Physiology and Pathophysiology of Wound Healing in Diabetes

7

Olivera Stojadinovic, Irena Pastar, Katherine A. Gordon, and Marjana Tomic-Canic

Abstract

Wound healing is an evolutionary conserved process that aims to restore the damaged barrier. This complex process involves many cellular responses including inflammation, proliferation, migration, angiogenesis, and tissue remodeling. Immediately after the injury, blood components are released into the wound site, activating the clotting cascade. The resulting clot induces hemostasis and provides a matrix for the influx of inflammatory cells. Inflammation is characterized by leukocyte migration and arrival to the site of injury. Neutrophils arrive first to remove contaminating bacteria (Singer and Clark, N Engl J Med 341(10):738–746, 1999) and are followed by monocytes, which differentiate into macrophages. Macrophages play an important role in augmenting the inflammatory response and tissue debridement. At the same time, many different cell types respond to initial inflammatory signals and start migrating to the wound site, including keratinocytes, endothelial cells, and circulating and local progenitor cells.

Keywords

Clotting cascade • Hemostasis • Cellular responses • Keratinocytes • Fibroblasts • Endothelial cells • Neutrophils • Macrophages • Stem and progenitor cells • Angiogenesis • Wound healing

Physiology of Wound Healing

Wound healing is an evolutionary conserved process that aims to restore the damaged barrier. This complex process involves many cellular responses including inflammation, proliferation, migration, angiogenesis, and tissue remodeling. Immediately after the injury, blood components

O. Stojadinovic • I. Pastar • K.A. Gordon • M. Tomic-Canic (✉)
Wound Healing and Regenerative Medicine Research Program, Department of Dermatology, University of Miami Miller School of Medicine, 1600 NW 10th Ave, RMSB 2023-A, Miami, FL 33136, USA
e-mail: MTCanic@med.miami.edu

A. Veves et al. (eds.), *The Diabetic Foot: Medical and Surgical Management*, Contemporary Diabetes,
DOI 10.1007/978-1-61779-791-0_7, © Springer Science+Business Media, LLC 2012

are released into the wound site, activating the clotting cascade. The resulting clot induces hemostasis and provides a matrix for the influx of inflammatory cells. Inflammation is characterized by leukocyte migration and arrival to the site of injury. Neutrophils arrive the first to remove contaminating bacteria [1] and are followed by monocytes, which differentiate into macrophages. Macrophages play an important role in augmenting the inflammatory response and tissue debridement. At the same time, many different cell types respond to initial inflammatory signals and start migrating to the wound site, including keratinocytes, endothelial cells (ECs), and circulating and local progenitor cells. Once they arrive they start to proliferate. Proliferation is characterized by re-epithelialization, neovascularization, and granulation tissue formation. Granulation tissue formation begins during the inflammation phase, forming a "beefy red" and highly vascular region of the healing tissue, predominantly relying on neovascularization [1]. During this phase, the immature fibrin matrix and granulation tissue are replaced by collagen and scar. Wound healing as a process does not end by wound closure, although this is the visible sign of complete healing. Upon closure, tissue is continuing with collagen deposition and cross-linking. During this remodeling phase, balance is established between collagen synthesis and destruction, whereby the scar gains its tensile strength [2]. Wound healing in adults results with a scar formation, fibrosis, and contracture. However, fetal skin, up to midway to the last trimester, heals without scar formation, using a regenerative pathway [3].

Cellular responses to injury involve direct cell–cell and cell–matrix interactions, as well as the indirect cross talk between different cell populations by soluble mediators. Thus, wound healing is orchestrated through the integration of multiple signals (growth factors, cytokines, and chemokines) released by participating cells: keratinocytes, fibroblasts, endothelial cells, macrophages, and platelets. The appropriate balance of these signaling factors as well as their spatio-temporal control is essential for successful wound healing [4–6]. Below we discuss in more detail functions of various contributing cells: keratinocytes, fibroblasts, endothelial cells, neutrophils, macrophages, and progenitor cells.

Cellular Components of Wound Healing

Keratinocytes

Keratinocytes play several critical roles in the wound healing process and are among the most important cells that respond to injury and accelerate healing. Under normal conditions, keratinocytes main role is barrier formation of the skin. During wound healing, keratinocytes play many important roles, including the release of cytokines and growth factors, which recruit other cell types and stimulate matrix formation and angiogenesis, respectively. Keratinocytes also migrate and proliferate within the wound bed to accelerate closure in a timely fashion.

In healthy skin, keratinocytes proliferate in the basal cell layer and differentiate in the suprabasal layers. Basal keratinocytes are mitotically active and help form the basement membrane by advancing cross talk with dermal fibroblasts, melanocytes, and Langerhans cells. When keratinocytes leave the basal cell layer, they change phenotypically and begin the process of differentiation. During this process, keratinocytes stop dividing, change their keratin production from K5/K14 to K1/K10, and begin producing a number of insoluble proteins. Terminal differentiation results in loss of nuclei and protein cross-linking, giving rise to a cornified layer and forming an epidermal barrier [7, 8]. This perpetual process of keratinocyte differentiation governs the maintenance of barrier.

Keratinocytes are responsible for barrier maintenance and they are equipped for rapid response to its damage. When the epidermal barrier is disrupted upon skin injury, keratinocytes release prestored interleukin-1 (IL-1), alerting surrounding cells to barrier damage [9, 10]. The signals released by keratinocytes act in both auto- and paracrine manners. This process, termed the "keratinocyte activation cycle," is characterized by changes in cellular behavior (migration and proliferation), induced secretion of a multitude of growth factors and cytokines and expression of

K6, K16, and K17 keratin proteins, which are often considered one of the first markers of epidermal healing [11, 12].

To close the gap in the epidermal barrier, keratinocytes at the wound edge first loosen their adhesion to each other and to the basal lamina. In addition, keratinocytes obtain the flexibility and ability to migrate over the extracellular matrix (ECM) deposited by dermal fibroblasts. This process requires rearrangement of integrin receptors and reassembly of the associated actin cytoskeleton and the keratin filament network [8]. Further, epidermal growth factor (EGF), keratinocyte growth factor (KGF), transforming growth factor alpha (TGF-α), fibroblast growth factor (FGF), IL-1, and Interleukin-6 (IL-6) have been shown to be among the important regulators of keratinocyte proliferation, migration, and re-epithelialization and communication with other cell types [5, 9].

Upon the advancement of the migrating epithelial tongue and first layer covering the wound, keratinocytes also start to proliferate to ensure an adequate supply of cells to encase the wound. Once the wound is healed, defined as being fully epithelialized with no drainage, and covered by a keratinocyte monolayer, the proliferation signals cease and a new stratification process begins. Thus, keratinocytes become "deactivated" and revert to their previous normal differentiation patterns.

Fibroblasts

Complex interactions and cross talk between fibroblasts, keratinocytes, and other cell types participating in wound healing is critical for successful wound closure. Fibroblasts play a vital role in wound healing as they migrate, proliferate, and supply an ECM during tissue repair. Under normal conditions, fibroblasts synthesize collagen and ECM, maintaining the structural integrity of the skin. Much like keratinocytes, fibroblasts' various roles are tightly regulated by cytokine and growth factor signaling over the course of wound healing. One of the many important roles of fibroblasts is to provide contractile properties to the wound as myofibroblasts. As an early response to wounding, dermal fibroblasts at the site of injury begin to proliferate. A few days upon wounding, fibroblasts begin migration into the provisional matrix of the wound clot to lay down their own collagen-rich matrix [13]. This ECM acts as a "scaffold" during tissue repair, providing structural support and attachment sites for cell surface receptors and it also works as a regulated "reservoir" for signaling molecules that modulate diverse processes such as angiogenesis, cell proliferation and migration, and inflammation [14]. In order to migrate into the clot, dermal fibroblasts must downregulate their collagen receptors and upregulate integrins that bind ECM proteins such as fibrin, fibronectin, and vitronectin [15]. During their migration, fibroblasts sense signals coming from both their matrix environment and from the growth factor milieu that surrounds them.

