Adipose-derived stem cells in wound healing: recent results in vitro and in vivo

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Abstract
Introduction
Chronic wounds represent a major problem in medicine today as their incidence is continuously increasing due to an ageing population and a rise in the incidence of underlying diseases. Cutaneous wound healing is a complex biological process. Chronic wounds are characterised by a prolonged inflammation, persistent infections, formation of drug-resistant microbial biofilms and the inability of dermal and/or epidermal cells to respond to regenerative stimuli. As conventional treatment strategies often fail, innovative therapies have been investigated over the last decade, including stem cell-based therapies. After the initial use of embryonic stem cells, the focus has been set on autologous mesenchymal stem cells over the past years. They can be isolated in large amounts from various tissues and hold no ethical concerns. A promising and cost-effective source of autologous mesenchymal stem cells is subcutaneous adipose tissue. Recent in vitro and in vivo studies have shown that adipose-derived stem cells have a positive impact on wound healing, as they are attracted to the wound site and influence regeneration processes via paracrine mechanisms. They are pluripotent and secrete a variety of growth factors. The aim of this critical review was to discuss adipose-derived stem cells in wound healing.

Conclusion
Mesenchymal stem cells-derived from adipose tissue are a promising alternative to embryonic or bone marrow-derived stem cells in the therapy of chronic wounds. Because the use of adipose-derived stem cells in wound healing applications is still limited by a lack of clinical data, further studies have to pave the way for their routine clinical application.

Introduction
Due to demographic changes the number of patients with multimorbidity is continuously increasing in western countries. One of the associated disorders is the chronic wound. This includes a rise in the incidence of impaired wound healing, causing a reduced patients’ quality of life and rising health care costs. Conventional therapies of chronic wounds are increasingly reaching their limits, motivating the search for alternative treatment options, including stem cell-based therapies1. This paper discusses the recent in vitro and in vivo results of adipose-derived stem cells (ASCs) in wound healing.

Physiological wound healing
Wound healing is a complex biological process consisting of the four different but partially overlapping steps coagulation, inflammation, formation of granulation tissue (proliferative phase) and remodelling or scar formation.

After injury, initiation of a blood-clotting cascade prevents excessive bleeding and provides temporary protection of the wound area. During this process platelet-derived growth factor and transforming growth factors A1 and 2 (TGF-A1 and TGF-2) are released, causing attraction of inflammatory cells such as leukocytes and macrophages.

Within a few days after injury, apoptosis of inflammatory cells occurs. Anti-inflammatory cytokines such as TGF-A1 and Interleukin (IL) 1 as well as bioactive lipids are assumed to be involved in this process (Figure 1).

In the following proliferative phase, production of growth factors and activation of dermal and epidermal cells lead to new tissue formation. Endothelial progenitor cells, which are essential for physiological wound healing, are mobilised by nitric oxide, vascular endothelial growth factor (VEGF) and matrix metalloproteinase (MMP9). Formation of extra-cellular matrix (ECM)-rich tissue occurs in response to stromal cell-derived factor (SDF)-1 and insulin-like growth factor (IGF).

In the last step of wound healing, matrix remodelling and/or scar formation through cellular migration, proliferation and angiogenic induction is initiated by TGF-A, MMPs and tumour necrosis factor (TNF)2.

Impaired wound healing
A chronic wound is defined as a wound that does not heal in a certain period of time in spite of appropriate therapy. However, the exact-time point is in discussion. Most authors refer to a chronic wound as a wound that exists for more than three months3. Impaired wound healing is associated with senescence, ischaemia and bacterial colonisation. Wound chronification is caused by local factors such as infection, venous insufficiency, mechanical trauma,

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Adipose-derived stem cells in regenerative medicine

After initially focussing on embryonic stem cells in regenerative medicine, the focus has been set on autologous mesenchymal stem cells (MSCs) over the last years. In contrast to embryonic stem cells MSCs can be isolated in large amounts from various tissues and hold no ethical concerns. According to the minimal criteria of the International Society for Cellular Therapy MSCs are plastic adherent in standard culture conditions, the phenotype is positive for CD73, CD90 and CD105 as well as negative to CD11b or CD14, CD19 or CD79α and HLA-DR. They are inducible to differentiate into adipocytes, osteoblasts and chondrocytes in vitro. A promising and cost-effective source of autologous MSCs is subcutaneous adipose tissue. ASCs are very stable under cell culture conditions, a normal haploid karyotype remains after 100 duplications. The content of ASCs per gram tissue is five-fold higher than in bone marrow. Adipose tissue can be extracted as solid adipose tissue or by liposuction, which is a less invasive and safer procedure than bone biopsies. For harvesting ASCs solid adipose tissue is mechanically chopped, enzymatically digested by collagenase and centrifuged according to specific protocols. Isolated stem cells can then be cultivated in cell culture. Using specific differentiation media, these cells can be differentiated into various cell types, which can be used to fill up leaks in bones and cartilage or to support healing of chronic ulcers.

