International Wound Journal ISSN 1742-4801

ORIGINAL ARTICLE

The role of adult tissue-derived stem cells in chronic leg ulcers: a systematic review focused on tissue regeneration medicine

Bruno Amato[1,2,†], Rita Compagna[1,2,†], Maurizio Amato[2], Lucia Butrico[3], Francesco Fugetto[4], Mariia D Chibireva[5], Andrea Barbetta[3], Marco Cannistrà[6], Stefano de Franciscis[1,3,‡] & Raffaele Serra[1,3,‡]

1 Interuniversity Center of Phlebolymphology (CIFL). International Research and Educational Program in Clinical and Experimental Biotechnology, Headquarters, University Magna Graecia of Catanzaro, Catanzaro, Italy
2 Department of Clinical Medicine and Surgery, University of Naples "Federico II", Naples, Italy
3 Department of Medical and Surgical Sciences, University of Catanzaro, Catanzaro, Italy
4 School of Medicine, University of Modena and Reggio Emilia, Modena, Italy
5 School of Medicine, Kazan State Medical University, Kazan, Tatarstan Republic, Russian Federation
6 Department of Surgery, Annunziata Hospital of Cosenza, Cosenza, Italy

Key words
Adult tissue-derived stem cells; Chronic leg ulcers; Stem cells; Wound healing

Correspondence to
Prof. R Serra, MD, PhD
Interuniversity Center of Phlebolymphology (CIFL)
International Research and Educational Program in Clinical and Experimental Biotechnology, Headquarters
University Magna Graecia of Catanzaro
Viale Europa
88100 Catanzaro
Italy
E-mail: rserra@unicz.it

Amato B, Compagna R, Amato M, Butrico L, Fugetto F, Chibireva MD, Barbetta A, Cannistrà M, de Franciscis S, Serra R. The role of adult tissue-derived stem cells in chronic leg ulcers: a systematic review focused on tissue regeneration medicine. Int Wound J 2015; doi: 10.1111/iwj.12499

Abstract

Wound healing is an articulated process that can be impaired in different steps in chronic wounds. Chronic leg ulcers are a special type of non-healing wounds that represent an important cause of morbidity and public cost in western countries. Because of their common recurrence after conventional managements and increasing prevalence due to an ageing population, newer approaches are needed. Over the last decade, the research has been focused on innovative treatment strategies, including stem-cell-based therapies. After the initial interest in embryonic pluripotent cells, several different types of adult stem cells have been studied because of ethical issues. Specific types of adult stem cells have shown a high potentiality in tissue healing, in both in vitro and in vivo studies. Aim of this review is to clearly report the newest insights on tissue regeneration medicine, with particular regard for chronic leg ulcers.

Introduction

Chronic leg ulcers (CLUs) affect 1% of the adult population and 3·6% of people older than 65 years representing one of the main cause of morbidity among older subjects, especially women in the western world; the prevalence of leg ulcers in Europe is about 0·18–1% (1–3).

CLUs occur more commonly in elderly people and their prevalence, in western countries, is rising due to an increase in both life expectancy and risk factors for atherosclerotic stenosis, that is smoking, obesity and diabetes (4). They are responsible for the high cost of caring for leg ulcers, including diagnosis, investigations, treatment, nursing care and rehabilitation: approximately 1% of the total health care costs in the western world are likely to be used for management of CLUs.

Key Messages

- chronic leg ulcers are a special type of non-healing wounds that represent an important cause of morbidity and public cost in western countries
- adult tissue-derived stem cells have a pivotal role in wound repair and regeneration and may be used to heal chronic leg ulcers
- considering the current available evidence regarding the therapeutic potential of adult stem cells in tissue healing, in the next future, they may represent an effective target in clinical practice

[†]These authors contributed equally to this work and share the first authorship.
[‡]These authors contributed equally to this work and share the senior authorship.

Venous ulcers are the most common type of leg ulcers, accounting for approximately 70% of cases. Arterial disease accounts for another 5–10% of leg ulcers; most of the others are due to either neuropathy (usually diabetic) or a combination of those diseases (5–8). They are characterised by significant morbidity, loss of productivity and reduced quality of life, especially among women (9). Furthermore, although the exact amount is not well established (10), the direct and the indirect social costs for the health care system associated with CLUs are very high, with the only diabetic ulcer costing $30 000 to $50 000 (11,12).

Various approaches have been developed for wound healing, but most of these have centred on one facet of wound healing, such as inflammation or growth factors (13–16). Furthermore, evidences have shown that stem cell therapy can be an excellent option for patients with CLUs (17–19): these therapies can provide a comprehensive solution by addressing multiple factors during wound healing, including cell proliferation, extracellular matrix (ECM) synthesis, growth factor release and vascularisation (20).

The aim of this study is to perform a systematic analysis of the most recent scientific literature on the role of adult tissue-derived stem cells in CLUs and the future prospects in regenerative medicine.

Materials and methods

PubMed and ScienceDirect databases were searched for articles using the terms: Chronic Leg Ulcers, Stem Cells Therapy, Angiogenesis, Wound Healing and Adult Tissue-Derived Stem Cells.

Only publications in English were included. Titles and abstracts were screened by one author (F. F.) to identify potentially relevant studies. All potentially eligible studies were subsequently evaluated in detail by one reviewer (F. F.) through consideration of the full text. Reference lists of retrieved articles were also searched for relevant publications.

Inclusion required clinical trials, case reports, meta-analysis and systematic reviews in which therapy with adult tissue-derived stem cells were provided in CLU patients. Studies were excluded if performed in languages other than English, if the patient cohort, in human studies, was defined by the presence of CLU and an additional confounding disease process or if CLU-specific results could not be distinguished from those of a larger population consisting of individuals without CLU. Studies were also excluded when the primary focus was other than chronic wounds.

Results

Study selection

Initial database searches yielded 34 studies from PubMed and 2302 from Science Direct in the last 5 years. We evaluated 115 eligible full text articles (Figure 1).

The pathophysiology of CLUs and their correlation with delayed wound healing, the current therapeutic approaches for CLUs found in literature, and the description of the application of the adult tissue-derived stem cell therapy in patients with CLUs are given below.

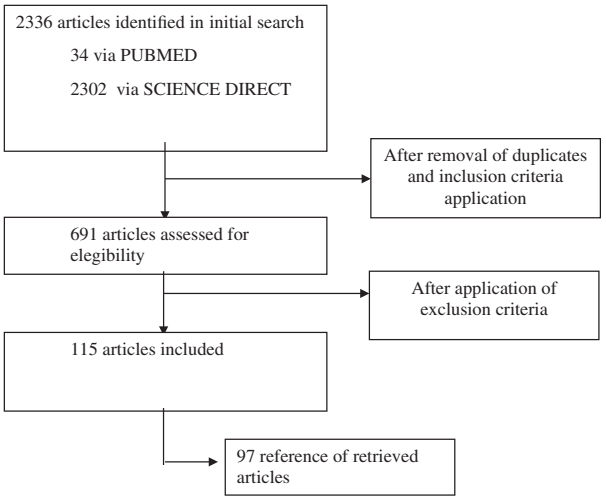

Figure 1 Flow of papers identified from search strategy.