About 1 week after wounding, the wound clot will be fully invaded by activated fibroblasts. These fibroblasts are stimulated by TGF-β1 and other growth factors to synthesize and remodel a new collagen-rich matrix [13]. At the same time, a proportion of the wound fibroblasts transforms into myofibroblasts, which express α-smooth muscle actin and resemble smooth muscle cells in their capacity for generating strong contractile forces [16].

Conversion from fibroblasts to myofibroblasts is triggered not only by growth factors such as TGF-β1 [16] but also by mechanical tension [17, 18]. The appearance of myofibroblasts coincides with a strong induction of contractile properties so that cells align parallel to mechanical tension that is building up in the granulation tissue. The various tensile forces acting on and exerted by wound fibroblasts before, during, and after contraction have been studied in collagen-gel models. A number of growth factors at the wound site are potent stimulators of fibroblast-driven gel contraction and presumably signal granulation tissue contraction in vivo [19]. Platelet-derived growth factor (PDGF)-AA and -BB isoforms and TGF-β1 led to efficient collagen-gel contraction [19–21]. IL-1α was shown to cause degradation of the collagen gels at later time points, most likely due to enhanced matrix metalloproteinase (MMP) activity [22].

Contraction stop signals are also being analyzed by releasing mechanically stressed anchored gels from their substrate attachments to simulate the loss of resistance after a wound has closed. Within minutes of release from resisting forces, PDGF and EGF receptors on the cell surface become deactivated [23] and the relaxed cells return to a quiescent state similar to that existing before the injury. Programmed cell death also occurs in the granulation tissue fibroblasts, triggered by TGF-β1 and FGF at the injury site, after wound contraction has ceased [24, 25].

Given the importance of fibroblasts and keratinocytes in proper wound healing, human skin substitutes have been developed as a wound treatment modality. Currently, a living skin equivalent, composed of living fibroblasts and keratinocytes in a native collagen matrix, is the only FDA-approved skin substitute [26]. Please see section on "Treatment for DFUs" for additional information.

Endothelial Cells

Additional responders to wound healing signals released by fibroblasts and keratinocytes are local endothelial cells. ECs are normally positioned within the vascular lumen and form the tubular structure of blood vessels. ECs act as a barrier between intraluminal blood and extravascular tissue. During angiogenesis, growth factors, cytokines, and cell–cell and cell–matrix interactions activate ECs. Activated ECs, platelets, macrophages, and fibroblasts release proangiogenic cytokines, leading to the invasion and migration of ECs into the ECM, EC proliferation, and new immature vascular formation [27].

Before ECs can begin angiogenesis, they must disrupt their interactions with neighboring ECs, digest the basement membrane and components of the ECM [27, 28]. Proteolytic enzymes, including serine proteases, urokinase plasminogen activator, and MMPs, are released by ECs to digest the basement membrane and ECM [29]. Once this is achieved, ECs are allowed to migrate to the site of new vessel formation [27]. MMPs digest the basement membrane and the ECM, ultimately allowing ECs to migrate and proliferate [29]. In a recent study, the addition of MMP synthetic inhibitor to EC cultures significantly decreased angiogenic activity [29]. ECs migrate to the site of new vessel formation by chemotaxis [27]. Further, specific adhesion molecules, integrins, mediate their cell–matrix interactions to ensure migration to the site of new vessel formation [27]. Integrins are adhesion molecules that are highly upregulated on ECs undergoing angiogenesis [30, 31].

Neutrophils

Inflammation in normal wound healing is essential, but must be tightly regulated both temporally and spatially by a variety of cell types. Immediately after injury extravagated blood constituents form a haemostatic plug. Platelets and polymorphonuclear leukocytes (also known as neutrophils or PMNs) entrapped and aggregated in the blood clot release a wide variety of factors that amplify the aggregation response, initiate a coagulation cascade, and/or act as chemoattractants for cells involved in the inflammatory phase [32]. At the same time, rapid activation of resident skin immune cells (mast cells, γδ T cells, and Langerhans cells) occurs [33–35]. The inflammatory phase continues with active recruitment of neutrophils and then macrophages from blood vessels, which is orchestrated by growth factor signals from the resident cells, mainly keratinocytes, and by foreign epitopes such as the lipopolysaccharides (LPS) of invading microorganisms [36]. Neutrophils arrive at the wound site within minutes of wounding and become the predominant cells in the wound for the first 2 days after the injury occurs, with especially high numbers on the second day. Extravasation of PMNs from blood vessels is activated by proinflammatory cytokines IL-1β, TNF-α, and IFNγ at the wound site, leading to expression of various classes of adhesion molecules essential for cell adhesion and diapedesis. Adhesion molecules crucial for neutrophil diapedesis include endothelial P- and E-selectins as well as ICAM-1, -2 [36]. PMNs have an important bactericidal role and kill invading microorganisms through several strategies, including bursts of reactive oxygen species (ROS) [32]. Inflammatory cells also exert their influence on the surrounding tissue by generating

nitric oxide (NO) and large amounts of ROS [37]. Chemokines are also very important mediators of neutrophil recruitment during tissue repair [38–40]. Gene expression profiles of wound PMNs suggested that these cells influence many other aspects of repair, such as resolution of the fibrin clot and provisional ECM, promotion of angiogenesis, and re-epithelialization [41]. The neutrophil infiltration ceases after a few days, and expended neutrophils are themselves phagocytosed by macrophages, which are present at the wound site within 2 days after injury.

Macrophages

Release of signals from keratinocytes and fibroblasts leads to recruitment of both local resident macrophages and those from the blood. Monocytes are drawn from the circulation somewhat later than neutrophils and their numbers peak a day or so after injury [36]. Once they leave the circulation, monocytes mature into macrophages and change their expression profiles and behavior according to the surroundings and growth factor stimuli [42]. At the wound site, they clear up matrix and cell debris, including spent neutrophils by phagocytosis [36].

Macrophage infiltration into the wound site is regulated by different chemotactic factors, including growth factors, proinflammatory cytokines, and chemokines (macrophage inflammatory protein 1α, MCP-1, RANTES) [4, 43–45]. Major sources of these chemoattractants at the wound site include platelets trapped in the fibrin clot at the wound surface, keratinocytes at the wound edge, fibroblasts, and leukocytes subsets. Both types of macrophages, classically activated (M1, proinflammatory) and alternatively activated (M2, anti-inflammatory and proangiogenic), are present in early phases of inflammation, but M2 macrophages predominate later in repair [46, 47]. In addition to their immunological functions as antigen-presenting cells and phagocytes during wound repair, macrophages also release a battery of growth factors and cytokines at the wound site, which further promotes cell proliferation and the synthesis of ECM molecules by resident skin cells [46, 47].

