.
[66] H. Mizuno, Adipose-derived stem cells for tissue repair and regeneration: ten years of research and a literature review, J. Nippon. Med. Sch. 76 (2009) 56–66.
[67] M. Rodbell, Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis, J. Biol. Chem. 239 (1964) 375–380.
[68] P. Zuk, Adipose-derived stem cells in tissue regeneration: a review, ISRN Stem Cell (2013) 1–35.
[69] H. Mizuno, Adipose-derived stem cells for tissue repair and regeneration: ten years of research and a literature review, J. Nippon. Med. Sch. 76 (2009) 56–66.
[70] A.C. Bouquest, A. Shahdadfar, J.E. Brinchmann, et al., Isolation of stromal stem cells from human adipose tissue, Methods Mol. Biol. 325 (2006) 35–46.
[71] M.P. Francis, P.C. Sachs, L.W. Elmore, S.E. Holt, Isolating adipose- derived mesenchymal stem cells from lipoaspirate blood and saline fraction, Organogenesis 6 (2010) 11–14.
[72] L. Aust, B. Devlin, S.J. Foster, et al., Yield of human adipose-derived adult stem cells from liposuction aspirates, Cytotherapy 6 (2004) 7–14.
[73] Y. Zhu, T. Liu, K. Song, et al., Adipose-derived stem cell: a better stem cell than BMSC, Cell Biochem. Funct. 26 (2008) 664–675.
[74] M.J. Oedayrajsingh-Varma, S.M. van Ham, M. Knippenberg, et al., Adipose tissue-derived mesenchymal stem cell yield and growth characteristics are affected by the tissue-harvesting procedure, Cytotherapy 8 (2006) 166–177.
[75] D.A. Banyard, A.A. Salibian, A.D. Widgerow, G.R. Evans, Implications for human adipose-derived stem cells in plastic surgery, J. Cell Mol. Med. 19 (2015) 21–30.
[76] E. Raposio, G. Caruana, S. Bonomini, G. Libondi, A novel and effective strategy for the isolation of adipose-derived stem cells: minimally manipulated adipose-derived stem cells for more rapid and safe stem cell therapy, Plast. Reconstr. Surg. 133 (2014) 1406–1409.
[77] E. Raposio, G. Caruana, M. Petrella, S. Bonomini, M.P. Grieco, A standardized method of Isolating adipose-derived stem cells for clinical applications, Ann. Plast. Surg. 76 (2016) 124–126.
[78] G. Caruana, N. Bertozzi, E. Boschi, M.P. Grieco, E. Grignaffini, E. Raposio, Role of adipose-derived stem cells in chronic cutaneous wound healing, Ann. Ital. Chir. 86 (2015) 1–4.
[79] E. Raposio, N. Bertozzi, S. Bonomini, et al., Adipose-derived stem cells added to platelet-rich plasma for chronic skin ulcer therapy, Wounds 28 (2016) 126–131.
[80] L. Reoseti, M. Serra, D. Tigani, et al., M. Cell manipulation in autologous chondrocyte implantation: from research to cleanroom, Chir. Organi Mov. 91 (2008) 147–151.
[81] U.S. Food and Drug Administration, CFR- Code of Federal Regulations Title Wq. Part 1271: Human Cells, Tissues and Cellular and Tissue-based Products, U.S. Food & Drug Administration, 2013.
[82] K. Yoshimura, T. Shiguera, D. Matsumoto, et al., Characterization of freshly isolated and cultured cells derived from the fatty and fluid portions of liposuction aspirates, J. Cell Phys. 208 (2006) 64–76.
[83] L.S. Baptista, R.F.J.C. Amaral, R.B.V. Carias, M. Aniceto, C.C. Silva, R. Borojevic, An alternative method for the isolation of mesenchymal stromal cells derived from lipoaspirate samples, Cytotherapy 11 (2009) 706–715.
[84] L. Fadel, B.R. Viana, M.L.T. Feitosa, et al., Protocols for obtainment and isolation of two mesenchymal stem cell sources in sheep, Acta Cir. Bras. 26 (2011) 267–273.