Pathophysiology of chronic wound and CLUs

Both local and systemic factors can be involved in chronic wound etiopathogenesis. Among local factors infection, ischaemia, arterial/venous insufficiency, local toxins, trauma and radiations are of great importance and inevitably characterise all the cases of CLUs in different amounts. Among systemic factors ageing, chronic diseases, alcoholism, smoking, drugs, nutritional deficiencies, chronic kidney disease and neuropathies appear to be the most important (21). Non-healing wounds usually result from an impairment of one or more of the four phases of normal healing (haemostasis, inflammation, proliferation and remodelling). They are characterised by an incessant inflammation of which neutrophils represent a marker (22). This chronic inflammatory state is the base of the ECM degradation and is due to loss of important wound healing products such as platelet-derived growth factor (PDGF) and hepatocyte growth factor (HGF), respectively broken down by reactive oxygen species or MMPs and elastases secreted by neutrophils (23). This picture is confirmed by the analysis of chronic wound fluid (CWF) that, when compared with acute wound fluid (AWF), presents enhanced pro-inflammatory cytokines, MMPs, neutrophil elastases along with reduced amount of growth factors (22,24,25) and characterises, in particular, both chronic diabetic and venous ulcers (26). Moreover, in case of chronic venous insufficiency, fibroblasts appear to be qualitatively altered (27,28).

Wound healing in diabetic ulcers appears to be affected in a more specific way. First of all, the cellular activity is altered, with keratinocytes, epidermal cells and fibroblasts showing increased level of apoptosis and impaired migration and functioning (29–31). In addition, epidermal stem cells present a lower capacity of differentiation (32), while adipose-derived stem cells (ADSCs) were not impaired. Because of their ability to produce growth factors, cytokines and type I collagen, the latter cells can represent a potential role in diabetic ulcers treatment (33). ECM synthesis is reduced in diabetic wounds, mainly because of an impaired fibroblast activity (34). In the same time, its degradation is faster because of the higher levels

of MMPs (31). Both angiogenesis and neovascularisation are impaired in diabetic wounds, the latter because of a senesce in endothelial progenitor cells (EPCs) (17). Macro and micro angiopathy further complicate this picture.

The dermal layer is the main source of keratinocytes (35). If this structure in the depth of the wound is destroyed (e.g. deep CLUs), the only source of new regenerating cells is the dermal region all around the injury and reepithelialisation is slow, uncompleted and complicated by scarring and the conventional treatment is more often failing (36).

Current treatments for CLUs

The treatment of chronic ulcers of the lower extremities presents a therapeutic challenge. First of all, it should be focused on the causal conditions. Sanitary measures together with both surgical and medical strategies represent the basic of a comprehensive management of CLUs. In particular (i) leg elevation, compression therapy and anticoagulant treatment and surgical reduction of reflux are employed in case of venous ulcers; (ii) revascularisation, antiplatelet medications and management of risk factors are the targets in case of arterial disease; (iii) neuropathic ulcers are managed with off-loading of pressure and with topical growth factors; (iv) debridement is frequently performed in diabetic ulcers; and (v) pressure ulcers require an off-loading of pressure and reduction of excessive moisture, sheer and friction along with adequate nutrition. However, ulcers frequently recur (9). Of note, although topically applied growth factors (e.g. PDGF, EFG and FGF) assist the chronic wound by speeding the formation of the granulation tissue or improving epidermal cell function and giving some benefits (37–39), these are frequently unsatisfactory probably because of the local degradation of such mediators due to the chronic inflammation (40).

CLUs surgery consists of: (i) skin transplantation, including skin autograft and allo/xenografts and (ii) tissue-engineered skin substitutes. Autografting is usually performed with a split-thickness skin graft (STSG), that is, a tangential excision of a skin graft that includes the epidermis and part of the dermis. The autologous origin of the graft guarantees a nil risk of rejection (41). However, although this procedure improves the early healing rate of the wound and the quality of life of the patient, the rate of success of such therapy is only partial (42) even if it can be improved by a topical application of PDGF (8). Allo- or xenografts are used as a temporary alternative to autografting and serve as barrier and potential source of tissue-healing factor. However, they are inevitably rejected by the host after 1 week (41). Another approach for the management of tissue injuries consists of tissue-engineered substitutes. An example is represented by the culture of allogeneic neonatal dermal fibroblasts on a polyglactin scaffold. These cells produce ECM proteins which, in turn, replace the previous mesh that is ultimately degraded. The final result is an allogenic dermal analogue that can be used to dress the wound. Being particularly used on diabetic foot ulcers, this allograft is punctually rejected, but appears to promote keratinocyte migration and restore of the dermis, with good outcomes. Another allogeneic skin graft consists of two layers, both dermis and epidermis, respectively obtained with fibroblast and keratinocytes taken from neonatal foreskin (43). As in the previous case, this skin substitute is also ultimately rejected. However, in recent years the treatment of CLUs has shown good clinical results (44). Despite their general good therapeutic outcomes, tissue-engineered skin substitutes are characterised by important limitations for clinical purposes. The specific disadvantages such as slow vascularisation with poor integration, rejection an high cost, poor handling properties, a short life and an inability to reconstitute skin appendages (45,46) make these strategies far from being the conclusive solution for wound healing. With such evidences, great interest has been directed towards potential application of stem cell biology in ulcer care.

Stem cells and CLUs

The most widely accepted stem cell definition is an undifferentiated cell with three unique capacities: (i) *self-renewal* (i.e. the ability to produce unaltered daughter cells by symmetric cell division), (ii) *long-term viability* and (iii) *potency* (i.e. the possibility to generate different specialised cell types) (47,48). Those cells that are capable of giving rise to a whole, intact organism (including both somatic and germal cell types) are defined as *totipotent*; *pluripotent* and *multipotent* (organ-specific) stem cells can instead give rise to cells belonging to all three germ layers or a single organ or tissue, respectively (49).

As they can be harvested from embryonic or adult tissues, two types of stem cells can be identified: (i) pluripotent embryonic stem cells (ESCs), derived from the inner mass of the blastocysts or primordial germ cells in the germinal ridges of later embryos and (ii) uni- or multipotent adult stem cells (ASCs), which reside in some differentiated, adult tissues, do not complete their differentiation programme and are able to give one or few cell lineages (50). These two categories can be recognised by different expression of cell surface receptors and transcription factor, along with morphological, cytological and histological characteristics.

After the initial enthusiasm due to the possibility to obtain epidermal and dermal components, ESCs had to face essential problems that have limited their clinical applicability. First, there are important ethical issues regarding the use of human embryos for cell harvesting. However, nowadays this aspect can be, at least in part, circumvented by using a single-cell biopsy and a single blastomer without interfering with the embryo's developmental potential (51). Second, as for the ESCs derived from other species, those obtained from human embryo are highly incapable of differentiating in specific tissues, both in vivo (52) and in vitro (50). The former phenomenon demonstrates that adult tissues cannot provide a complete environment to direct the site-specific differentiation of ESCs (50). Nevertheless, reports have recently showed a successful differentiation of ESC-derived skin in vitro, giving hope and drive for future researches in this field (53). Third, teratocarcinomas have arisen from the ESCs (54). Fourth, and may be more importantly, ESC-derived skin still represents an allogeneic substitute and cannot guarantee permanent wound coverage. As allogeneic and xenogeneic grafts are already available at more moderate cost, the clinical advantage of using ESC-derived skin in not clear.

Table 1 Stem cells and their therapeutic effects

Cell type	Cell markers	Role in wound healing
BM-MSCS	CD105+, CD73+, CD90+, CD45−, CD34−, CD14−, CD11b−, CD79 alpha, CD19− and HLA-DR−	Increase cell proliferation, collagen synthesis, growth factor release, wound contraction, neovascularisation and cellular recruitment to wounds
ADSCs	CD31−, CD34+/−, CD45−, CD90+, CD105− and CD146−	Promote cell proliferation, collagen synthesis, promote neovessel formation and tissue remodelling
EPCs	CD34+, VEGFR-2+ and CD133+	Promote vascularisation secrete proangiogenic growth factors and cytokines, and differentiate into endothelial cells
BM-MNCs	haematopoietic progenitor cell markers: CD133+, CD117+ and CD34 MSCs markers and endothelial progenitor population: CD34+/−, CD133+ and VEGFR2+	Secrete angiogenic growth factors decrease local inflammation, and promote vascularisation differentiate into endothelial cells
Fibrocytes	CD 34+, CD11b+, CD13+, MHC II+, CD86+, CD45+, collagen-1+, procollagen-1+, CD3−, CD4−, CD8−, CD19− and CD25−	Increasing cell proliferation ECM deposition, wound contraction and vascularisation. Secrete of growth factors and chemokines

ADSCs, adipose-derived stem cells; BM-MNCs, bone-marrow-derived mononuclear cells; BM-MSCs, bone-marrow-derived mesenchymal stem cells; EPCs, endothelial progenitor cells.