Inflammatory cells also exert their influence on the surrounding tissue by generating nitric oxide (NO) and large amounts of ROS [37]. NO and ROS are known to drive certain aspects of repair [48] but at the same time affected wound cells must protect themselves by detoxifying programs [37]. NO is a very transitory molecule, whose levels together with inducible NO synthase (iNOS) activity shows a distinct time course during normal healing [49, 50]. Although the issue of whether inflammatory cells are an essential requirement for repair remains controversial [51], it is clear that these cell populations exert a profound influence on all other cells within the wound and in the surrounding tissue. One of the important roles of inflammatory cytokines is to regulate angiogenesis, which they accomplish in concert with signals from other wound cells and from serum (see section on “Angiogenesis”). However, nonhealing wounds fail to progress through the normal phases of wound repair, but instead remain in a chronic inflammatory state. Imbalances in wound proteases and their inhibitors in chronic wounds, because of sustained production of inflammatory mediators and influx of inflammatory cells, prevent matrix synthesis and remodeling, essential for progression to a healed wound [52–56].

The inflammatory phase of wound healing has been studied in detail, but most of the research efforts were focused on the onset of inflammation and little is known about inflammation resolution. Better understanding of how inflammation resolves will provide a basis for novel treatment modalities favoring the closure of chronic wounds.

Stem and Progenitor Cells in Wound Healing

In order to have sustained healing without scarring, Fu et al. suggested combining growth factors with stem cell therapy so that sweat glands, sebaceous glands, and hair follicles could be reconstituted with a more functional integument [6]. Therefore, stem and progenitor cells are of great interest to wound healing as active participants as well as potential therapeutic approach.

The ability of the skin to replenish itself and contribute to the maintenance of tissue renewal

and homeostasis relies on resident populations of stem cells (SCs) [57–59]. To date, SCs of the skin have been identified to occupy at least three distinct niches: the bulge of the hair follicle, the base of the sebaceous gland, and the basal layer of the epidermis [59–64]. Whereas the SCs of the sebaceous gland niche and the interfollicular basal layer niche have only been proven to behave unipotently exclusively maintaining homeostasis of their respective tissue, the SCs of the hair bulge have been long thought to maintain a multipotent nature: serving as a reservoir for renewal of not only hair but also sebaceous glands in conditions of hyperproliferation and interfollicular epidermis subsequent to wounding [65]. This is not surprising as it is known that the basal layer and the hair follicle outer root sheath are not only connected but also biochemically similar [66]. If stimulated adequately epidermal SCs have even the potential to develop additional cell types and tissues [67–70]. In the healthy skin, SCs are quiescent [71]. However, in response to injury, SCs niches lose their quiescence and resident SCs are recruited to replace the damaged tissue [72–74]. SCs of the hair bulge are required for regenerating the interfollicular epidermis in response to wounding [75] and the most recent studies have shown major contribution of hair follicle in anagen phase during tissue repair [76]. Importantly, in addition to the hair follicle bulge SCs, recent studies discovered other populations of epithelial SCs within distinct regions of the hair follicle [77]. Although epidermal SCs have been characterized largely by their functional properties and marker expression [78, 79], their full therapeutic capacity is still elusive.

Amnion-derived multipotent progenitor cells (AMP cells) provide another avenue for therapeutic approach to wound healing as well as diabetic foot ulcers (DFUs). AMP cells display many favorable characteristics of stem cells, including the ability to differentiate to many cell types such as skin, hair, neurons, cardiac muscle, liver, pancreas, and possibly vascular tissue [80, 81]. They are isolated from the full-term placenta, which makes them abundantly available. From a safety standpoint, the low antigenicity of amnion [82] and documented nontumorigenicity [81] is an advantage for use as a cell replacement therapy. Amniotic membrane and human amniotic epithelial cells are used on skin wounds, burn injuries, and chronic leg ulcers and to prevent adhesions in surgical procedures [83–91]. Amniotic membrane is also used in ocular surface reconstruction to promote development of normal corneal or conjunctival epithelium [92]. Human amniotic membrane and hAEC have been shown to survive for prolonged times in immunocompetent animals, including rabbits [92], rats [93], guinea pigs [94], and bonnet monkeys [95]. In addition, long-term engraftment was observed after i.v. injection of heterogeneous human amniochorionic cells into newborn swine and rats, with human microchimerism detected in bone marrow, brain, lung, and thymus [96], suggesting active migration and integration into specific organs and indicating active tolerance of the xenogeneic cells. Amnion-derived cells have been shown to secrete many cytokines that are associated with wound healing and some have been credited with contributing to scarless healing in the fetus [97–107].

These therapies have also been important in demonstrating that local therapy is clinically effective in the treatment of DFUs and will be useful approach to implement findings from this project into future treatments. Adult bone marrow (BM) is well known source of multipotent adult progenitor cells that can differentiate into many adult tissue types in vivo and in vitro when placed in the proper cytokine environment [108, 109] and may provide a alternative for progenitor cell therapy approach. Multipotent adult progenitor cells could also home to injured tissues and participate in the repair and regeneration [110, 111]. It has been known that bone marrow (BM) provides inflammatory cells and endothelial progenitor cells (EPCs) during normal wound healing. However, recent studies strongly suggest that the BM contributes not only to inflammation and angiogenesis, but also to keratinocytes and fibroblast-like cells [112–114]. Most importantly, wounding can stimulate the engraftment of bone marrow-derived mesenchymal stem cells (BM-MSCs) to the skin promoting wound healing [115].

BM-MSCs are self-renewing SCs characterized by specific markers—CD105, CD73, and CD9 [116]. They represent about 0.001–0.01% of the nucleated BM cells, but the fact that they are expandable in culture and capable of differentiating into several cell types [117] makes them very attractive for therapeutic purposes. A number of animal studies have shown that BM-MSCs contribute to the repair/regeneration of a variety of injured tissues including the myocardium [118, 119], bone and cartilage [120], tendon [121], and most importantly skin [121, 122]. Moreover, topically applied autologous BM-MSCs have shown potential to heal human chronic wounds that are recalcitrant to other treatments [123, 124]. Many other mesenchymal tissues also contain committed lineage-directed mesenchymal precursor cells, which participate in local regeneration. MSCs from the skin and other tissues, like adipose tissue, muscles, and scalp tissue resemble BM-MSCs and express similar markers [125–127]. Skin fibroblasts are also a useful source of cells from which pluripotent SCs may be generated [128]. Another type of circulating bone marrow-derived progenitor cells, called fibrocytes, have been suggested to migrate into the wound and contribute to the formation of the myofibroblastic population of granulation tissue [129]. Fibrocytes participate in tissue remodeling by producing ECM proteins (i.e., collagen I and collagen III) and by secreting MMPs [130].

Bone marrow-derived EPCs are the essential cells for vasculogenesis [131]. Vasculogenesis likely begins when BM multipotent progenitor cells differentiate into early EPCs [109, 132], at which time the cells acquire hematopoietic endothelial lineage specific cell surface markers [133, 134]. EPCs are undifferentiated in the BM and in a quiescent state in two zones [27]. One zone, the osteoblastic zone, maintains EPCs in the GO phase of the cell cycle and keeps the EPCs in close proximity with stromal cells [132, 135]. The second zone, the vascular zone, maintains EPCs in the S phase or G2/M phase of the cell cycle, which is readily available to differentiate into tissue-specific progenitor cells and enter the peripheral circulation [132]. EPC mobilization from the BM into the circulation is thought to occur via cytokine-mediated pathways [133]. These cytokines include the vascular endothelial growth factor (VEGF) family and stromal-cell-derived factor-1 (SDF1α) [133]. Early EPCs that exit the bone marrow are positive for CD133, CD34, and VEGF-R2, which are specific to EPCs determined to become endothelial cells. Next, EPCs enter the peripheral circulation and migrate to areas of vasculogenesis. In the circulation, the early EPCs differentiate into late EPCs by losing CD133 and gaining other, more specific EC surface markers [136]. In the circulation, EPCs constitute 0.002% of mononuclear cell fraction of whole blood [137]. This pool of circulating EPCs is increased when vasculogenic stimuli are released for neovascularization. EPCs play an important role in normal wound healing [138–140]. Multiple studies have shown that EPCs derived from diabetic mice exhibited impaired vascularization and wound healing which could be reversed by ischemia-induced upregulation of SDF-1α [2, 138]. Consistent with the effect of EPCs on wound healing in animal models, impaired function and reduced numbers of circulating EPCs have been described in both type 1 and type 2 diabetic patients [141, 142], suggesting that modulation of EPC numbers and function has a potential for therapy for DFUs.