[85] F. Al Battah, J. De Kock, E. Ramboer, et al., Evaluation of the multipotent character of human adipose tissue-derived stem cells isolated by Ficoll gradient centrifugation and red blood cell lysis treatment, Tox Vitro 25 (2011) 1224–1230.
[86] F. Bianchi, M. Maioli, E. Leonardi, et al., A new nonenzymatic method and device to obtain a fat tissue derivative highly enriched in pericyte-like elements by mild mechanical forces from human lipoaspirates, Cell Transpl. 22 (2013) 2063–2077.
[87] A.J. Singer, R.A. Clark, Cutaneous wound healing, N. Engl. J. Med. 341 (1999) 738–746.
[88] R. Goldman, Growth factors and chronic wound healing: past, present, and future, Adv. Skin. Wound Care 17 (2004) 24–35.
[89] S. Barrientos, O. Stojadinovic, M.S. Golinko, et al., Growth factors and cytokines in wound healing, Wound Rep. Regen. 16 (2008) 585–601.
[90] H. Lawall, P. Bramlage, B. Amann, Stem cell and progenitor cell therapy in peripheral artery disease, Thromb. Haemost. 103 (2010) 696–709.
[91] W.H. Hassan, U. Udo Greiser, W. Wang, Role of adipose-derived stem cells in wound healing, Wound Rep. Reg. 22 (2014) 313–325.
[92] M. Cherubino, P.J. Rubin, N. Natasa Miljkovic, et al., Adispose-derived stem cells for wound healing applications, Ann. Plast. Surg. 66 (2011) 210–215.
[93] J. Rehman, D. Traktuev, J. Li, et al., Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells, Circulation 109 (2004) 1292–1298.
[94] A. Miranville, C. Heeschen, C. Sengenes, C.A. Curat, R. Busse, A. Bouloumie, Improvement of postnatal neovascularization by human adipose tissue-derived stem cells, Circulation 110 (2004) 349–355.
[95] F. Lu, H. Mizuno, A.C. Uysal, X. Cai, R. Ogawa, H. Hyakusoku, Improved viability of random pattern skin flaps through the use of adipose-derived stem cells, Plast. Reconstr. Surg. 121 (2008) 50–58.
[96] M. Nambu, S. Kishimoto, S. Nakamura, et al., Accelerate wound healing in healing-impaired db/db mice by autologous adipose tissue-derived stromal cells combined with atelocollagen matrix, Ann. Plast. Surg. 62 (2009) 317–321.
[97] G. Marino, M. Moraci, E. Armenia, et al., Therapy with autologous adipose-derived regenerative cells fot the care of chronic ulcer of lower limbs in patients with peripheral arterial disease, J. Surg. Res. 185 (2013) 36–44.
[98] H. Lawall, P. Bramlage, B. Amann, Stem cell and progenitor cell therapy in peripheral artery disease, Thromb. Haemost. 103 (2010) 696–709.
[99] A. Germani, G. Di Campli, G. Pompilio, et al., Regenerative therapy in peripheral artery disease, Cardiovasc Ther. 27 (2009) 289–304.
[100] M. Blanton, I. Hadad, B. Johnstone, et al., Adipose stromal cells and platelet-rich plasma therapies synergistically increase revascularization during wound healing, Plast. Reconstr. Surg. 123 (2009) 56S–64S.
[101] M. Cherubino, K. Marra, Adipose-derived stem cells for soft tissue reconstruction, Regen. Med. 4 (2009) 109–117.
[102] C. Nie, G. Zhang, D. Yang, et al., Targeted delivery of adipose-derived stem cells via acellular dermal matrix enhances wound repair in diabetic rats, J. Tissue Eng. Reg. Med. 9 (2015) 224–235.
[103] C. Nie, D. Yang, S. Morris, Local delivery of adipose-derived stem cells via acellular dermal matrix as a scaffold: a new promising strategy to accelerate wound healing, Med. Hypotheses 72 (2009) 679–682.
