STEM CELL THERAPIES FOR DIABETIC WOUND HEALING

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ABSTRACT

Despite the major improvements in drug therapy over the past decades, effective treatments for diabetic wounds are still lacking. Adult Stem Cells (ASCs) are multipotent cells with the ability to differentiate into a diverse range of specialised cell types. This evidence provides the rationale for the use of ASCs in diabetic complications such as foot ulcers.

Mesenchymal stem cells (MSCs) are specific ASCs and seem to be promising for diabetic foot treatment and, so far without any major adverse side effects. MSCs promotes acceleration of wound closure by modulating the inflammatory environment, recruiting inflammatory cells into the wounds, promoting neovascularization and regeneration of appendages. The benefit of ASCs therapy in wound healing is unlikelly to be related to the route of administration, and a number of different delivery systems have already been successfully tested.

The aim of this work is to present a systematic review of both pre-clinical and clinical research regarding ASCs use in diabetic ulcers and the future directions of this therapy. It is clear that there is an extensive investigation on this field with some ongoing trials addressing stem cells treatments. However, further evidence from long-term studies is required before the widespread use of MSCs in wound healing.

Keywords: Dermal regeneration; Clinical translation; Wound healing; Diabetic ulcer; Adult stem cells; Mesenchymal stem cells

CURRENT KNOWLEDGE

Adult stem cells are multipotent cells with the ability to renew them and to differentiate into a diverse range of specialised cell types such as fibroblasts, endothelial cells and keratinocytes[1].

Adult stem cells comprise three different groups: the bone marrow stem cells (BM-SC), the circulating pool of stem/progenitor cells (which are also derived from the bone marrow), and the tissue-resident stem cells. BM-SC

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can be categorized into multipotent adult progenitor cells, mesenchymal stem cells (MSCs), and hematopoietic stem cells[1]. The circulating pool of stem/progenitor cells includes different types of cells, among which the most studied for the setting of vascular complications are the endothelial progenitor cells (EPCs).

The past decade has provided new and fascinating *in vitro* and *in vivo* data supporting the use of mesenchymal stem cells (MSC) and endothelial progenitor cells (EPC) for the treatment of diabetic complications. However, one of the issues raised is the possible contribution of these cells for atheroma formation, neointimal hyperplasia, and retinal aberrant angiogenesis, as well as the potential risk of malignant transformation. Therefore, this subject will require further long-term analysis[1].

The worldwide increase in the prevalence of diabetes mellitus (DM) justifies the efforts of the scientific community not only to prevent this disease but also to oppose the development and progression of its well-known complications[3]. One of the investigation focuses on stem cells, where experimental evidence suggests that cell-based therapies might represent a new and promising strategy for the treatment of diabetic vascular complications, namely on mesenchymal stem cells and endothelial progenitor cells. Despite this strategic and promising investment, there are concerns about how the diabetic environment affects these cells and, consequently additional challenges include making these cells resistant to the diabetic environment and thus increasing their clinical efficacy[4].

Several experimental works have shown that DM affects the mobilization and the functions of adult stem cells and therefore they provide the rationale for the use of adult stem cells for diabetic complications such as macro and microvascular complications and wound healing[1].

Wound healing normally results from a combined effort of inflammatory and non-inflammatory cells recruited to the injured site[5]. Recent studies suggest that MSC and EPC represent a significant proportion of the non-inflammatory cells that migrate to the skin. Albierto et al[6] showed that in DM, the number of EPC within the granulation tissue is significantly reduced with respect to non-diabetic controls. Furthermore local increased apoptosis and decreased proliferation of these cells have been reported. The MSC have been associated to acceleration of wound closure by differentiating into fibroblasts and keratinocytes, promoting neovascularization and regeneration of appendages and recruiting inflammatory cells into wounds[7,8].

Diabetic foot ulcers (DFU) are a significant and rapidly growing complication of diabetes. Stem cells are a promising treatment for DFUs as they are capable of targeting, as well as bypassing the underlying abnormal healing mechanisms and deranged cell signalling in diabetic wounds, thereby promoting healing[9].

THE THERAPEUTIC EFFECT OF DIFFERENT TYPES OF STEM CELLS (PRE-CLINICAL RESULTS)

EPCs are able to form new blood vessels and promote neovascularisation after ischemia. Using a model of

diabetic mice with hind limbs ischemia-reperfusion (I/R) injury the investigators observed that diabetic rats were completely unable to mobilize EPC after I/R injury, compared to the controls. After insulin administration and premedication with granulocyte-colony stimulating factor (G-CSF) and other stem cells factors, they achieved a partial recovery in post-ischemic EPC mobilization[10]. This study suggests that mobilization mechanism is sensitive to chronic hyperglycaemia and early on remains reversible. Despite the impaired mobilization of adult stem cells, diabetic patients also present dysfunctional circulating progenitor cells. A growing body of evidence demonstrates that DM is associated with a generalized reduction in circulating EPC and this decline is linearly correlated with the severity of DM, in terms of HbA1c and blood glucose, whereas it is inversely related to blood glucose control.