In summary, there is a profound therapeutic potential of progenitor cells and a great interest in current developments of stem or progenitor cells therapy for treatment of wound healing disorders, including DFUs.

Pathophysiology of Wound Healing in Diabetes Mellitus

Over 170 million patients worldwide are affected by diabetes, with an estimated 20.8 million affected in the USA [143]. By 2030, these numbers are projected to double [144]. DFUs occur in 15% of patients with diabetes and are a leading cause of hospital admissions for people with diabetes in the developed world [145, 146]. DFUs precede 84% of all diabetes-related leg amputations [146] and lead to pain, suffering, and poor quality of life [2].

As mentioned earlier, wound healing is a dynamic process involving overlapping inflammatory, proliferating, and remodeling phases. It engages the coordinated action of both resident and migratory cell populations within the ECM environment. However, in individuals suffering from DM, wounds fail to heal in a timely and orderly manner. The pathophysiologic relationship between diabetes and impaired healing is multifactorial. Vascular, neuropathic, immune functions and biochemical abnormalities each contribute to the altered tissue repair in diabetic patients.

Extrinsic factors such as callus formation, excessive pressure, and wound infection also play a role in healing impairment. In addition, lack of glucose control impairs local leukocyte defenses and persisting hyperglycemia contributes to the metabolic pathophysiology of diabetes-related complications.

From the extensive research conducted so far, it appears that diabetes negatively affects majority of cellular processes in wound healing. Studies show that prolonged inflammatory phase in diabetic wounds causes delay in the formation of mature granulation tissue and subsequently reduction in wound tensile strength [147]. Diabetic wounds show decreased number and function of neutrophils and macrophages. Macrophage efferocytosis is dysfunctional (e.g., efficient dead cell clearance at the wound site), thus resulting in increased apoptotic cell burden and higher expression of proinflammatory and lower expression of anti-inflammatory cytokines [148–150]. For example, increased levels of tumor necrosis factor alpha (TNF-α) found in diabetic wounds may lead to decreased fibroblast proliferation and increased apoptosis by inducing caspase activity [150]. On the other hand, sustained inflammatory response and deregulated expression of cytokines may amplify caspase activity as well [151, 152].

Fibroblasts from diabetic wounds look different than healthy fibroblasts. They are usually large and inflated compared to the spindle-shaped morphology of the fibroblasts in age-matched controls. Fibroblasts from diabetic wounds show numerous vesicular bodies, dilated endoplasmic reticulums, and lack of microtubular structure [153]. In addition to a reduced number and morphological changes, fibroblasts from diabetic wounds exhibit diminished proliferative capacity that contributes to a decreased production of ECM proteins, delayed wound contraction, and impaired wound healing [153, 154]. On the contrary, activity of some MMPs is found increased in diabetic wounds when compared to acute wound healing [155]. Increased expression of MMP8 and MMP26 was found in tissue from DFUs [156]. MMP2 and MMP9 show sustained overexpression in chronic nonhealing DFUs [157]. The latest report implicates MMP9 levels in wound fluid as a predictor of poor wound healing in DFUs [158]. The ratio of MMP and tissue inhibitor of metalloproteinases (TIMP), which in normal physiological conditions maintains the proteolytic balance, is found to be disturbed in DFUs. High MMP1/TIMP1 ratio has been shown as a predictor of wound healing in DFUs [159]. In contrast, high MMP9/TIMP1 ratio predicts poor wound healing [158] (Table 7.1). The combination of increased concentrations of MMP2, MMP9, MMP14 with decreased concentrations of TIMP2 in DFUs suggests that the increased proteolytic environment significantly reduces the formation of new connective tissue matrix and contributes to the failure of diabetic wounds to heal. Overall, these changes lead to decreased tensile strength in diabetic wounds [152, 160].

The decrease in growth factors responsible for tissue repair such as TGF-β may explain deregulation of MMPs [161]. It is known that most MMP genes have TGF-β inhibitory element in their promoter regions and thus a possible explanation for deregulation of MMPs is that reduced levels of TGF-β lower down the inhibitory regulatory effect on MMP genes and cause overexpression of MMPs [162, 163]. However, the exact mechanism responsible for increased MMP activity in diabetes is still to be elucidated. The lack of TGF-β signaling in chronic wounds could also lead to the increased iNOS activity and greater NO synthesis [164], since TGF-β1 has been demonstrated to downregulate iNOS activity in macrophages and epithelial cells [165, 166]. Although NO can stimulate angiogenesis, excessive amounts may have an inhibitory

Table 7.1 Deregulated matrix metalloproteinase (MMPs) and their inhibitors (TIMPs) in DFUs

↑	MMP2
↑	MMP8
↑	MMP9
↑	MMP14
↑	MMP26
↓	TIMP1
↓	TIMP2
↑	MMP1/TIMP1 Predictor of healing ulcers
↑	MMP9/TIMP1 Predictor of poor healing

effect by decreasing endothelial cell and lymphocyte proliferation, and possibly inhibiting platelet and leukocyte activation [167–169]. In patients with diabetes, elevated levels of plasma NO have found to be associated with recurrent ulcers [170]. In addition to lower concentrations of growth factors, diabetic wounds contain fibroblasts that show diminished response to growth factors such as EGF, IGF-I, bFGF, PDGF-AB, GM-CSF, and VEGF [153, 166, 171–173]. We have shown that fibroblasts cultured from the different wound locations (e.g., nonhealing edge, wound base, and adjacent skin) show differences in the response to the various growth factors [154].

Similar to fibroblasts, epidermal keratinocytes display dysfunction in DFUs. One study found that epidermal keratinocytes at the edge of DFUs express pathogenic markers beta-catenin, and c-myc and show abnormal localization of EGF receptor (Fig. 7.1). Keratinocytes appear to be trapped between proliferation and differentiation. Epidermis comprising nonhealing edges of DFUs is acanthotic, hyper-, and para-keratotic [174, 175]. Hyperproliferative keratinocytes show an activated phenotype and are negative when stained for keratins involved in epidermal differentiation. In addition to deregulated proliferation and differentiation, there is a reduced expression of a key molecule present on migrating epithelium LM-3A32 (uncleaved, precursor of the α3 chain of laminin 5) contributes to impaired migratory capacity of these cells [176, 177]. Over expression of EGF, GM-CSF, and TGF-β1 in DFU

