TOPICAL REVIEW

Does cell therapy and tissue engineering represent a promising treatment of diabetic foot ulcers?

Ulicna M, Danisovic L, Vojtassak J

Institute of Medical Biology, Genetics and Clinical Genetics, Faculty of Medicine, Comenius University, Bratislava, Slovakia. marcela.ulicna@fmed.uniba.sk

Abstract: Diabetes mellitus is one of the most severe and costly chronic disease of our time. Approximately 2–3 % of diabetic patients have an active foot ulcer, and 15 % of all patients with diabetes will develop an ulcer during their lifetime. Treatment of foot complications is one of the main items in the absorption of enormous economic and health resources addressed to the diabetic patients. Advances in basic science, tissue culture techniques and cell therapy promise to improve the treatment of diabetes as well as its complications, i.e. also the ischemic ulcers of the foot. At present, the isolation of any specific type of cells, their in vitro expansion and biological characterization of acquired cell population are possible. For the healing process in ischemic diabetic ulcers, stem cells, endothelial progenitor cells and fibroblasts, both in suspension or placed on an extracellular scaffold are used. This process is focused on stimulating the new blood vessels formation. This is stimulated by the paracrine secretion of multiple growth factors and their receptors. Verified are the vascular endothelial growth factor and its receptor, fibroblast growth factor, interleukin-8 and proangiogenic cytokines (Ref. 62).

Key words: diabetic foot ulcer, cell therapy, tissue engineering, clinical trials.

As the incidence of diabetes mellitus is increasing it is deemed to represent one of the major health problems in the 21st century. Indeed, the total number of diabetic patients has been projected to increase from 171 million in 2000 (prevalence 2.8 %) to 366 million in 2030 (prevalence 4.4 %) (1). The incidence of diabetes mellitus is increasing globally, and the complications related to this endocrine disorder are also mounting. The annual incidence of foot ulcers among people with diabetes has been variously estimated to be between 1 % and 4.1 %, and the annual incidence of amputation is in range of 0.21–1.37 % (2). Diabetes-induced limb amputations are associated with an increased risk of additional amputations and result in a 5-year mortality rate in range of 39 to 69 % (3, 4).

A chronic wound is any interruption in the continuity of the skin and integrity of the tissue that requires a prolonged time (>8 weeks) to heal, does not heal, or recurs (5). The most prevalent forms of chronic wounds are leg ulcers caused by vascular insufficiency (6, 7), and foot ulcerations associated with diabetic complications (8, 9).

Critical limb ischemia has been defined as chronic ischemic rest pain, ulcer or gangrene attributable to objectively proven arterial occlusive disease (10). Risk factors of critical limb ischemia include diabetes, hypercholesterolemia, hypertension, smoking and genetic predisposition (11). Diabetic foot disease is a major health problem involving 15 % of the 200 million patients with diabetes worldwide. The predisposing factors include abnormal plantar pressure points, foot deformities, and minor trauma (6). The prognosis is poor after the clinical manifestation appears; less than 50 % of patients will avoid death or major amputation in 1 year. Mortality is the major problem. After 1 year, it represents 25 %, after 2 years 31.6 % and after 3 years 60 %. Amputation is also associated with high perioperative mortality, 5–10 % of cases with below-the-knee amputation and 15–20 % of cases with above-the-knee amputation (11). More than 60 % of non-traumatic amputations in the western world are performed in the population of diabetics. Major amputations increase morbidity and mortality and reduce the patient’s quality of life. It is clear that effective treatment can reduce the number of major amputations (12).

Standard principles for diabetic foot ulcer treatment include debridement, off-loading, dressing, control of infections, revascularization and amputation.

Pathophysiology of diabetic foot ulcer

The pathophysiology of diabetic foot ulcers and delayed healing has been well described. Mustoe et al propose a unifying hypothesis of chronic wound pathogenesis based on four main causative factors: local tissue hypoxia, bacterial colonization of the wound, repetitive ischemia-reperfusion injury, and an altered cellular and systemic stress response in the aged patient (13). Contributing factors include progressive development of sensory,
vasomotor, and autonomic neuropathy leading to loss of protective sensation; joint and bone deformities that increase plantar foot pressure; and alterations in autoregulation of dermal blood flow. Diabetic patients show an earlier development and progression of lower extremity peripheral arterial occlusive disease (PAD) with a predilection for blockages at the trifurcation level of vessels just distal to the knee (14).