In view of these evidences, research has largely focused its attention on ASCs. ASCs can have an endodermal, mesodermal or ectodermal origin and reside in several tissues such as central nervous system, epidermis, intestine, liver, lung and retina (55). The primary function of these cells is to serve as self-renewing stem cells and regenerate site-specific tissues in case of both physiological and pathological stimuli.

The rationale of the speculated employment of such cells in the clinical practice of CLUs is that: (i) despite traditional comprehensive wound management, including vascular reconstruction, many patients present non-responding wounds, which often resulting in amputation; (ii) ASCs could help replace lost tissues as well as create those skin appendages missing in the tissue-engineered skin substitutes (45,46).

We will now focus on ASC therapies, including mesenchymal stem cells (MSCs), EPCs, bone-marrow-derived mononuclear cells (BM-MNCs) and fibrocytes (Table 1). The large majority of preclinical studies regarding MSCs and CLUs have been conducted on murine diabetic wounds because of the more feasible nature of such models.

Mesenchymal stem cells

Mesenchymal stem cells (MSCs), also called mesenchymal stromal cells, are a group of non-haematopoietic ASCs that have a mesodermal origin. First described in 1966 by Friedenstein *et al.* (56), they are capable of differentiating in a far greater number of lineages than their normal mesoderm fate and can give arise to endodermic and ectodermic cells, skin included (57–60). MSCs can be found in almost every tissue (periosteum, tendon, muscle, synovial membrane and normal skin among the others) (61). To date, neither surface nor stemness marker allowing an accurate classification of these cells have been found, and the exact identity of MSCs in vivo is not yet clear (62). The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy defines MSCs as cells that (i) are plastic adherent in standard culture conditions; (ii) express CD105, CD73 and CD90 while lacking CD45, CD34, CD14 or CD11b, CD79 alpha or CD19 and HLA-DR molecules; (iii) can differentiate into chondroblasts, osteoblasts and adipocytes in vitro. This definition is the most commonly used in research (63). Their wide distribution along with multipotency firmly suggests an important role for MSCs in wound healing and replacement of cells that are lost in both physiological and pathological conditions. They also contribute to the digestive system, liver, musculoskeletal system, periodontal tissue and neurological homeostasis (64).

An important characteristic of MSCs is their capacity to be home to the damaged tissue sites, even when administered from an exogenous source. Central in this phenomenon is the inflammation at the site of wound, with chemokines (e.g. CXCL12, CXCL4 and CCR2) (65,66), adhesion molecules (such as P-selectin and VCAM-1) (67) and matrix metalloproteinases (MMPs, such as MMP-2) being the most implicated. Furthermore, all these molecules are induced by inflammatory cytokines such as TNF and IL-1 (68,69), which ultimately control stem cells' contribution at the site of injury.

Along with a multilineage differentiation potentiality, MSCs are involved in all four phases of wound repair. First, they can interact with cells of both the innate and adaptive immune systems and possess anti-inflammatory responses (70–72). In one study, the application of MSCs to an active inflammatory site resulted in a decrease of pro-inflammatory cytokines (such as TNF-α and interferon-γ) with a concomitant increase of anti-inflammatory cytokines (namely IL-10 and IL-4) and T-reg cells (73). Moreover, MSCs possess an anti-microbial activity that is of great importance in wound and CLUs healing. This is mediated by both direct (i.e. the secretion of anti-microbial factors) and indirect (i.e. the enhancement of the immune response of the host) mechanisms (74). The secretion of paracrine mediators at the site of inflammation is another way of the mesenchymal cell support in wound healing. In particular, growth factors (such as VEGF, PDGF, EGF, bFGF, FGF-23 and TGF-β) (75,76) and cytokines (such as IL-6 and CCL-2) (77–79) are responsible for angiogenesis and both recruitment and functioning of macrophages, endothelial cells, keratinocytes and fibroblasts, which are the main actors of

the physiological wound healing process. Of note, the secretion of VEGF and HGF, together with the maintenance of a good balance between tween TGF-β1 and TGF-β3 makes MSCs important in prevention of scarring (80–82). Although capable of transdifferentiating into vascular endothelial cells and skin components (83), it is currently believed that the paracrine activity of MSCs represents the primary mechanism by which these cells contribute to tissue healing mainly because of poor engraftment and survival of MSCs at the site of injury (75).

The unique anti-inflammatory activity of MSCs is capable of limiting the host immunoreaction against themselves in case of allogeneic transplantation. In addition, although presenting MHC Class I alloantigens, MSCs are characterised by minimal levels of surface immunostimulatory antigens such as MHC Class II alloantigens and co-stimulatory molecules including CD80 (B7-1), CD86 (B7-2) and CD40 (84). These evidences support a low immunogenicity and a high anti-rejection activity of the allogeneic MSCs, at least in the short term and in particular transplanting routes and microenvironments (84–86) and little or no rejection was observed after transplantation when allogeneic MSCs were administered systemically (87,88).

In view of the above, MSCs have been employed in tissue regeneration medicine in two different ways: (i) replacing the lost tissue, via transplantation or construction of bio-engineered tissues and (ii) attracting in vivo resident stem cells of the patient to the site of injury.

MSCs can easily be obtained from the bone marrow, adipose tissue, umbilical cord, human placenta, muscle, dermis, nerve tissue and lung, and can be further expanded in vitro and cryopreserved (87,89–96). Thus, at least in theory, all the above can be used in tissue regenerative medicine. However, because of practical and ethical issues, most of the preclinical and clinical studies were conducted on bone-marrow-derived mesenchymal stem cells (BM-MSCs) and ADSCs and to date there is a huge amount of data exalting their important contribution to tissue healing, including limb ulcer models, in every route of administration (97–106).

BM-MSCs, also known as marrow stromal cells, are self-renewing stem cells that are localised in the bone marrow. They represent a rare population of bone marrow cells (0·001–0·01% of the nucleated figures and 1/10 of HSCs), but are expandable in vitro (83). Although there is still a paucity of clinical data, their contribution to CLU healing is easily conceivable in light of the above. In their study, Kwon *et al.* demonstrated that systemic and local administration of BM-MSCs improved wound healing in a diabetic rat; this was mainly because of an increased production of collagen types I and V at the site of injury (107).

ADSCs, also known as adipose-derived stromal cells, adipose-derived mesenchymal progenitor cells and processed lipoaspirate cells (PLAs), have such variegated nomenclature mainly because of a lack of consensus and a still changing knowledge of both phenotype and function of these cells (17). However, as reported in Table 1, the International Society for Cellular Therapy considers both CD34+ and CD34 as ADSCs (108). Recent evidences suggest that CD34+ ADSCs can be characterised as having a greater proliferative potentiality, while CD34− ADSCs have a greater differentiating capacity (108,109).

As they can be extracted in large amounts with minor donor site morbidity and they have major proliferative capacities when compared to BM-MSCs, ADSCs represent an intriguing tool for both chronic wound and CLU treatment. However, clinical trials on CLUs are still lacking (110,111).