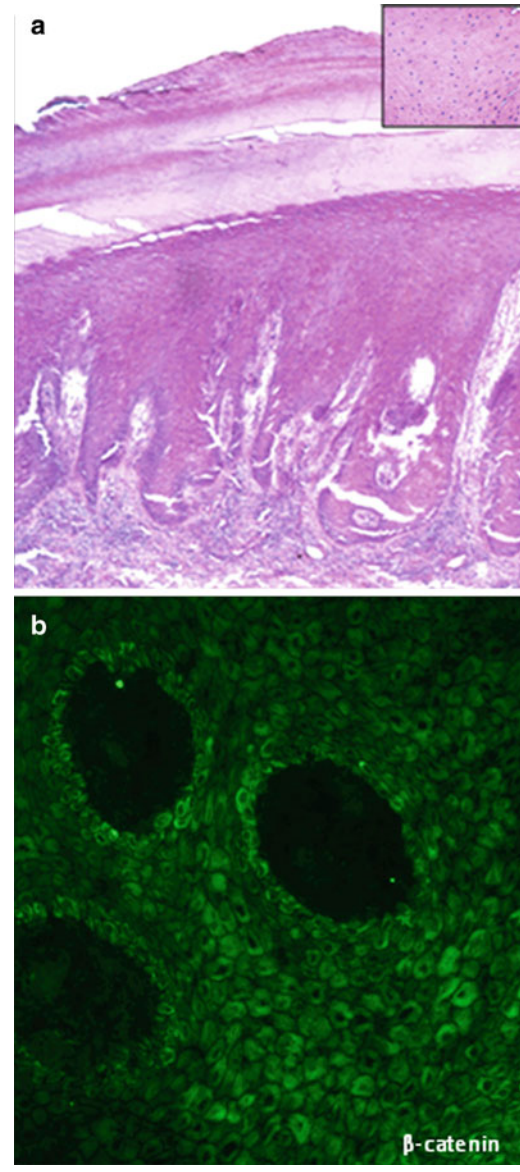

Fig. 7.1 A typical nonhealing edge of a DFU shows hyperproliferative epidermis with nuclei present in cornified layer (*inset*) (**a**). Immunofluorescence with a beta-catenin specific antibody. Beta-catenin is present in the nuclei of a nonhealing DFU epidermis (**b**)

epidermis is also postulated to play a role in deregulated keratinocyte proliferation, lack of keratinocyte apoptosis, and migration in these ulcers [161]. Tissue from DFUs show accumulation of CD1a+ Langerhans cells (LC) in epidermis compared to normal skin and insufficient upregulation of beta-defensin-2 (hBD2) [178].

Many other factors such as decrease in heat shock protein expression, decreased chemotaxis, less antioxidant synthesis, and increased oxygen free radical generation have been shown to play a role in pathogenesis of diabetic healing [179].

The local environment of the diabetic wound is healing impaired due to high bacterial burden and the barrier to diffusion of growth factors and cytokines important for healing. In addition, prolonged hypoxia correlates with healing inability [139]. Hypoxia is pathologically increased in diabetic wound healing [139]. Oxygen tension is positively correlated with collagen production [180–182] and bacterial killing [183, 184].

Diabetics are at an increased risk for infection due to high bacterial burden. It has been reported that diabetic patients have a 25% chance of developing a DFU and a greater than 50% chance of these ulcers becoming infected [185, 186]. Further, diabetic patients have a tenfold increased chance of being hospitalized with a bone or soft tissue infection than those without diabetes [185–187]. Infection and subsequent biofilm production undermines healing in DFUs. Biofilms are polymicrobial populations of cells encased in hydrated extracellular polymeric substances and attached to a surface (e.g., tissue) [188] It has been proven that one of the greatest barriers to healing in chronic wounds is biofilm due to polymicrobial infections [189–191]. Furthermore, it has been shown that biofilms are more prevalent in chronic, nonhealing wounds, and rare in acute, healing wounds [192].

One of the most significant risk factors for the development of DFUs is diabetic neuropathy leading to amputations, infections, morbidity, and mortality. The prevalence of diabetic neuropathy ranges from 7% within 1 year of diagnosis to 50% for those with diabetes for >25 years [193]. Thus, as patients age, diabetic neuropathy prevalence increases; it is present to some degree in >50% of patients over 60 years [145, 194]. Diabetic neuropathy increases the risk of foot ulceration by sevenfold [195]. Diabetic neuropathy predisposes the diabetic foot to various complications including ulceration [195] and disabling joint deformity [196]. One of the ways diabetic neuropathy causes damage is via altered autonomic regulation of cutaneous blood flow [197]. Further, motor neuropathy leads to atrophic changes in the foot musculature, leading to foot deformity and decreased joint mobility [196]. Ultimately, these complications further the risk for DFU. Therefore, identifying at-risk diabetic patients is crucial for the prevention of foot ulceration and various screening methods are used for this, including evaluation of vibration perception threshold [195, 198], plantar foot pressure measurements [199], and joint mobility [200].

Angiogenesis

Angiogenesis and vasculogenesis form the mature circulatory system, which is one of the first organs to form and maintains metabolic homeostasis by supplying oxygen and nutrients and removing waste products [27, 131]. An imbalance of the two interrelated processes of angiogenesis and vasculogenesis contributes to the pathogenesis of numerous malignant, inflammatory, ischemic, infectious, immune, and wound healing disorders [131]. In angiogenesis, endothelial cells develop from preexisting blood vessels and migrate and proliferate into a cord-like structure [27] (Table 7.2). Intussusceptive microvascular growth (whereby a mature vessel lumen is divided by the ingrowths of cellular columns) is another component of angiogenesis [27]. Vasculogenesis is the de novo formation of immature vascular structures from the differentiation of progenitor cells [27] (Table 7.3). These newly formed vascular structures mature into capillaries, arterioles, arteries, venules, and veins [27, 108, 109, 201, 202].

Angiogenesis is capillary formation from preexisting ones, which first requires destabilization of the preexisting endothelial tubular structure [27, 211–213]. Often, angiogenesis is caused by tissue injury or neoplastic transformation [27, 214, 215]. During wound healing, neovascularization is new capillary formation to replace damaged capillaries and reestablish the supply of oxygen and nutrients to the wound [27]. During the proliferation phase of wound healing, angiogenesis re-establishes the supply of oxygen and

Table 7.2 Major differences between normal angiogenesis and angiogenesis seen in DFUs

Normal angiogenesis	Angiogenesis in DFU
Proangiogenic cytokines (including VEGF) are released from platelets, monocytes, and fibroblasts	Fibroblasts may become senescent in chronic wounds and lose their ability to provide angiogenic functions [27]
Endothelial cells (ECs) disrupt their interactions with neighboring ECs	Resident ECs of the chronic wound may lose their ability to support new vessel formation [27]
ECs digest the basement membrane and extracellular matrix (ECM) components (via MMPs)	Impaired balance between the accumulation of ECM components and their remodeling by MMPs [160]
ECs, fibroblasts, platelets, smooth muscle cells, and monocytes release more proangiogenic cytokines	The fluid of chronic wounds block cellular proliferation and angiogenesis [203, 204] Impairment of leukocyte function and proliferation occur in hyperglycemia [205]
ECs invade ECM and migrate/proliferate to new vessels	Disruption of new vessel formation disrupts healing at the level of the peripheral wound [27] Hypoxia impairs angiogenesis [206, 207]

Table 7.3 Major differences between normal and vasculogenesis in DFUs

Normal vasculogenesis	Vasculogenesis in DFU
Multipotent adult progenitor cells (MAPCs) differentiate into hematopoietic precursor cells or early endothelial progenitor cells (EPCs) in the bone marrow	Impaired VEGF-induced proliferation response in EPCs [208]
Increased vascular endothelial growth factor-A (VEGF-A) induces vascular endothelial growth factor receptor-1 (VEGF-R1) activation and subsequently increased matrix metalloproteinase-9 (MMP9) secretion	Hyperglycemia-mediated inhibition of VEGF [209]
Increased MMP9 mediates the conversion of membrane-bound kit ligand (mKitL) to soluble kit ligand (sKitL), which mobilizes EPCs from the bone marrow to circulation	Decreased number and function of circulating EPCs impairs healing [131]
Early EPCs in the circulation further differentiate to late EPCs and gain specific endothelial cell (EC) surface markers	Diminished blood supply to peripheral wound [131]
Late EPCs arrive to the site of the new vessel formation and further differentiate into mature ECs or act as a source of proangiogenic cytokines	EPCs demonstrate abnormal mobilization and homing mechanisms in diabetics [210]

nutrients to the wound. Vasculogenesis is the de novo formation of blood vessels from the differentiation of bone marrow-derived precursor cells. Vasculogenesis occurs during both fetal development and in the adult [27]. In the formation of fetal vasculature, primitive mesodermal cells called hemangioblasts form blood islands. These are spatially arranged with cells that differentiate into endothelial cells, or angioblasts, at the periphery. Other cells included in this process are hematopoietic stem cells [27]. An imbalance of angiogenesis or vasculogenesis contributes to numerous pathologies, including malignancies, inflammatory or ischemic, infectious, and immune diseases.