In diabetes, myriad of factors, including intrinsic factors (neuropathy, vascular problems, and other complicating systemic effects due to diabetes) and extrinsic factors (wound infection, callus formation, and excessive pressure to the site) can impair the wound healing. It is well known that peripheral vascular disease (macroangiopathy and microangiopathy), along with diabetic neuropathy, plays a major role in diabetic foot ulceration. About 20% of diabetic lower extremity ulcers have arterial low insufficiency as their primary aetiology, 50% will have primary diabetic neuropathy, and 30% will have both conditions (15). In addition, the impairment of host responses to infection and other cellular dysfunctions contributes to the refractory nature of diabetic wounds.

**Neo (re) vascularization – the crucial problem**

In recent studies, the main accent is given to starting the healing process in diabetic ulcers and wound closure by stimulating the new blood vessels formation. Neo (re) vascularisation occurs by two processes, angiogenesis and vasculogenesis (16). Angiogenesis occurs when endothelial cells sprout from pre-existing blood vessels and then migrate and proliferate to form a cord-like structure (17, 18). Vasculogenesis is the de novo formation of immature cords from the differentiated progenitor cells. For the initiation of these processes, fibroblasts, embryonic and adult stem cells (alone or placed on an extracellular matrix as a graft) are used.

**Fibroblasts**

Fibroblasts are cells ubiquitous in connective tissue that makes and secretes collagen. Fibroblasts are normally attracted into the wound environment by chemotactic factors released by platelets and macrophages following tissue injury. The fibroblasts migrate into the wound bed, proliferate and deposit matrix components (19). Dermal fibroblasts then synthesize an array of cytokines, interleukins and angiogenic factors. However, the crucial problem in the pathogenesis of chronic ulcers is the lack of response to growth factors or receptors. Fibroblasts cultured from chronic venous ulcers do not respond to stimulatory activity of TGF-β1 because there is a reduced level of TGF-β1 type II receptor. In addition, EGF, FGF-2, PDGF and TGF-β levels are lower in chronic wounds than in acute wounds (20, 21). Even the study of fluid from acute wounds demonstrates a proliferative effect on fibroblasts, keratinocytes and endothelial cells (22). However, the fluid of chronic wounds has increased levels of some harmful matrix metalloproteinases that brake down the ECM proteins and growth factors, and block the cellular proliferation and angiogenesis (23). Even if growth factors are available, they may not reach the intended cells. The leakage of macromolecules, such as fibrinogen, α1 macroglobulin, and albumin leads to the binding of growth factors, making them unavailable for wound healing (24). The unresponsiveness to growth factors in chronic wounds may translate into diminished fibroblast activity, poor cell migration, insufficient angiogenesis, and shortage of cellular activities that are useful in wound healing (25).

To bypass these problems, new materials have been prepared with the help of tissue engineering and commercially produced as grafts for advanced diabetic ulcers treatment, namely membranes colonized by allogeneic cells, fibroblasts and/or keratinocytes.

**Dermagraft – Human fibroblast-derived dermis**

Dermagraft (Advanced Tissue Science/Smith & Nephew) is a tissue-engineered dermal replacement. Human newborn dermal fibroblasts are cultured in three dimensions, using a specially designed bioreactor, on knitted lactate/glycolate copolymer scaffolds with periodic changes in growth medium. During culturing, the fibroblasts secrete a three-dimensional extracellular matrix comprised largely of collagen but also proteoglycans and other proteins. Histologically, the three-dimensional cultures contain fine collagen bundles with interspersed cells resembling the papillary dermis. Its single major component is collagen type I comprising about 70% of protein and 10% of wet weight, although collagens III and V can be detected immunohistochemically. The fibroblasts deposit several other major extracellular matrix components including fibronectin, tenasin and proteoglycan decorin. Following their implantation into a wound these three-dimensional fibroblast cultures are capable of supplying the wound bed with live fibroblasts that secrete a variety of growth factors and cytokines that are capable of controlling the cell proliferation, inducing the angiogenesis and modifying the inflammatory processes (26, 27).