An important limitation of MSC employment in both chronic wound and ulcer management is represented by the long duration and complex procedures required for their expansion in vitro (17).

Endothelial progenitor cells

Human EPCs are a subset of circulating bone-marrow-derived figures that have been generally defined as cells (i) expressing a surface antigenic panel similar to that characterising the vascular endothelial cells; (ii) capable of adhering to the endothelium at the site of hypoxia/ischaemia; and (iii) capable of participating in neovascularisation (112). To date no specific marker has been known by which EPCs can be defined, although they express CD34, KDR (VEGFR-2) and CD133 markers (17).

Since EPCs can be recruited from bone marrow and peripheral blood to the sites of hypoxia/ischaemia and are able to participate in neovascularisation processes, it is currently believed that these cells can be important actors in tissue healing and numerous preclinical studied have been published to this effect (113,114). They indirectly participate in wound healing by secreting important mediators such as VEGF, hepatic growth factor (HGF), angiogenin 1, stroma derived factor (SDF)-1α, insulin-like growth factor (IGF)-1, along with inducing endothelial nitric oxide synthase (eNOS)/inducible nitric oxide synthase (iNOS) (115).

Clinical data regarding EPCs and leg ulcers is still lacking. Several works performed in murine models of diabetic wounds have found that both augmented neovascularisation and re-epithelialisation can be linked to the direct and indirect effects of ESC-derived EPCs applied on wounds (116).

However, EPCs are characterised by similar problems as in MSCs when applied to clinical practice (117).

Bone-marrow-derived mononuclear cells

The term BM-MNCs identifies a wide group of cells encompassing both staminal and differentiated figures in which haematopoietic stem cells, MSCs, EPCs and precursor cells along with their progeny are included (118). Because of their abundance in both peripheral blood and bone marrow, MNCs do not need in vitro expansion and are therefore a feasible source of staminal cells (118).

Because of their heterogeneity, several cell markers characterise BM-MNCs. Two cell sets are mostly involved in the angiogenetic process: (i) haematopoietic progenitor cells, which are CD133+, CD117+ and CD34+, and MSCs, particularly the endothelial progenitor population composed mainly of CD34−/CD133+/VEGFR2+ and CD34+/CD133+/VEGFR2+ cells (119).

Several clinical trials firmly demonstrate that MNCs improve leg ulcers (120,121). However, the specific therapeutic mechanisms still remain unknown. One hypothesis suggests that an augmented angiogenesis represents a central point in

MNC-mediated wound healing. Such speculation is supported by the elevated expression of angiogenic growth factors found after MNC transplantation (17). It appears that MNCs can even differentiate into endothelial cells, thus improving the neovascularisation at the wound site (119,122–124). Finally, an anti-inflammatory role of MNCs has been invoked (83). It can be concluded that although the complex makeup of MNCs makes it difficult to study them in a detailed manner, these cells represent a practical future tool for the clinical setting mainly because of their avoidance of an in vitro expansion.

Fibrocytes

In 1994, Bucala *et al.* (125) found that circulating, bone-marrow-derived 'fibrocytes' had the ability to adopt a mesenchymal phenotype and participated in scar formation. Fibrocytes represent a small subset (0·1–0·5%) of leukocytes and can be mostly found in the peripheral blood (126). They are characterised by a spindle shape when cultured in vitro and present a combination of markers (such as CD34, CD11b+, CD13+, MHC II+, CD86+ and CD45+) which is common to both fibroblasts and monocytes. Stromal cell markers (like collagen I, vimentin and fibronectin) further distinguish these cells (17).

Fibrocytes showed a great capacity to migrate to wound or chronic inflamed sites and localise to areas of ongoing ECM deposition (127) and an important role of these cells in wound healing is supported by several works. In some studies fibrocytes showed increase in ECM deposition, vascularisation and wound contraction (128). Moreover, they have been found capable of improving reepithelialisation, angiogenesis and local cell proliferation (127). A paracrine secretion is also speculated, with growth factors (VEGF, bFGF, TGF-β, ODGF), chemokines and ECM augmented in wounds treated with fibrocytes (127–129). Although differentiation into mesenchymal cells and contractile myofibroblast has been reported (126,127), their ability to do it in vivo is still controversial.

Fibrocytes are currently studied in several diseases, such as human hypertrophic scars, nephrogenic systemic fibrosis, human atherosclerotic pulmonary diseases characterised by repeated cycles of inflammation and repair (such as asthma), chronic pancreatitis, chronic cystitis and tumour metastasis (126,127). To date there are still few studies exploring the therapeutic potential of circulating fibrocytes in CLUs. However, in their study, Behjati *et al.* (130) were not able to use the patient's fibrocytes on leg diabetic ulcers because of the rarity of such cells in the peripheral blood.

Stem cells and the future of regenerative medicine

The aim of the novel field of regenerative medicine is to restore structure and function of damaged tissues, and stem cells represent a promising approach to wound healing through the release of soluble mediators that modulate chronic inflammation thereby speeding up healing processes. However, significant drags remain on improving progenitor cell selection and tissue delivery. Innovative techniques such as microfluidic single-cell characterisation seem to be promising for identifying and isolating the most appropriate cells for therapeutic use, as well as new and effective delivery vehicles in order to ameliorate the targeting of damaged tissues (131).

In the new era of regenerative medicine a new class of stem cells, has recently been discovered, the induced pluripotent stem cells (iPSCs). The use of iPSCs may allow the generation of autologous pluripotent stem cell population derived from differentiated adult tissues, being also non-immunogenic. In this context, iPSCs have at the same time combined advantages of the pluripotency of ESCs and the availability of ASCs, but still some concerns remain with the utilisation of ASCs: difficulties with genetic manipulation, safety profile, efficiency and cost-effectiveness (131,132).

Conclusions

Chronic leg ulceration still represents an important problem, especially in the western countries, and new therapeutic strategies are needed. The stem-cell-based tissue regeneration medicine is proving its potentiality in tissue healing and regeneration. Although functional stem cell units have been described throughout all layers of human skin, other niches can be found throughout the body. Both bone marrow and adipose tissue derived stem cells appear to be important in tissue healing, but a necessity of a long-lasting and complicated in vivo expansion still limits their clinical practice. BM-MNCs are easily found in the peripheral blood, do not need a culture and are now extensively evaluated for leg ulcer treatment. Finally, more studies are needed to completely understand the physiological and pathological role of EPC fibrocytes and the new promising iPSCs. Considering the current available evidence regarding therapeutic potential of ASCs in tissue healing, we are strongly convinced that, in the next future, they will represent a reality in clinical practice of leg ulceration.

References

1. Serra R, Grande R, Butrico L, Montemurro R, De Caridi G, Fugetto F, Dominijanni A, Gallelli L, Greto Ciriaco A, Vitagliano T, Greco M, de Franciscis S. Skin grafting and topical application of platelet gel in the treatment of vascular lower extremity ulcers. *Acta Phlebol* 2014;**15**:129–36.
2. Serra R, Buffone G, de Franciscis A, Mastrangelo D, Vitagliano T, Greco M, de Franciscis S. Skin grafting followed by low-molecular-weight heparin long-term therapy in chronic venous leg ulcers. *Ann Vasc Surg* 2012;**26**:190–7.
3. Serra R, Butrico L, Ruggiero M, Rossi A, Buffone G, Fugetto F, De Caridi G, Massara M, Falasconi C, Rizzuto A, Settimio UF, Perri P, Dardano G, Grande R, de Franciscis S. Epidemiology, diagnosis and treatment of chronic leg ulcers: a systematic review. *Acta Phlebol* 2014;**15**:1–2.
4. Serra R, Grande R, Butrico L, Rossi A, Settimio UF, Caroleo B, Amato B, Gallelli L, de Franciscis S. Chronic wound infections: the role of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *Expert Rev Anti Infect Ther* 2015;**13**:605–13.
5. Mekkes JR, Loots MA, Van Der Wal AC, Bos JD. Causes, investigation and treatment of leg ulceration. *Br J Dermatol* 2003;**148**:388–401.
6. de Franciscis S, Gallelli L, Battaglia L, Molinari V, Montemurro R, Stillitano DM, Buffone G, Serra R. Cilostazol prevents foot ulcers in diabetic patients with peripheral vascular disease. *Int Wound J* 2015;**12**:250–3.