Angiogenesis in Diabetes

During the last decade, the incidence of microvascular complications in DM has rapidly increased [213]. Dysfunctional angiogenesis has been suggested as a common origin for retinopathy, nephropathy, neuropathy, and impaired wound healing [213, 216–220], although the complex pathogenesis of diabetic microvascular complications is still largely unknown [221–223].

VEGF is a key player in a number of diabetes-related pathologies [224]. In some organ systems, elevated VEGF levels act as a pathologic angiogenic stimulus (i.e., ocular neovascularization)

[225], whereas in others, low levels of VEGF activity leads to pathology (i.e., nephropathy, peripheral neuropathy, and wound healing) [140, 226, 227].

Angiogenesis-related complications are implicated in a number of diabetic complications, including diabetic retinopathy, diabetic nephropathy, diabetic neuropathy, coronary artery disease (CAD), and impaired wound healing. As the number of microvascular complications in DM continue to rise, a better understanding of dysfunctional angiogenesis becomes more critical. Further understanding of the role of angiogenesis in these pathologies could provide novel treatments and improve the lives of the millions of patients suffering with diabetes.

Diabetic retinopathy (DR) is one of the leading causes of blindness worldwide [228]. Vision loss occurs due to retinal ischemia, retinal vascular exudation, intraocular hemorrhage, and ultimately, fibrotic complications [229]. Nearly all patients with type 1 DM and over 60% of patients with type 2 DM develop retinopathy during the first two decades of the disease [229]. DR is characterized by abnormal angiogenesis, leading to new vessels that are often immature and play a pathological role in retinopathy, contributing to both vitreous hemorrhage and fibrosis [230]. Increased vascular permeability leads to plasma leakage and the development of macula edema [230]. Diabetic macular edema and retinal neovascularization represent two of the most serious pathological changes in DR [219]. Previous studies have shown that angiogenic factors, including VEGF, play a key role in the development of these two changes [225]. Elevated levels of VEGF in ocular fluids of patients with proliferative DR have been shown [231]. Chronic hyperglycemia increases the synthesis of VEGF (a normally proangiogenic cytokine), contributing to the microvascular abnormalities in DR [232]. Inhibition of VEGF diminishes the microvascular complications seen in experimental animal models [232]. Retinal hypoxia/ischemia upregulates the production of VEGF, which results in abnormal and deregulated angiogenesis [233]. The growth of new vessels from the retina or optic nerve occurs as a result of VEGF release into the vitreous cavity [232]. Further, injection of VEGF into normal primate eyes induces the same pathologic processes seen in diabetic retinopathy, including microaneurysm formation and increased vascular permeability [232]. A key target of current clinical trials is VEGF [225]. Anti-VEGF treatments may represent an alternative adjunctive treatment for proliferative DR. Currently, there are three anti-VEGF agents available: pegaptanib, bevacizumab, and ranibizumab [219].

Diabetic nephropathy (DN) is one of the leading causes of end-stage renal disease [234]. In a pathogenesis similar to DR, abnormal angiogenesis also occurs in diabetic nephropathy [234]. VEGF-A is involved in the normal physiological processes of the kidney [234]. VEGF-A has been shown to be upregulated in the early stages of DN, likely leading to excessive blood vessel formation [235]. However, a decline of VEGF-A in the later phase of DN has also been shown [235]. Two studies have shown beneficial effects of anti-VEGF antibody treatments [236, 237]. However, some theorize that VEGF-A inhibitors could lead to endothelial injury because endothelial cells require VEGF-A in physiological conditions [234]. While anti-angiogenic treatments have prevented the progression of animal models of diabetic nephropathy, further studies are needed before these treatments can be applied to a clinical setting [234].

Diabetes mellitus is one of the greatest cardiovascular risk factors and leads to vascular dysfunction and atherosclerotic disease. The formation of coronary collateral vessels is of functional importance in patients with CAD and is a compensatory mechanism secondary to repetitive or chronic myocardial ischemia [238]. DM was recently shown to be one of the first negative predictors of collateral vessel formation [239]. This reduced collateral circulation in diabetic patients likely contributes to their increased morbidity and mortality [238]. So far, research has shown that coronary collateral vessel formation depends on monocyte function, which is impaired in diabetic patients and VEGF-related signal transduction defects may be the basis of impaired monocyte function in diabetics [240].

Thus, VEGF-A may be a potential therapeutic strategy for reduced coronary collateral circulation in diabetic patients [240].

Treatment for DFUs

Standardized treatment of DFUs includes glycemic control, debridement of necrotic tissue, control of infection, use of moist dressings, protection from pressure or trauma related to ambulation, and adjuvant hyperbaric oxygen (HBO) therapy [131]. In the setting of arterial insufficiency in diabetes, revascularization with return of delivery of oxygen or nutrients is essential and can be accomplished by surgical bypass or percutaneous angioplasty [27]. Unfortunately, this is only feasible at the level of large- and medium-sized arteries and not at the microvascular level [27]. Currently, the only FDA-approved growth factor and cell therapies for DFUs are not routinely used, making management very difficult [2]. Recent study documented that the sooner advanced biological therapies were used, the better the outcome of healing is achieved [241].

Surgical debridement has been a standard of therapy in the treatment of DFUs. Theoretically, surgical debridement aids wound healing by removing necrotic tissue and optimizing the healing capacity of surrounding viable tissue. Despite the fact that surgical debridement is routine practice in DFU treatment, there is incomplete evidence-based science supporting its role [242]. Debridement may work in synergy with other treatment approaches such as cell therapy or growth factors.

Growth factors are promising biological therapies for DFUs and have been useful in combination with surgical debridement. Granulocyte macrophage colony stimulating factor (GM-CSF) and VEGF-A have been considered as treatments to stimulate the bone marrow release of EPCs for wound healing but risks such as acute arterial thrombosis, angina, hypotension, sepsis, and death have complicated their development as treatment modalities [243–248]. Other recombinant angiogenic growth factors have been tested with positive results; these include EGF, FGF, and PDGF (which is an FDA-approved topical wound ointment) [27]. Another study, a randomized placebo-controlled trial, found that granulocyte-colony stimulating factor (G-CSF) increases the release of neutrophils from the bone marrow and improves neutrophil function in DFUs [249]. VEGF-A is an endothelial cell mitogen [250–255], chemotactic agent [256, 257], and inducer of vascular permeability [258–263], and as such is a promising candidate for treatment of chronic wounds. HBO therapy is an adjunctive therapy used to stimulate wound healing when the microvasculature has become compromised but the larger vessels remain open or have been re-vascularized [131]. Tissue-level hyperoxia is the outcome of HBO treatments and it has been supported by many studies [264–269]. In an experimental model of diabetic wound healing, HBO has been shown to work in synergy with chemokines produced by keratinocytes and fibroblasts. Together, HBO and these chemokines recruit endothelial progenitors to circulation and home them to the site of the injury. Thus, adequate presence of chemokines released by keratinocytes and myofibroblasts is important component of successful of HBO therapy [2, 138]. Many current potential treatments are still under research. Gene therapy and stem cell therapy, including a gene encoding VEGF-A, has been reported to enhance healing and angiogenesis in ischemic ulcers in a mouse model of diabetes [270]. Fibroblasts are also a potential therapeutic target: augmentation of fibroblast endogenous cytokine production via transient vector transfection could activate the local angiogenic cascade and promote wound healing [27].