Several works have shown that MSCs can promote wound healing by modulating the inflammatory environment, promoting the formation of a well vascularized granulation matrix, encouraging the migration of keratinocytes and inhibiting apoptosis of wound healing cells[11]. These trophic effects appear to be useful in diabetic ulcers, helping the difficult regeneration of tissue in this specific group of patients.

In vivo, MSCs seem to enhance the regenerative potential of multiple tissues types as a result of various trophic mechanisms that become activated when exposed to the biochemical factors that are characteristic of an injury environment. The inflammatory mediators interferon-γ (IFNγ) and tumour necrosis factor-α (TNFα) can regulate homing and migration of MSCs through the extracellular matrix (ECM), and MSCs have demonstrated chemotaxis toward a variety of wound healing cytokines in vitro[11].

Once they are localized to the wound bed, MSCs can enhance dermal regeneration during all phases of the wound healing process. In the inflammation phase, proinflammatory mediators, such as IFNγ,TNFα, and interleukin-1β (IL1β), can activate regulatory functions in MSCs. Therefore, MSCs can attenuate the acute immune response to injury by inhibiting the recruitment, proliferation, and biological activity of mast cells, T cells, B cells, and natural killer cells. MSCs continue to support tissue regeneration during the proliferation phase contributing to generation of a high-quality, well-vascularized granulation tissue, enhances re-epithelialization of the wound, and attenuates the formation of fibrotic scar tissue[11].

Angiogenesis is necessary in this phase and MSCs express several factors, including basic fibroblast growth factor (bFGF), vascular endothelial growth factor-A (VEGF-A), and adrenomedullin, which promotes the proliferation of microvascular endothelial cells, vascular stability, and the development of a long-lasting functional vascular network [12]. MSCs also secrete a variety of cytokines and growth factors with antifibrotic properties, including hepatic growth factor (HGF), IL10, adrenomedullin, and MMP-9, which promotes turnover of the ECM, keratinocyte proliferation, and inhibition of myofibroblast differentiation.

In addition to their differentiation potential, MSCs exhibit substantial

trophic support to regenerating tissues. As described in the previous section, MSCs appear to be an attractive cell type for a cell-based therapy to promote dermal regeneration.

Chen et al[13] has demonstrated that the cytokines and growth factors secreted by murine MSCs are sufficient for improved wound healing by applying concentrated MSC-conditioned medium directly to a full-thickness excisional wound in mice via injection and topical administration. The conditioned medium encouraged the migration of macrophages and endothelial cells into the wound space and promoted neovascularization in the regenerating tissues, thereby accelerating the rate of wound closure. In a similar study, murine MSCs were applied to excisional wounds in mice, and syngeneic and allogeneic cells demonstrated an equivalent ability to migrate through the tissue and to attenuate the local inflammatory response, as compared with the control allo-fibroblasts, which were restricted to the sites of injection[14]. In a rabbit model[15] human bone marrow-derived MSCs were injected into an incisional wound, and the cellular therapy resulted in improved wound closure with better wound tensile strength and with a significant reduction in the appearance of a scar.

The trophic functions of MSCs in wound healing environment have been further elucidated using diabetic animal models to evaluate cells behaviour in the context of impaired cellular metabolism and its consequences on microvascular function[16]. Using the db/db diabetic mouse model of impaired wound healing, murine bone marrow-derived MSCs were applied directly to a full-thickness excisional wound, promoting the formation of a well-vascularized granulation tissue, more rapid re-epithelialization, and better gap closure, thereby preventing the development of a chronic non-healing wound.

Adipose-derived stem cells (ASCs) hold great promise for wound healing, because they are multipotential stem cells capable of differentiation into various cell lineages and secretion of angiogenic growth factors. Nie et al[17] demonstrated that ASCs secret angiogenic cytokines in vitro and in vivo, including VEGF, HGF, and FGF2, thereby increasing neovascularization and accelerating the time lag for wound closure. In this study, there was also evidence that the ASCs differentiated directly into endothelial and epithelial cell types and were integrated directly into the regenerated tissue.

In another study, the authors have shown that ASCs accelerated wound healing rate in diabetic rats, but did not increase length and volume density of the vessels and volume density of the collagen fibers[18], decreasing the numerical density of fibroblasts. Consequently the investigators concluded that ASCs enhance diabetic wound healing rate probably by other mechanisms rather than enhancing angiogenesis or accumulating collagen fibers.