7. Serra R, Grande R, Scarcello E, Buffone G, de Franciscis S. Angiosome-targeted revascularisation in diabetic foot ulcers. *Int Wound J* 2013. DOI: 10.1111/iwj.12162.
8. Serra R, Buffone G, Dominijanni A, Molinari V, Montemurro R, de Franciscis S. Application of platelet-rich gel to enhance healing of transmetatarsal amputations in diabetic dysvascular patients. *Int Wound J* 2013;**10**:612–5.
9. Agale SV. Chronic leg ulcers: epidemiology, aetiopathogenesis, and management. *Ulcers* 2013;**413604**:9.
10. Nelzen O. Leg ulcers: economic aspects. *Phlebology* 2000;**15**:110–4.
11. Beckrich K, Aronovitch SA. Hospital-acquired pressure ulcers: a comparison of costs in medical versus surgical patients. *Nurs Econ* 1999;**17**:263–71.
12. Boyce ST, Warden GD. Principles and practices for treatment of cutaneous wounds with cultured skin substitutes. *Am J Surg* 2002;**183**:445–56.
13. Serra R, Grande R, Buffone G, Gallelli L, de Franciscis S. The effects of minocycline on extracellular matrix in patients with chronic venous leg ulcers. *Acta Phlebol* 2013;**14**:99–107.
14. Serra R, Grande R, Butrico L, Buffone G, Caliò FG, Squillace A, Rizzo BA, Massara M, Spinelli F, Ferrarese AG, De Caridi G, Gallelli L, de Franciscis S. Effects of a new nutraceutical substance on clinical and molecular parameters in patients with chronic venous ulceration. *Int Wound J* 2014. DOI: 10.1111/iwj.12240.
15. Serra R, Gallelli L, Buffone G, Molinari V, Stillitano DM, Palmieri C, de Franciscis S. Doxycycline speeds up healing of chronic venous ulcers. *Int Wound J* 2015;**12**:179–84.
16. Serra R, Gallelli L, Conti A, De Caridi G, Massara M, Spinelli F, Buffone G, Caliò FG, Amato B, Ceglia S, Spaziano G, Scaramuzzino L, Ferrarese AG, Grande R, de Franciscis S. The effects of sulodexide on both clinical and molecular parameters in patients with mixed arterial and ulcers of lower limbs. *Drug Des Devel Ther* 2014;**8**:519–27.
17. Yang M, Sheng L, Zhang TR, Li Q. Stem cell therapy for lower extremity diabetic ulcers: where do we stand? *Biomed Res Int* 2013;**2013**:462179.
18. Compagna R, Amato B, Massa S, Amato M, Grande R, Butrico L, de Franciscis S, Serra R. Cell Therapy in patients with Critical Limb Ischemia. *Stem Cells Int* 2015;**2015**:931420.
19. Sorrell JM, Caplan AI. Topical delivery of mesenchymal stem cells and their function in wounds. *Stem Cell Res Ther* 2010;**1**:30.
20. Amato B, Compagna R, Amato M, Grande R, Butrico L, Rossi A, Naso A, Ruggiero M, de Franciscis S, Serra R. Adult vascular wall-resident multipotent vascular stem cells, matrix metalloproteinases and arterial aneurysms. *Stem Cells Int* 2015;**2015**:434962.
21. Robertson L, Lee AJ, Gallagher K, Carmichael SJ, Evans CJ, McKinstry BH, Fraser SC, Allan PL, Weller D, Ruckley CV, Fowkes FG. Risk factors for chronic ulceration in patients with varicose veins: a case control study. *J Vasc Surg* 2009;**49**:1490–8.
22. Diegelmann RF, Evans MC. Wound healing: an overview of acute, fibrotic and delayed healing. *Front Biosci* 2004;**9**:283–9.
23. Amato B, Coretti G, Compagna R, Amato M, Buffone G, Gigliotti D, Grande R, Serra R, de Franciscis S. Role of matrix metalloproteinases in non-healing venous ulcers. *Int Wound J* 2013. DOI: 10.1111/iwj.12181.
24. Nwomeh BC, Liang HX, Cohen IK, Yager DR. MMP-8 is the predominant collagenase in healing wounds and nonhealing ulcers. *J Surg Res* 1999;**81**:189–95.
25. Yager DR, Zhang LY, Liang HX, Diegelmann RF, Cohen IK. Wound fluids from human pressure ulcers contain elevated matrix metalloproteinase levels and activity compared to surgical wound fluids. *J Invest Dermatol* 1996;**107**:743–8.
26. Loots MA, Lamme EN, Zeegelaar J, Mekkes JR, Bos JD, Middelkoop E. Differences in cellular infiltrate and extracellular matrix of chronic diabetic and venous ulcers versus acute wounds. *J Invest Dermatol* 1998;**111**:850–7.
27. Mendez MV, Stanley A, Phillips T, Murphy M, Menzoian JO, Park HY. Fibroblasts cultured from distal lower extremities in patients with venous reflux display cellular characteristics of senescence. *J Vasc Surg* 1998;**28**:1040–50.
28. Raffetto JD, Mendez MV, Marien BJ, Byers HR, Phillips TJ, Park HY, Menzoian JO. Changes in cellular motility and cytoskeletal actin in fibroblasts from patients with chronic venous insufficiency and in neonatal fibroblasts in the presence of chronic wound fluid. *J Vasc Surg* 2001;**33**:1233–41.
29. Usui ML, Mansbridge JN, Carter WG, Fujita M, Olerud JE. Keratinocyte migration, proliferation, and differentiation in chronic ulcers from patients with diabetes and normal wounds. *J Histochem Cytochem* 2008;**56**:687–96.
30. Desta T, Li J, Chino T, Graves DT. Altered fibroblast proliferation and apoptosis in diabetic gingival wounds. *J Dent Res* 2010;**89**:609–14.
31. Lerman OZ, Galiano RD, Armour M, Levine JP, Gurtner GC. Cellular dysfunction in the diabetic fibroblast: impairment in migration, vascular endothelial growth factor production, and response to hypoxia. *Am J Pathol* 2003;**162**:303–12.
32. Zhong QL, Liu FR, Liu DW, Peng Y, Zhang XR. Expression of β-catenin and cyclin D1 in epidermal stem cells of diabetic rats. *Mol Med Rep* 2011;**4**:377–81.
33. Nambu M, Ishihara M, Kishimoto S, Yanagibayashi S, Yamamoto N, Azuma R, Kanatani Y, Kiyosawa T, Mizuno H. Stimulatory effect of autologous adipose tissue-derived stromal cells in an atelocollagen matrix on wound healing in diabetic db/db mice. *J Tissue Eng* 2011;**2011**:158105.
34. Lateef H, Stevens MJ, Varani J. All-trans-retinoic acid suppresses matrix metalloproteinase activity and increases collagen synthesis in diabetic human skin in organ culture. *Am J Pathol* 2004;**165**:167–74.
35. Werner GR. Regulation of wound healing by growth factors and cytokines. *Physiol Rev* 2003;**83**:835–70.
36. Chen M, Przyborowski M, Berthiaume F. Stem cells for skin tissue engineering and wound healing. *Crit Rev Biomed Eng* 2009;**37**:399–421.
37. Wieman TJ, Smiell JM, Su Y. Efficacy and safety of a topical gel formulation of recombinant human platelet-derived growth factor-BB (becaplermin) in patients with chronic neuropathic diabetic ulcers. A phase III randomized placebo-controlled double-blind study. *Diabetes Care* 1998;**21**:822–7.
38. Falanga V, Eaglstein WH, Bucalo B, Katz MH, Harris B, Carson P. Topical use of human recombinant epidermal growth factor (h-EGF) in venous ulcers. *J Dermatol Surg Oncol* 1992;**18**:604–6.
39. Loot MA, Kenter SB, Au FL, van Galen WJ, Middelkoop E, Bos JD, Mekkes JR. Fibroblasts derived from chronic diabetic ulcers differ in their response to stimulation with EGF, IGF-I, bFGF and PDGF-AB compared to controls. *Eur J Cell Biol* 2002;**81**:153–60.
40. Bennett SP, Griffiths GD, Schor AM, Leese GP, Schor SL. Growth factors in the treatment of diabetic foot ulcers. *Br J Surg* 2003;**90**:133–46.
41. Tzaneva S, Heere-Ress E, Kittler H, Böhler K. Surgical treatment of large vascular leg ulcers: a retrospective review evaluating risk factors for healing and recurrence. *Dermatol Surg* 2014;**40**:1240–8.
42. Kirsner RS, Eaglstein WH, Kerdel FA. Split-thickness skin grafting for lower extremity ulcerations. *Dermatol Surg* 1997;**23**:85–91.
43. Saap LJ, Donohue K, Falanga V. Clinical classification of bioengineered skin use and its correlation with healing of diabetic and venous ulcers. *Dermatol Surg* 2004;**30**:1095–100.
44. Jones JE, Nelson EA, Al-Hity A. Skin grafting for venous leg ulcers. *Cochrane Database Syst Rev* 2013;**31**:CD001737.
45. Hassan WU, Greiser U, Wang W. Role of adipose-derived stem cells in wound healing. *Wound Repair Regen* 2014;**22**:313–25.
46. Burd A, Ahmed K, Lam S, Ayyappan T, Huang L. Stem cell strategies in burns care. *Burns* 2007;**33**:282–91.
47. Weissman IL. Stem cells: units of development, units of regeneration, and units in evolution. *Cell* 2000;**100**:157–68.
48. Cha J, Falanga V. Stem cells in cutaneous wound healing. *Clin Dermatol* 2007;**25**:73–8.