Discussion

The authors have referenced some of their own studies in this review. The protocols of these studies have been

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Figure 1: Simplified view of physiological and impaired wound healing. The processes of physiological and impaired wound healing and some of the involved factors are represented schematically. In contrast to an acute wound (left side) the chronic wound (right side) is characterised by a persistent inflammation as well as an impaired extra-cellular matrix synthesis and neovascularisation which lead to an impaired healing process. EPC, endothelial progenitor cell; FGF, fibroblast growth factor; IGF, insulin-like growth factor; IL, Interleukin; MMP, matrix metalloproteinase; PDGF, platelet-derived growth factor; ROS, reactive oxygen species; TGF, transforming growth factors; TNF, tumour necrosis factor; VEGF vascular endothelial growth factor.

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Chronic wounds are a rising problem in daily clinical routine. As conventional treatment strategies often fail, stem cell-based therapies have been investigated over the last decade. Recent in vitro and in vivo studies have shown that ASCs are a desirable alternative to embryonic or bone marrow-derived stem cells.

ASCs have already been shown to have a positive impact on wound healing, as they are attracted to the wound site and influence wound healing processes via paracrine mechanisms as well as fusion and differentiation, for example, into keratinocytes or fibroblasts. Recent in vitro and in vivo experiments have shown that ASCs can exhibit antioxidative effects, which are mainly mediated via paracrine mechanisms by the activation of dermal fibroblasts and keratinocytes. ASCs secrete a variety of growth factors such as basic fibroblast growth factor (bFGF), keratinocyte growth factor, TGF-β, hepatocyte growth factor (HGF) and VEGF. An overview of ASC functions in wound healing is summarised in Figure 2.

**Figure 2:** ASC function in wound healing and influencing conditions. ASCs are shown to have a positive impact on healing of chronic wounds via paracrine mechanisms, as scaffold material as well as fusion and differentiation, for example, into keratinocytes or fibroblasts (green). Success of ASC application in chronic wound treatment can be influenced by several conditions (yellow) such as donor/host specificity, chronic wound fluid and further aspects. ASC, adipose-derived stem cells.

**Homing of adipose-derived stem cells to wound site**

ASC migration and attachment to endothelial cells are prerequisites for entering the target tissue and therefore the positive effects of ASC at the site of injury. After tissue injury several cytokines are released. As ASCs express a variety of chemokine receptors including CXCR4, chemottractants such as SDF-1 induce their migration to the site of injury. The exact mechanisms by which ASCs enter the target tissue are not fully understood yet. But leucocyte migration into inflammatory tissue seems to involve adhesion molecules such as P-selectin.

**Recent work in vitro**

The stimulatory effects of ASCs on regeneration processes are thought to be mainly mediated by paracrine mechanisms. Lee et al. treated immortalised human keratinocytes (HaCaT cells) and human dermal fibroblasts with 50% conditioned medium of human ASCs (ASC-CM). Proliferation of HaCaTs and fibroblasts as well as in vitro wound healing of HaCaTs was promoted by ASC-CM. Their results demonstrate an improved keratinocyte and fibroblast function in wound healing via ASCs in a paracrine fashion.

To utilise paracrine effects of ASCs in a low cytotoxic way for wound healing applications, Hassan et al. generated a living dressing system. They encapsulated human ASCs in situ in a water-soluble, thermo responsive hyper-branched polyethylene glycol-based copolymer. Cell viability in this hydrogel was high for up to 7 days under cell culture conditions. While cellular secretion of growth factors such as VEGF and placental-derived growth factor production increased over 7 days, cellular proliferation was inhibited and IL-2 and IFNγ release were unaffected.

In a recent study it has been shown that the bacterial strains *Escherichia coli*, *Staphylococcus aureus*...
and Streptococcus pyogenes have no considerable cytotoxic effects on ASCs. Exposure of ASCs to these bacteria and their components has no negative effect on ASC proliferation. Therefore Fiedler et al.15 presumed, that ASCs can support wound healing even at bacterially infected wound sites.

Recent work in vivo
In an animal-based model it has already been shown that transplanted MSCs can migrate to impaired tissues and activate regeneration processes16. Hong et al.17 showed in a rabbit ear in vivo model that topically delivered rabbit ASCs are engrafted and proliferate in wounds, where they exhibited an activated fibroblast phenotype. Furthermore, ASCs led to increased endothelial cell and macrophage recruitment. In contrast to bone marrow-derived MSCs and dermal fibroblasts they increased granulation tissue formation17. Improved skin regeneration after ASC transplantation in vivo has been shown by Sheng et al.18 correspondingly. The presence of ASCs induced expression of epidermal and VEGF and increased cell proliferation and neovascularisation. The regenerated skin was much thicker compared with controls. The transplanted ASCs were detectable in subcutaneous tissue, vascular vessels and hair follicles for 4 weeks18.

Similar to healthy animal models positive effects of ASCs on wound healing could also be shown for animals with chronic diseases or artificially induced impaired wound healing. As, consequence of ASC transfer an increase in capillary density, collagen intensity, VEGF and TGF-β3 expression was displayed. Animals with autologous ASC transplantation on wounds showed significantly increased survival, angiogenesis and epithelialisation19. ASCs accelerated wound healing of radiation ulcers in a modified rat model, where they were co-localised with endothelial cell markers in ulcerated tissues. The treatment with ASCs initiated smaller wound sizes and was associated with the development of new blood vessels20.