**Graftskin – Apligraf; Human skin equivalent (HSE)**

Graftskin (Apligraf; Organogenesis, Canton, MA, and Novartis Pharmaceuticals, East Hanover, NJ) is an allogeneic bi-layered cultured skin equivalent that is currently available in the U.S. for the treatment of venous ulcers (28, 29). Apligraf is a composite graft composed of a cultured living dermis and sequentially cultured epidermis, and is derived from neonatal foreskin. The manufacturing process involves cell bank production of fibroblasts mixed with type I of bovine collagen to produce a cast matrix. Cell bank keratinocytes are added approximately 1 week later to form the epidermal layer. The dermal fibroblasts and keratinocytes are separated from normally discarded infant human foreskin. Apligraf consists of four components: extracellular matrix, viable allogeneic dermal fibroblasts, epidermal keratinocytes and stratum corneum. The extracellular matrix consists of type I bovine collagen organized into fibrils and fibroblast-produced proteins (30). Unlike human skin, Graftskin does
not contain structures such as blood vessels, hair follicles, or sweat glands or other cell types such as melanocytes, macrophages, or lymphocytes (29). Although HSE is FDA-approved for treatment of diabetic foot and venous stasis ulcers, its clinical efficacy remains limited because the molecular mechanisms underlying its therapeutic effect are not fully understood. HSE cellular components were determined to express 15 different growth factors/cytokine genes known to promote wound healing. Brem et al hypothesized that bi-layered HSE generates its effect by means of local synthesis and release of multiple growth factors in specific combination and concentration that improve the impaired reparative process in chronic wounds (31).

Clinical trials with Graftskin

In their randomized prospective trial, Veves et al assessed the effectiveness of Graftskin, in the treatment of noninfected, nonischemic chronic plantar diabetic foot ulcers. In 24 centres in the U.S., 208 patients were randomly assigned to ulcer treatment with either Graftskin or saline-moistened gauze (control group). At the 12-week follow-up visit, 56% of Graftskin-treated patients achieved complete wound healing compared with 38% in the control group. Application of Graftskin results in a higher healing rate when compared with state-of-the-art currently available treatment and is not associated with any significant side effects (32).

Also Edmonds et al. suggested in a similar study that the use of Graftskin resulted in wound closure by 12 weeks (33).

Stem cells

Stem cells (SCs) are characterized as undifferentiated cells that have been derived from embryonic, foetal and adult organisms (34–36). SCs have a potential of self-renewing and differentiating to mature cell types, as well as long-term in vivo repopulation potential. Stem cells can be isolated from a large number of tissues including bone marrow, peripheral blood, adipose tissue, umbilical cord blood, periosteum, synovial membrane, muscle, dermis, dental pulp, pericytes, trabecular bone, pancreas, and articular cartilage (37).

Bone marrow stem cells

Bone marrow with peripheral blood is one of the best sources of stem cells. Bone marrow contains multipotent stem cells (MSC), which after entering the microenvironment of a specific tissue (niche) can differentiate into both, haematopoietic and nonhematopoietic cells, including endothelial cells, osteoblasts, neural cells, fibroblasts and keratinocytes (38). MSC are also able to induce angiogenesis, both in vivo (39) and in vitro (40). Kinnaird et al. demonstrated that MSCs are secreting also a large number of arteriogenic and angiogenic cytokines contributing to collateral remodelling in ischemic limb via paracrine mechanisms (41). They include vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), Angiopoetin-1 (Ang-1), matrix metalloproteinase (MMPs) and transforming growth factor β (TGF-β). According to newly obtained results, the release of angiogenic factors rather than endothelial transdifferentiation is accountable for MSCs-mediated strengthened angiogenesis (41).

Endothelial progenitor cells (EPCs)