49. Sharma RK, John JR. Role of stem cells in the management of chronic wounds. *Indian J Plast Surg* 2012;**45**:237–43.
50. Stocum DL. Stem cells in regenerative biology and medicine. *Wound Repair Regen* 2001;**9**:429–42.
51. Klimanskaya I, Chung Y, Becker S, Lu SJ, Lanza R. Human embryonic stem cell lines derived from single blastomeres. *Nature* 2006;**444**:481–5.
52. Moodley Y, Thompson P, Warburton D. Stem cells: a recapitulation of development. *Respirology* 2013;**18**:1167–76.
53. Coraux C, Hilmi C, Rouleau M, Spadafora A, Hinnrasky J, Ortonne JP, Dani C, Aberdam D. Reconstituted skin from murine embryonic stem cells. *Curr Biol* 2003;**13**:849–53.
54. Evans MJ, Kaufman M. Pluripotential cells grown directly from normal mouse embryos. *Cancer Surv* 1983;**2**:185–208.
55. Can A. A concise review on the classification and nomenclature of stem cells. *Turk J Hematol* 2008;**25**:57–9.
56. Friedenstein AJ, Piatetzky-Shapiro II, Petrakova KV. Osteogenesis in transplants of bone marrow cells. *J Embryol Exp Morphol* 1966;**16**:381–90.
57. Bianchi G, Borgonovo G, Pistoia V, Raffaghello L. Immunosuppressive cells and tumour microenvironment: focus on mesenchymal stem cells and myeloid derived suppressor cells. *Histol Histopathol* 2011;**26**:941–51.
58. Prockop DJ. Marrow stromal cells as stem cells for nonhematopoietic tissues. *Science* 1997;**276**:71–4.
59. Nakagawa H, Akita S, Fukui M, Fujii T, Akino K. Human mesenchymal stem cells successfully improve skin-substitute wound healing. *Br J Dermatol* 2005;**153**:29–36.
60. Krause DS, Theise ND, Collector MI, Henegariu O, Hwang S, Gardner R, Neutzel S, Sharkis SJ. Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell* 2001;**105**: 369–77.
61. Sellheyer K, Krahl D. Cutaneous mesenchymal stem cells. Current status of research and potential clinical applications. *Hautarzt* 2010;**61**:429–34.
62. Lv FJ, Tuan RS, Cheung KM, Leung VY. Concise review: the surface markers and identity of human mesenchymal stem cells. *Stem Cells* 2014;**32**:1408–19.
63. Tsai TL, Wang B, Squire MW, Guo LW, Li WJ. Endothelial cells direct human mesenchymal stem cells for osteo- and chondro-lineage differentiation through endothelin-1 and AKT signaling. *Stem Cell Res Ther* 2015 May 1;**6**:88.
64. Wei X, Yang X, Han ZP, Qu FF, Shao L, Shi YF. Mesenchymal stem cells: a new trend for cell therapy. *Acta Pharmacol Sin* 2013;**34**:747–54.
65. Wynn RF, Hart CA, Corradi-Perini C, O'Neill L, Evans CA, Wraith JE, Fairbairn LJ, Bellantuono I. A small proportion of mesenchymal stem cells strongly expresses functionally active CXCR4 receptor capable of promoting migration to bone marrow. *Blood* 2004;**104**:2643–5.
66. Belema-Bedada F, Uchida S, Martire A, Kostin S, Braun T. Efficient homing of multipotent adult mesenchymal stem cells depends on FROUNT-mediated clustering of CCR2. *Cell Stem Cell* 2008;**2**:566–75.
67. Ruster B, Gottig S, Ludwig RJ, Bistrian R, Muller S, Seifried E, Gille J, Henschler R. Mesenchymal stem cells display coordinated rolling and adhesion behavior on endothelial cells. *Blood* 2006;**108**:3938–44.
68. Shi M, Li J, Liao L, Chen B, Li B, Chen L, Jia H, Zhao RC. Regulation of CXCR4 expression in human mesenchymal stem cells by cytokine treatment: role in homing efficiency in NOD/SCID mice. *Haematologica* 2007;**92**:897–904.
69. Ren G, Zhao X, Zhang L, Zhang J, L'Huillier A, Ling W, Roberts AI, Le AD, Shi S, Shao C, Shi Y. Inflammatory cytokine-induced intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 in mesenchymal stem cells are critical for immunosuppression. *J Immunol* 2010;**184**:2321–8.
70. Newman RE, Yoo D, LeRoux MA, Danilkovitch-Miagkova A. Treatment of inflammatory diseases with mesenchymal stem cells. *Inflamm Allergy Drug Targets* 2009;**8**:110–23.
71. Uccelli A, Moretta L, Vito PV. Mesenchymal stem cells in health and disease. *Nat Rev Immunol* 2008;**8**:726–36.
72. Singer NG, Caplan AI. Mesenchymal stem cells: mechanisms of inflammation. *Annu Rev Pathol* 2011;**6**:457–78.
73. Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood* 2005;**105**:1815–22.
74. Maxson S, Lopez EA, Yoo D, Danilkovitch-Miagkova A, Leroux MA. Concise review: role of mesenchymal stem cells in wound repair. *Stem Cells Transl Med* 2012;**1**:142–9.
75. Gnecchi M, Zhang Z, Ni A, Dzau VJ. Paracrine mechanisms in adult stem cell signaling and therapy. *Circ Res* 2008;**103**:1204–19.
76. Chen L, Tredget EE, Wu PY, Wu Y. Paracrine factors of mesenchymal stem cells recruit macrophages and endothelial lineage cells and enhance wound healing. *PLoS One* 2008;**3**:e1886.
77. Caplan AI, Dennis JE. Mesenchymal stem cells as trophic mediators. *J Cell Biochem* 2006;**98**:1076–84.
78. Xu G, Zhang Y, Zhang L, Ren G, Shi Y. The role of IL-6 in inhibition of lymphocyte apoptosis by mesenchymal stem cells. *Biochem Biophys Res Commun* 2007;**361**:745–50.
79. Liu Y, Han ZP, Zhang SS, Jing YY, Bu XX, Wang CY, Sun K, Jiang GC, Zhao X, Li R, Gao L, Zhao QD, Wu MC, Wei LX. Effects of inflammatory factors on mesenchymal stem cells and their role in the promotion of tumor angiogenesis in colon cancer. *J Biol Chem* 2011;**286**:25007–15.
80. Ono I, Yamashita T, Hida T, Jin HI, Ito Y, Hamada H, Akasaka Y, Ishii T, Jimbow K. Combined administration of basic fibroblast growth factor protein and the hepatocyte growth factor gene enhances the regeneration of dermis in acute incisional wounds. *Wound Repair Regen* 2004;**12**:67–79.
81. Shah M, Foreman DM, Ferguson MW. Neutralisation of TGF-beta 1 and TGF-beta 2 or exogenous addition of TGF-beta 3 to cutaneous rat wounds reduces scarring. *J Cell Sci* 1995;**108**:985–1002.
82. Colwell AS, Beanes SR, Soo C, Dang C, Ting K, Longaker MT, Atkinson JB, Lorenz HP. Increased angiogenesis and expression of vascular endothelial growth factor during scarless repair. *Plast Reconstr Surg* 2005;**115**:204–12.
83. Wu Y, Chen L, Scott PG, Tredget EE. Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. *Stem Cells* 2007;**25**:2648–59.
84. Klyushnenkova E, Mosca JD, Zernetkina V, Majumdar MK, Beggs KJ, Simonetti DW, Deans RJ, McIntosh KR. T cell responses to allogeneic human mesenchymal stem cells: immunogenicity, tolerance, and suppression. *J Biomed Sci* 2005;**12**:47–57.
85. Gu LH, Zhang TT, Li Y, Yan HJ, Qi H, Li FR. Immunogenicity of allogeneic mesenchymal stem cells transplanted via different routes in diabetic rats. *Cell Mol Immunol* 2015;**12**:444–55.
86. Griffin MD, Ryan AE, Alagesan S, Lohan P, Treacy O, Ritter T. Anti-donor immune responses elicited by allogeneic mesenchymal stem cells: what have we learned so far? *Immunol Cell Biol* 2013;**91**:40–51.
87. Mansilla E, Marin GH, Sturla F, Drago HE, Gil MA, Salas E, Gardiner MC, Piccinelli G, Bossi S, Salas E, Petrelli L, Iorio G, Ramos CA, Soratti C. Human mesenchymal stem cells are tolerized by mice and improve skin and spinal cord injuries. *Transplant Proc* 2005;**37**:292–4.
88. Liu H, Kemeny DM, Heng BC, Ouyang HW, Melendez AJ, Cao T. The immunogenicity and immunomodulatory function of osteogenic cells differentiated from mesenchymal stem cells. *J Immunol* 2006;**176**:2864–71.
89. Bianco P, Robey PG, Simmons PJ. Mesenchymal stem cells: revisiting history, concepts, and assays. *Cell Stem Cell* 2008;**2**:313–9.
90. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. *Science* 1999;**284**:143–7.