Recent clinical studies
Since the end of 19th century autologous transplantation of fatty tissue was used as filler in plastic surgery. A continuous improvement of methods and purification of material through specific centrifugation protocols increased the predictability and stability of results. Techniques based on the use of stem cell niches from adipose tissue are already important applications in plastic and cosmetic surgery21. Gir et al.22 listed 174 published cases of clinical applications of ASCs in plastic surgery involving soft-tissue augmentation, wound healing and tissue engineering. No adverse effects have been reported in these studies.

Akita et al.23 described the successful use of non-cultured autologous ASCs for treatment of chronic radiation injury. In the excised irradiated skin defect ASCs were applied together with bFGF and an artificial dermis.

Improving aspects for adipose-derived stem cells
Jiang et al.24 demonstrated that a surface carrier of medical-grade silicone coated by plasma polymerisation with a thin layer of acrylic acid allows high-efficiency transfer of ASCs to wounds in an in vivo murine model. ASCs delivered by the carrier significantly accelerated wound healing similar to those delivered by intradermal injection24.

To improve their therapeutic potential, ASCs were genetically modified. Treated cells showed an extended proliferation period, an increased secretion of VEGF, a higher migration potential and after injection into a mouse wound model, a promoted wound healing25. Furthermore, overexpression of the SDF-1 receptor CXCR4 has been shown to improve ASC motility, retention and proliferation19.

Limitations of the use of adipose-derived stem cells
Donor specificity is a well-known phenomenon. Recent studies have investigated ASC function in the context of donor’s age and gender. Guercio et al.26 have shown a higher proliferation capacity of ASCs of younger dogs than older animals. Donor specificity of human ASCs has been shown by Shu et al.27, who observed a relation of donor age and cell differentiation as well as anti-apoptosis ability. Another study with human ASCs showed that, independently from donor's age, equal amounts of ASCs could be isolated. However, infant-derived cells showed different morphology and enhanced angiogenic and osteogenic capabilities28.

So far, there is little evidence on the effects of gender on ASC potential. Fossett and Khan29 summarised that females have a significantly higher yield of MSCs than males and that oestrogens have an excitatory role controlling levels of cytokines and growth factor production29. Moreover, gender of ASC donors influenced the proliferation, differentiation, paracrine and anti-apoptosis abilities of human ASCs27.

Besides age and gender, body mass index, chronic diseases, western lifestyle and many other features cause donor-specific differences. For progenitor cells it has been shown that cells harvested from patients with chronic diseases have a reduced regenerative potential30,31. Whether these individual factors have an influence of the regeneration potential of ASC is still unclear and has to be focused in future studies.

ASCs from streptozotocin-induced type 1 diabetic mice showed a decreased proliferative potential and reduced migration. Additionally, a reduction of stem cell marker-positive cells was shown. The release of the
growth factors HGF, VEGF-A and IGFl-1 was significantly reduced in supernatants of ASCs-derived from diabetic mice12.

Another influencing factor on ASC applicability in chronic wound treatment is the composition of wound fluid, which significantly differs between acute and chronic wounds. In an *in vitro* wound model they have been shown to influence ASCs function inversely. Whereas AWF has a strong chemotactic impact and stimulates ASC proliferation, CWF has an inhibiting effect on ASC migration and proliferation. CWF strongly induces expression of bFGF, VEGF and MMP933.

Further aspects on practicality of ASC application are handling and commercialisation, which are difficult as seen from the industrial side. Moreover, the risk of inducing cancer by transplantation of ASCs is not fully excluded yet11 (Figure 2).

**Future aspects**

Currently, there are no standardised protocols for the clinical application of ASCs and no consensus of the number of cells is required for different treatment options. To ensure the safety and efficacy of ASC application standardised protocols and larger randomised controlled trials are needed22. Furthermore, donor specificity has to be investigated in detail and the underlying reasons of different efficacy have to be elucidated. However, within the scope of personalised medicine, application of autologous ASCs in chronic wound treatment has to be considered individually.

**Conclusion**

MSCs derived from adipose tissue, called ASCs, are a promising alternative to embryonic or bone marrow-derived stem cells in the therapy of chronic wounds. They are less invasive to harvest, migrate to the wound site through paracrine effects and catalyse wound healing via paracrine mechanisms as well as fusion and differentiation, for example, into keratinocytes or fibroblasts.

Because the use of ASCs in wound healing applications is still limited by a lack of clinical data, further studies have to pave the way for their routine clinical application.

**Abbreviations list**

ASC, adipose-derived stem cell; AWF, acute wound fluid; bFGF, basic fibroblast growth factor; CWF, chronic wound fluid; ECM, extra-cellular matrix; HGF, hepatocyte growth factor; IGF, insulin-like growth factor; IL, Interleukin; MMP, matrix metalloproteinase; MSC, mesenchymal stem cells; SDF, stem cell-derived factor; TGF, transforming growth factors; TNF, tumour necrosis factor; VEGF, vascular endothelial growth factor.

**References**