Cell therapy, including cryopreserved human fibroblast-derived dermal substitute, composed of fibroblasts, ECM, and a bioabsorbable scaffold or living skin equivalent composed of keratinocytes and fibroblasts in a native collagen matrix are FDA-approved for the treatment of DFUs. It has been shown that cell therapy promotes healing via the release of various cytokines and growth factors into the local wound milieu [271–273]. This approach to healing is most effective when coupled with surgical debridement of the chronic wound.

Off-loading is a treatment modality that aims to redistribute pressure away from the area of ulceration to improve wound healing in DFUs. The incorporation of pressure-relieving properties in a wound care dressing for the treatment of DFUs is another newer option for treatment [274], where the wound dressing effectively reduces pressure at individual metatarsal heads in patients at risk of diabetic foot ulceration.

Dressings of various types are useful in the management of DFUs. Among the many dressing options, three were recently tested on DFUs in a large randomized controlled trial. The four treatment options (nonadherent, knitted, viscose filament gauze, an iodine-impregnated dressing, both traditional dressings combined, or a new antimicrobial dressing) were found to be similar in efficacy [275]. Collagen-alginate topical wound dressing was found to be more effective than saline-soaked gauze in another randomized controlled trial [276]. The proper dressing modalities, when used in combination with off-loading, debridement, and/or the other more sophisticated biological therapies, are crucial to the treatment of DFUs.

In conclusion, the pathophysiology of wound healing in DM is complex and represents the deregulation and dysfunction of all the phases and cell types involved in the process. In spite of the recent advances, the most effective clinical protocols for the treatment of DFU are yet to be determined. Thus, further research in this area should yield optimization of future therapies for this devastated complication of diabetes.

References

1. Singer AJ, Clark RA. Cutaneous wound healing. N Engl J Med. 1999;341(10):738–46.
2. Brem H, Tomic-Canic M. Cellular and molecular basis of wound healing in diabetes. J Clin Invest. 2007;117(5):1219–22.
3. Larson BJ, Longaker MT, Lorenz HP. Scarless fetal wound healing: a basic science review. Plast Reconstr Surg. 2010;126(4):1172–80.
4. Werner S, Grose R. Regulation of wound healing by growth factors and cytokines. Physiol Rev. 2003; 83(3):835–70.
5. Werner S, Krieg T, Smola H. Keratinocyte-fibroblast interactions in wound healing. J Invest Dermatol. 2007;127(5):998–1008.
6. Fu X, Li X, Cheng B, Chen W, Sheng Z. Engineered growth factors and cutaneous wound healing: success and possible questions in the past 10 years. Wound Repair Regen. 2005;13(2):122–30.
7. Blumenberg M, Tomic-Canic M. Human epidermal keratinocyte: keratinization processes. EXS. 1997;78:1–29.
8. Raja SK, Garcia MS, Isseroff RR. Wound re-epithelialization: modulating keratinocyte migration in wound healing. Front Biosci. 2007;12:2849–68.
9. Barrientos S, Stojadinovic O, Golinko MS, Brem H, Tomic-Canic M. Growth factors and cytokines in wound healing. Wound Repair Regen. 2008;16(5): 585–601.
10. Tomic-Canic M, Agren MS, Oscar MA. Epidermal repair and the chronic wound. In: Rovee DT, Maibach H, editors. The epidermis in wound healing. Boca Raton, FL: CRC; 2004. p. 25–57.
11. Freedberg IM, Tomic-Canic M, Komine M, Blumenberg M. Keratins and the keratinocyte activation cycle. J Invest Dermatol. 2001;116(5):633–40.
12. Kupper TS. The activated keratinocyte: a model for inducible cytokine production by non-bone marrow-derived cells in cutaneous inflammatory and immune responses. J Invest Dermatol. 1990;94(6 Suppl): 146S–50.
13. Martin P. Wound healing – aiming for perfect skin regeneration. Science. 1997;276(5309):75–81.
14. Badylak SF. The extracellular matrix as a scaffold for tissue reconstruction. Semin Cell Dev Biol. 2002;13(5):377–83.
15. Ffrench-Constant C, Van de Water L, Dvorak HF, Hynes RO. Reappearance of an embryonic pattern of fibronectin splicing during wound healing in the adult rat. J Cell Biol. 1989;109(2):903–14.
16. Desmouliere A, Geinoz A, Gabbiani F, Gabbiani G. Transforming growth factor-beta 1 induces alpha-smooth muscle actin expression in granulation tissue myofibroblasts and in quiescent and growing cultured fibroblasts. J Cell Biol. 1993;122(1):103–11.
17. Hinz B, Mastrangelo D, Iselin CE, Chaponnier C, Gabbiani G. Mechanical tension controls granulation tissue contractile activity and myofibroblast differentiation. Am J Pathol. 2001;159(3):1009–20.
18. Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C, Brown RA. Myofibroblasts and mechano-regulation of connective tissue remodelling. Nat Rev Mol Cell Biol. 2002;3(5):349–63.
19. Montesano R, Orci L. Transforming growth factor beta stimulates collagen-matrix contraction by fibroblasts: implications for wound healing. Proc Natl Acad Sci USA. 1988;85(13):4894–7.
20. Clark RA, Folkvord JM, Hart CE, Murray MJ, McPherson JM. Platelet isoforms of platelet-derived growth factor stimulate fibroblasts to contract collagen matrices. J Clin Invest. 1989;84(3):1036–40.
21. Jiang H, Rhee S, Ho CH, Grinnell F. Distinguishing fibroblast promigratory and procontractile growth factor environments in 3-D collagen matrices. FASEB J. 2008;22(7):2151–60.