[104] D. Beniker, D. McQuillan, S. Livesey, et al., The use of acellular dermal matrix as a scaffold for periosteum replacement, Orthopedics 26 (2003) S591–S596.
[105] A. Altman, N. Matthias, Y. Yan, et al., Dermal matrix as a carrier for in vivo delivery of human adipose-derived stem cells, Biomaterials 29 (2008) 1431–1442.
[106] W.S. Kim, B.S. Park, J.H. Sung, The wound-healing and antioxidant effects of adipose-derived stem cells, Expert Opin. Biol. Ther. 9 (2009) 879–887.
[107] T. Ebrahimian, F. Pouzoulet, C. Squiban, V. Buard, M. André, B. Cousin, et al., Cell therapy based on adipose tissue-derived stromal cells promotes physiological and pathological wound healing, Arterioscler. Thromb. Vasc. Biol. 29 (2009) 503–510.
[108] S.P. Huang, C.H. Huang, J.F. Shyu, et al., Promotion of wound healing using adipose-derived stem cells in radiation ulcer of a rat model, J. Biomed. Sci. 20 (2013) 51–61.
[109] C. Nie, D. Yang, J. Xu, et al., Locally administered adipose-derived stem cells accelerate wound healing through differentiation and vasculogenesis, Cell Transpl. 20 (2011) 205–216.
[110] D.O. Trakutev, S. Merfeld-Clauss, J. Li, et al., A population of multipotent CD34- positive adipose stromal cells share pericyte and mesenchymal surface markers, reside in a periendothelial location, and stabilize endothelial networks, Circ. Res. 102 (2008) 77–85.
[111] V. Trottier, G. Marceau-Fortier, L. Germain, C. Vincent, J. Fradette, IFATS collection: using human adipose-derived stem/stromal cells for the production of new skin substitutes, Stem Cells 26 (2008) 2713–2723.
[112] W.S. Kim, B.S. Park, J.H. Sung, J.M. Yang, S.B. Park, S.J. Kwak, et al., Wound healing effect of adipose-derived stem cells: a critical role of secretory factors on human dermal fibroblasts, J. Dermatol. Sci. 48 (2007) 15–24.
[113] E.H. Javazon, S.G. Keswani, A.T. Badillo, et al., Enhanced epithelial gap closure and increased angiogenesis in wounds of diabetic mice treated with adult murine bone marrow stromal progenitor cells, Wound Rep. Reg. 15 (2007) 350–359.
[114] G.D. Yancopoulos, S. Davis, N.W. Gale, J.S. Rudge, S.J. Wiegand, J. Holash, Vascular-specific growth factors and blood vessel formation, Nature 407 (2000) 242–248.
[115] R.D. Galiano, O.M. Tepper, C.R. Pelo, et al., Topical vascular endothelial growth factor accelerates diabetic wound healing through increased angiogenesis and by mobilizing and recruiting bone marrow-derived cells, Am. J. Pathol. 164 (2004) 1935–1947.
[116] A. Zografou, O. Papadopoulos, C. Tsigris, et al., Autologous transplantation of adipose-derived stem cells enhances skin graft survival and wound healing in diabetic rats, Ann. Plast. Surg. 71 (2013) 225–232.
[117] B. Puissant, C. Barreau, P. Bourin, et al., Immunomodulatory effect of human adipose tissue-derived adult stem cells: comparison with bone marrow mesenchymal stem cells, Br. J. Haematol. 129 (2005) 118–129.
[118] H. Li, X. Fu, Mechanisms of action of mesenchymal stem cells in cutaneous wound repair and regeneration, Cell Tissue Res. 348 (2012) 371–377.
[119] F. Simonacci, N. Bertozzi, M.P. Grieco, E. Grignaffini, E. Raposio, Autologous fat transplantation for breast reconstruction: a literature review, Ann. Med. Surg. (Lond) 12 (2016) 94–100.