91. Anjos-Afonso F, Bonnet D. Nonhematopoietic/endothelial SSEA-1+ cells define the most primitive progenitors in the adult murine bone marrow mesenchymal compartment. *Blood* 2007;**109**:1298–306.
92. In't Anker PS, Scherjon SA, Kleijburg-van der Keur C, de Groot-Swings GM, Claas FH, Fibbe WE, Kanhai HH. Isolation of mesenchymal stem cells of fetal or maternal origin from human placenta. *Stem Cells* 2004;**22**:1338–45.
93. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, Benhaim P, Lorenz HP, Hedrick MK. Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng* 2001;**7**:211–28.
94. Lu D, Chen B, Liang Z, Deng W, Jiang Y, Li S, Xu J, Wu Q, Zhang Z, Zie B, Chen S. Comparison of bone marrow mesenchymal stem cells with bone marrow-derived mononuclear cells for treatment of diabetic critical limb ischemia and foot ulcer: A double-blind, randomized, controlled trial. *Diabetes Res Clin Pract* 2011;**92**:26–36.
95. Badiavas EV, Falanga V. Treatment of chronic wounds with bone marrow-derived cells. *Arch Dermatol* 2003;**139**:510–6.
96. Vojtassák J, Danisovic L, Kubes M, Bakos D, Jarábek L, Ulicná M, Blasko M. Autologous biograft and mesenchymal stem cells in treatment of the diabetic foot. *Neuro Endocrinol Lett* 2006;**27**(Suppl 2):134–7.
97. Falanga V, Iwamoto S, Chartier M, Yufit T, Butmarc J, Kouttab N, Shrayer D, Carson P. Autologous bone marrow-derived cultured mesenchymal stem cells delivered in a fibrin spray accelerate healing in murine and human cutaneous wounds. *Tissue Eng* 2007;**13**:1299–312.
98. Yoshikawa T, Mitsuno H, Nonaka I, Sen Y, Kawanishi K, Inada Y, Takakura Y, Okuchi K, Nonomura A. Wound therapy by marrow mesenchymal cell transplantation. *Plast Reconstr Surg* 2008;**121**:860–77.
99. Dash NR, Dash SN, Routray P, Mohapatra S, Mohapatra PC. Targeting nonhealing ulcers of lower extremity in human through autologous bone marrow-derived mesenchymal stem cells. *Rejuvenation Res* 2009;**12**:359–66.
100. Kesting MR, Loeffelbein DJ, Steinstraesser L, Muecke T, Demtroeder C, Sommerer F, Hoelzle F, Wolff KD. Cryopreserved human amniotic membrane for soft tissue repair in rats. *Ann Plast Surg* 2008;**60**:684–91.
101. Kim WS, Park BS, Sung JH, Yang JM, Park SB, Kwak SJ, Park JS. Wound healing effect of adipose-derived stem cells: a critical role of secretory factors on human dermal fibroblasts. *J Dermatol Sci* 2007;**48**:15–24.
102. Blanton MW, Hadad I, Johnstone BH, Mund JA, Rogers PI, Eppley BL, March KL. Adipose stromal cells and platelet-rich plasma therapies synergistically increase revascularization during wound healing. *Plast Reconstr Surg* 2009;**123**(2 Suppl):56S–64.
103. Kim WS, Park BS, Park SH, Kim HK, Sung JH. Antiwrinkle effect of adipose-derived stem cell: activation of dermal fibroblast by secretory factors. *J Dermatol Sci* 2009;**53**:96–102.
104. Nambu M, Ishihara M, Nakamura S, Mizuno H, Yanagibayashi S, Kanatani Y, Hattori H, Takase B, Ishizuka T, Kishimoto S, Amano Y, Yamamoto N, Azuma R, Kiyosawa T. Enhanced healing of mitomycin C-treated wounds in rats using inbred adipose tissue-derived stromal cells within an atelocollagen matrix. *Wound Repair Regen* 2007;**15**:505–10.
105. Uysal AC, Mizuno H, Tobita M, Ogawa R, Hyakusoku H. The effect of adipose-derived stem cells on ischemia-reperfusion injury: immunohistochemical and ultrastructural evaluation. *Plast Reconstr Surg* 2009;**124**:804–15.
106. Amos PJ, Kapur SK, Stapor PC, Shang H, Bekiranov S, Khurgel M, Rodeheaver GT, Peirce SM, Katz AJ. Human adipose-derived stromal cells accelerate diabetic wound healing: impact of cell formulation and delivery. *Tissue Eng Part A* 2010;**16**:1595–606.
107. Kwon DS, Gao X, Liu YB, Dulchavsky DS, Danyluk AL, Bansal M, Chopp M, McIntosh K, Arbab AS, Dulchavsky SA, Gautam SC. Treatment with bone marrow-derived stromal cells accelerates wound healing in diabetic rats. *Int Wound J* 2008;**5**:453–63.
108. Lee SH, Jin SY, Song JS, Seo KK, Cho KH. Paracrine effects of adipose-derived stem cells on keratinocytes and dermal fibroblasts. *Ann Dermatol* 2012;**24**:136–43.
109. Bailey AM, Kapur S, Katz AJ. Characterization of adipose-derived stem cells: an update. *Curr Stem Cell Res Ther* 2010;**5**:95–102.
110. Lee HC, An SG, Lee HW, Park JS, Cha KS, Hong TJ, Park JH, Lee SY, Kim SP, Kim YD, Chung SW, Bae YC, Shin YB, Kim JI, Jung JS. Safety and effect of adipose tissue-derived stem cell implantation in patients with critical limb ischemia. *Circ J* 2012;**76**:1750–60.
111. Sarasua JG, Lopez SP, Viejo MA, Basterrechea MP, Rodriguez AF, Gutierrez AF, Gala JG, Menendez YM, Augusto DE, Arias AP, Hernandez JO. Treatment of pressure ulcers with autologous bone marrow nuclear cells in patients with spinal cord injury. *J Spinal Cord Med* 2011;**34**:301–7.
112. Yoder MC. Human endothelial progenitor cells. *Cold Spring Harb Perspect Med* 2012;**2**:a006692.
113. Deng X, Szabo S, Chen L, Paunovic B, Khomenko T, Tolstanova G, Tarnawski AS, Jones MK, Sandor Z. New cell therapy using bone marrow-derived stem cells/endothelial progenitor cells to accelerate neovascularization in healing of experimental ulcerative colitis. *Curr Pharm Des* 2011;**17**:1643–51.
114. Suh W, Kim KL, Kim JM, Shin IS, Lee YS, Lee JY, Jang HS, Lee JS, Byun J, Choi JH, Jeon ES, Kim DK. Transplantation of endothelial progenitor cells accelerates dermal wound healing with increased recruitment of monocytes/macrophages and neovascularization. *Stem Cells* 2005;**23**:1571–8.
115. Asahara T, Kawamoto A, Masuda H. Concise review: circulating endothelial progenitor cells for vascular medicine. *Stem Cells* 2011;**29**:1650–5.
116. Lee MJ, Kim J, Lee KI, Shin JM, Chae JI, Chung HM. Enhancement of wound healing by secretory factors of endothelial precursor cells derived from human embryonic stem cells. *Cytotherapy* 2011;**13**:165–78.
117. Sheng L, Yang M, Du Z, Yang Y, Li Q. Transplantation of stromal vascular fraction as an alternative for accelerating tissue expansion. *J Plast Reconstr Aesthet Surg* 2013;**66**:551–7.
118. Yang M, Sheng L, Li H, Weng R, Li QF. Improvement of the skin flap survival with the bone marrow-derived mononuclear cells transplantation in a rat model. *Microsurgery* 2010;**30**:275–81.
119. Murphy MP, Lawson JH, Rapp BM, Dalsing MC, Klein J, Wilson MG, Hutchins GD, March KL. Autologous bone marrow mononuclear cell therapy is safe and promotes amputation-free survival in patients with critical limb ischemia. *J Vasc Surg* 2011;**53**:1565–74.e1.
120. Jain P, Perakath B, Jesudason MR, Nayak S. The effect of autologous bone marrow-derived cells on healing chronic lower extremity wounds: results of a randomized controlled study. *Ostomy Wound Manage* 2011;**57**:38–44.
121. Yamaguchi Y, Yoshida S, Sumikawa Y, Kubo T, Hosokawa K, Ozawa K, Hearing VJ, Yoshikawa K, Itami S. Rapid healing of intractable diabetic foot ulcers with exposed bones following a novel therapy of exposing bone marrow cells and then grafting epidermal sheets. *Br J Dermatol* 2004;**151**:1019–28.
122. Pedroso DC, Tellechea A, Moura L, Fidalgo-Carvalho I, Duarte J, Carvalho E, Ferreira L. Improved survival, vascular differentiation and wound healing potential of stem cells co-cultured with endothelial cells. *PLoS One* 2011;**6**:e16114.
123. Okuno Y, Nakamura-Ishizu A, Kishi K, Suda T, Kubota Y. Bone marrow-derived cells serve as proangiogenic macrophages but not endothelial cells in wound healing. *Blood* 2012;**117**:5264–72.
124. Walter DH, Krankenberg H, Balzer JO, Kalka C, Baumgartner I, Schlüter M, Tonn T, Seeger F, Dimmeler S, Lindhoff-Last E, Zeiher AM, PROVASA Investigators. Intraarterial administration of bone marrow mononuclear cells in patients with critical limb ischemia: a randomized-start, placebo-controlled pilot trial (PROVASA). *Circ Cardiovasc Interv* 2011 Feb 1;**4**:26–37.