A number of delivery systems have been tested demonstrating the versatility of the treatment with stem cells to fulfil the clinical needs for dermal regeneration. For example, human bone marrow-derived MSCs have been incorporated into a fibrin spray and used as a regenerative dressing for full-thickness wounds[19]. This biologic wound dressing was

sufficient to stimulate complete closure of full-thickness excisional wounds in diabetic mice, whereas controls remained in a chronic, non-healing state. MSCs can be administered systemically during dermal wound healing, and they will home to the location of injury to impart their regenerative effects. In a bleomycin model of injury to lung epithelial tissues in mice, it has been established that MSCs will home to the site of inflammation, attenuate the acute inflammatory response, and decrease ECM deposition, thereby minimizing the extent of pulmonary fibrosis[20]. These findings have been extended to dermal wound healing as systemically injected murine MSCs engrafted at the site of an excisional wound model in mice and appeared to transdifferentiate into keratinocytes, endothelial cells, and pericytes and to accelerate the rate of wound healing.

Finally, in a study of Huang et al[21] the authors explore the role of MSCs on repairing skin appendages in renewal tissues, to assess whether engrafted bone-marrow-derived mesenchymal stem cells via a delivery system can participate in cutaneous wound healing and sweat-gland repair in mice. Epidermal growth factor (EGF) microspheres were used to support MSCs and, after 3 weeks, MSC-engineered skin (EGF loaded) treated wounds exhibited accelerated healing with increased re-epithelialization rates and less skin contraction, suggesting that MSCs delivered by this EGF microspheres-based engineered skin model may be a promising strategy to repair sweat glands and improve cutaneous wound healing after injury.

SUMMARY OF THE CLINICAL TRIALS

One clinical trial has shown improvements of microcirculation and complete wound healing both among patients transplanted with bone marrow mononuclear cells and with expanded bone marrow cells enriched in CD90+ cells. Diabetic patients with diabetic foot related to chronic limb ischemia and without the option for surgical or interventional revascularization were recruited and randomized to the transplant groups or the control group. The intervention group was divided into 1) bone marrow cells administered intramuscular or 2) intra-arterial or 3) expanded bone marrow cells administered intramuscular or 4) intra-arterial. Patients were evaluated for ankle brachial index (ABI), transcutaneous oxygen partial pressure (TcPO2), and reactive hyperaemia (Blood Oxygen Level Dependent [BOLD]). Patients also underwent imaging with angiographic methods. Furthermore, this study proved that transplantation of these cells is safe and feasible[22].

Jain and colleagues have performed a prospective, randomized, clinical study comparing the rate of healing of chronic lower limb wounds in patients with diabetes mellitus whose wounds were treated with topical application of BM-SC to whole blood (control). Forty-eight patients were enrolled in this study, 25 were randomized to study treatment and 23 to control treatment. At 2 weeks, the average decrease in wound area was 17.4% (39.6-43.4 cm2) in the treatment group compared to 4.84% (41.6-42.8 cm2) in the control group and at the 12th week, the average de-

crease in wound area was 36.4% (SD 0.48) in the treatment group compared to 27.32% (SD 0.32) in the control group[23].

At the present there is an ongoing trial addressing whether the implantation of BM-SC aspirate concentrate can be clinically effective in treating critical limb threatening ischemia so that the number of amputations can be reduced. It is a multicentre randomized double-blind study comparing the efficiency of concentrated BM-SC injected into the critically ischemic limb to a placebo procedure where only saline is injected.

There are three further clinical trials registered at the site clinicaltrials.gov pertaining to the treatment of cutaneous wounds with stem cells in diabetic patients: 1) Endogenous Progenitors Cell Therapy for Diabetic Foot Ulcers; 2) Umbilical Cord Mesenchymal Stem Cells Injection for Diabetic Foot; 3) Safety Study of Stem Cells Treatment in Diabetic Foot Ulcers. However, none of these trials has already started recruiting patients.

FUTURE DIRECTIONS

Several important conceptual and technical advances have converged to allow us to consider the possibility of using MSCs as starting material for tissue repair protocols.

Stem cell therapy represents a fascinating new approach for the management of wound healing, and there is a significant interest in the clinical translation of mesenchymal stem cells-based therapy to promote dermal regeneration.

Recent preclinical and clinical research[24-26] has shown some exciting results in the absence of any major adverse side effects, however, many unresolved questions about experimental and clinical issues are still open for future research, especially many basic problems concerning, among others, the method of delivering the cells. Although systemic delivery is a good option, the engraftment efficiency can be difficult to evaluate and predict without rigorous methods to ensure that the cells will home to the lesion site. Skin wounds are excellent candidates for another delivery method: the direct application of therapeutic cells to site of injury[27]. This delivery method for mesenchymal stem cells may require an appropriate carrier to ensure that the cells conserve their potential benefits and can efficiently migrate into the wound bed. Additional research is needed in order to develop strategies that ensure that these cells reach wound sites in sufficient numbers to maximize their therapeutic benefits.

Further studies are required to clarify the interactions between these therapeutic cells and the heterogeneous immune population present in the wound site. It is clear that much more work is needed and evidence from long-term studies is required before the widespread use of MSCs as a wound healing therapy. What is of utmost importance is that the strong partnerships that have come about between scientists and clinicians are nurtured and encouraged so that stem cell therapy for wound healing will continue to set the example of how to deliver true translational science.

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