EPCs are responsible for postnatal vasculogenesis in physiological and pathological neovascularization by secreting angiogenic growth factors in a paracrine manner (14, 42). EPCs are immature cells, which have the capacity to proliferate, migrate, and differentiate into endothelial lineage cells but have not yet acquired the characteristics of mature endothelial cells. EPCs and embryonic hematopoietic stem cells (HSCs) are derived from a common precursor (hemangioblast) and share many surface marker antigens such as platelet endothelial cell-adhesion molecule-1 (PECAM-1/CD31), vascular endothelial growth factor receptor 2 (VEGFR2, also known as KDR or Flk1), von Willebrand factor (vWF), vascular endothelial cadherin (VE-cadherin), Tie-2, c-Kit, Sca-1, AC133, and CD34 (43–48). Actually, there is no simple definition of EPCs because no marker expression is strictly specific. The term EPC therefore encompasses a group of cells that exist in a variety of stages ranging from hemangioblasts to fully differentiated ECs (46). Upon the arrival at the target tissue, EPCs enter the tissue. Some differentiate into mature ECs and other act as a source of proangiogenic cytokines (49). Ischemia and tissue injury are potent stimuli for neovascularization. It has been estimated that EPCs contribute up to 25% of endothelial cells in newly formed vessels in animal models (50). Since then, based on increasing evidence it has been suggested that BM-derived EPCs can functionally contribute to neovascularisation and wound healing, limb ischemia (51), post myocardial infarction (52), endothelialisation of vascular grafts (53), atherosclerosis (54), retinal and lymphoid organ neovascularisation (55), vascularisation during neonatal growth (56), and tumour growth (57).

Clinical trials

A novel marker for human hematopoietic stem and progenitor cells is represented by CD133+ cells (58). The healing potential of human foetal aorta-derived CD133+ stem cells in a new mouse model of ischemic diabetic ulcer was tested (59). Wound was covered with 2x104 of collagen containing CD133+ foetal cells. These cells expressed high levels of wingless (Wnt) genes, which had been down-regulated following the differentiation into CD133+ cells along with up-regulation of Wnt antagonists. The CD133+ cells accelerated wound closure and promoted reparative angiogenesis through stimulation of endothelial cell proliferation, migration, and survival by paracrine effects. The CD133+ cells secreted high levels of vascular endothelial growth factor (VEGF)-A and interleukin (IL)-8. Obtained results are very promising.

Gu et al compared the effectiveness of autologous implantaion between bone marrow stem cells and peripheral blood stem
cells for the treatment of lower limb ischemia. A series of subjective indexes (such as improvement in pain and cold sensation and numbness) and objective indexes such as increase in ankle brachial index (ABI), transcutaneous oxygen pressure (TcpO₂), angiography, amputation rate, and improvement in foot-wound healing were used to evaluate the effect. They conclude that bone marrow stem cell graft and peripheral blood stem cell graft are all effective in treating lower limb ischemia (60).

In their trial, Procházka et al monitored 37 patients who failed to respond to previous therapeutic strategies in the treatment of ulcerated limbs (e.g. surgical revascularization and endovascular repair) and underwent local transplantation of autologous bone marrow stem cells (BMSCs). The efficacy of this therapy was assessed by using several endpoints such as prevention of amputation, wound healing and the degree of angiogenesis. In order to assess the limb ischemia and hypoxia, several tests and measurements were performed prior to and after the transplantation. The measurements included toe pressure, skin perfusion pressure, ABI, laser doppler baseline and heat perfusion assessment, TcpO₂ without and with O₂ provocation inhalation test. All parameters adjusted and confirmed a very good tissue vasoreactivity after BMSCs transplantation (61).

The combination of autologous BMSC in suspension and dermal graft composed of autologous fibroblasts on collagen membrane (Coladerm) in combination with autologous mesenchymal stem cells (MSC) derived from the patient’s bone marrow represents a new technique for the treatment of chronic non-healing wound (diabetic ulcer) (62). The patient’s bone marrow aspirate was applied directly to the wound and injected into the edges of the wound and finally covered with the prepared autologous biograft. The next two applications of cultured MSC were carried out on days 7 and 17. After 29 days of combined treatment, the wound showed an overall decrease in wound size, an increase in vascularity of the dermis and dermal thickness of the wound bed.

Conclusion

During the past few years, a significant increase in the incidence of diabetes and diabetic foot ulcers has been observed in developed countries. In effect of their high frequency, an enormous scientific activity has been developed to recognize the physiopathological molecular pathways in diabetic ischemic ulcer. The processes and main factors involved in the new vessels formation were identified. The development of bioengineered skins together with a proangiogenic effect of fibroblasts and stem cells is likely to represent an advanced approach in treating the foot ulcers.

References


41. Kinnaird T, Stabile E, Burnett MS, Lee CW, Barrs S, Fuchs S, Epstein SE. Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote in vitro and in vivo arteriogenesis through paracrine mechanisms. Circul Res 2004; 94: 678—685.


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