125. Bucala R, Spiegel LA, Chesney J, Hogan M, Cerami A. Circulating fibrocytes define a new leukocyte subpopulation that mediates tissue repair. *Mol Med* 1994;**1**:71–81.
126. Quan TE, Cowper S, Wu SP, Bockenstedt LK, Bucala R. Circulating fibrocytes: collagen-secreting cells of the peripheral blood. *Int J Biochem Cell Biol* 2004;**36**:598–606.
127. Bellini A, Mattoli S. The role of the fibrocyte, a bone marrow-derived mesenchymal progenitor, in reactive and reparative fibroses. *Lab Invest* 2007;**87**:858–70. [Epub 2007 Jul 2].
128. Kao HK, Chen B, Murphy GF, Li Q, Orgill DP, Guo L. Peripheral blood fibrocytes: enhancement of wound healing by cell proliferation, re-epithelialization, contraction, and angiogenesis. *Ann Surg* 2011;**254**:1066–74.
129. Hartlapp I, Abe R, Saeed RW, Peng T, Voelter W, Bucala R, Metz CN. Fibrocytes induce an angiogenic phenotype in cultured endothelial cells and promote angiogenesis in vivo. *FASEB J* 2001;**15**:2215–24.
130. Behjati M, Hashemi M, Shoarayenejati A, Karbalaie K, Nasr-Esfahani MH. Safety, efficacy and pitfalls of fibrocyte application in the treatment of diabetic foot ulcer. *Int Wound J* 2015;**12**:27–31.
131. Duscher D, Barrera J, Wong VW, Maan ZN, Whittam AJ, Januszyk M, Gurtner GC. Stem cells in wound healing: the future of regenerative medicine? a mini-review. *Gerontology* 2015. DOI: 10.1159/000381877.
132. Mizuno H, Tobita M, Uysal AC. Concise review: adipose-derived stem cells as a novel tool for future regenerative medicine. *Stem Cells* 2012;**30**:804–10.