22. Tingstrom A, Heldin CH, Rubin K. Regulation of fibroblast-mediated collagen gel contraction by platelet-derived growth factor, interleukin-1 alpha and transforming growth factor-beta 1. J Cell Sci. 1992;102(Pt 2):315–22.
23. Lin YC, Grinnell F. Treatment of human fibroblasts with vanadate and platelet-derived growth factor in the presence of serum inhibits collagen matrix contraction. Exp Cell Res. 1995;221(1):73–82.
24. Akasaka Y, Ono I, Kamiya T, Ishikawa Y, Kinoshita T, Ishiguro S, Yokoo T, Imaizumi R, Inomata N, Fujita K, Akishima-Fukasawa Y, Uzuki M, Ito K, Ishii T. The mechanisms underlying fibroblast apoptosis regulated by growth factors during wound healing. J Pathol. 2010;221(3):285–99.
25. Desmouliere A, Redard M, Darby I, Gabbiani G. Apoptosis mediates the decrease in cellularity during the transition between granulation tissue and scar. Am J Pathol. 1995;146(1):56–66.
26. Zaulyanov L, Kirsner RS. A review of a bi-layered living cell treatment (Apligraf) in the treatment of venous leg ulcers and diabetic foot ulcers. Clin Interv Aging. 2007;2(1):93–8.
27. Bauer SM, Bauer RJ, Velazquez OC. Angiogenesis, vasculogenesis, and induction of healing in chronic wounds. Vasc Endovascular Surg. 2005;39(4): 293–306.
28. Sephel GC, Kennedy R, Kudravi S. Expression of capillary basement membrane components during sequential phases of wound angiogenesis. Matrix Biol. 1996;15(4):263–79.
29. Burbridge MF, Coge F, Galizzi JP, Boutin JA, West DC, Tucker GC. The role of the matrix metalloproteinases during in vitro vessel formation. Angiogenesis. 2002;5(3):215–26.
30. Brooks PC, Clark RA, Cheresh DA. Requirement of vascular integrin alpha v beta 3 for angiogenesis. Science. 1994;264(5158):569–71.
31. Hynes RO, Bader BL, Hodivala-Dilke K. Integrins in vascular development. Braz J Med Biol Res. 1999;32(5):501–10.
32. Dovi JV, Szpaderska AM, DiPietro LA. Neutrophil function in the healing wound: adding insult to injury? Thromb Haemost. 2004;92(2):275–80.
33. Noli C, Miolo A. The mast cell in wound healing. Vet Dermatol. 2001;12(6):303–13.
34. Jameson JM, Sharp LL, Witherden DA, Havran WL. Regulation of skin cell homeostasis by gamma delta T cells. Front Biosci. 2004;9:2640–51.
35. Cumberbatch M, Dearman RJ, Griffiths CE, Kimber I. Langerhans cell migration. Clin Exp Dermatol. 2000;25(5):413–8.
36. Eming SA, Krieg T, Davidson JM. Inflammation in wound repair: molecular and cellular mechanisms. J Invest Dermatol. 2007;127(3):514–25.
37. Schafer M, Werner S. Oxidative stress in normal and impaired wound repair. Pharmacol Res. 2008;58(2): 165–71.
38. Engelhardt E, Toksoy A, Goebeler M, Debus S, Brocker EB, Gillitzer R. Chemokines IL-8, GROalpha, MCP-1, IP-10, and Mig are sequentially and differentially expressed during phase-specific infiltration of leukocyte subsets in human wound healing. Am J Pathol. 1998;153(6):1849–60.
39. Gillitzer R, Goebeler M. Chemokines in cutaneous wound healing. J Leukoc Biol. 2001;69(4):513–21.
40. Esche C, Stellato C, Beck LA. Chemokines: key players in innate and adaptive immunity. J Invest Dermatol. 2005;125(4):615–28.
41. Theilgaard-Monch K, Knudsen S, Follin P, Borregaard N. The transcriptional activation program of human neutrophils in skin lesions supports their important role in wound healing. J Immunol. 2004;172(12):7684–93.
42. Martinez FO, Gordon S, Locati M, Mantovani A. Transcriptional profiling of the human monocyte-to-macrophage differentiation and polarization: new molecules and patterns of gene expression. J Immunol. 2006;177(10):7303–11.
43. DiPietro LA, Burdick M, Low QE, Kunkel SL, Strieter RM. MIP-1alpha as a critical macrophage chemoattractant in murine wound repair. J Clin Invest. 1998;101(8):1693–8.
44. Frank S, Kampfer H, Wetzler C, Stallmeyer B, Pfeilschifter J. Large induction of the chemotactic cytokine RANTES during cutaneous wound repair: a regulatory role for nitric oxide in keratinocyte-derived RANTES expression. Biochem J. 2000;347 (Pt 1):265–73.
45. Wetzler C, Kampfer H, Stallmeyer B, Pfeilschifter J, Frank S. Large and sustained induction of chemokines during impaired wound healing in the genetically diabetic mouse: prolonged persistence of neutrophils and macrophages during the late phase of repair. J Invest Dermatol. 2000;115(2):245–53.
46. Deonarine K, Panelli MC, Stashower ME, Jin P, Smith K, Slade HB, Norwood C, Wang E, Marincola FM, Stroncek DF. Gene expression profiling of cutaneous wound healing. J Transl Med. 2007;5:11.
47. Shaw TJ, Martin P. Wound repair at a glance. J Cell Sci. 2009;122(Pt 18):3209–13.
48. Sen CK, Roy S. Redox signals in wound healing. Biochim Biophys Acta. 2008;1780(11):1348–61.
49. Reichner JS, Meszaros AJ, Louis CA, Henry Jr WL, Mastrofrancesco B, Martin BA, Albina JE. Molecular and metabolic evidence for the restricted expression of inducible nitric oxide synthase in healing wounds. Am J Pathol. 1999;154(4):1097–104.
50. Lee RH, Efron D, Tantry U, Barbul A. Nitric oxide in the healing wound: a time-course study. J Surg Res. 2001;101(1):104–8.
51. Martin P, Leibovich SJ. Inflammatory cells during wound repair: the good, the bad and the ugly. Trends Cell Biol. 2005;15(11):599–607.
52. Pilcher BK, Wang M, Qin XJ, Parks WC, Senior RM, Welgus HG. Role of matrix metalloproteinases

and their inhibition in cutaneous wound healing and allergic contact hypersensitivity. Ann N Y Acad Sci. 1999;878:12–24.
53. Nagaoka T, Kaburagi Y, Hamaguchi Y, Hasegawa M, Takehara K, Steeber DA, Tedder TF, Sato S. Delayed wound healing in the absence of intercellular adhesion molecule-1 or L-selectin expression. Am J Pathol. 2000;157(1):237–47.
54. Madlener M, Parks WC, Werner S. Matrix metalloproteinases (MMPs) and their physiological inhibitors (TIMPs) are differentially expressed during excisional skin wound repair. Exp Cell Res. 1998;242(1):201–10.
55. Parks WC. Matrix metalloproteinases in repair. Wound Repair Regen. 1999;7(6):423–32.
56. Mast BA, Schultz GS. Interactions of cytokines, growth factors, and proteases in acute and chronic wounds. Wound Repair Regen. 1996;4(4):411–20.
57. Cotsarelis G. Epithelial stem cells: a folliculocentric view. J Invest Dermatol. 2006;126(7):1459–68.
58. Kaur P. Interfollicular epidermal stem cells: identification, challenges, potential. J Invest Dermatol. 2006;126(7):1450–8.
59. Watt FM, Lo Celso C, Silva-Vargas V. Epidermal stem cells: an update. Curr Opin Genet Dev. 2006;16(5):518–24.
60. Fuchs E. Skin stem cells: rising to the surface. J Cell Biol. 2008;180(2):273–84.
61. Potten CS. The epidermal proliferative unit: the possible role of the central basal cell. Cell Tissue Kinet. 1974;7(1):77–88.
62. Bickenbach JR. Identification and behavior of label-retaining cells in oral mucosa and skin. J Dent Res. 1981;60(Spec No C):1611–20.
63. Lavker RM, Sun TT. Heterogeneity in epidermal basal keratinocytes: morphological and functional correlations. Science. 1982;215(4537):1239–41.
64. Morris RJ, Potten CS. Highly persistent label-retaining cells in the hair follicles of mice and their fate following induction of anagen. J Invest Dermatol. 1999;112(4):470–5.
65. Taylor G, Lehrer MS, Jensen PJ, Sun TT, Lavker RM. Involvement of follicular stem cells in forming not only the follicle but also the epidermis. Cell. 2000;102(4):451–61.
66. Tumbar T, Guasch G, Greco V, Blanpain C, Lowry WE, Rendl M, Fuchs E. Defining the epithelial stem cell niche in skin. Science. 2004;303(5656):359–63.
67. Morasso MI, Tomic-Canic M. Epidermal stem cells: the cradle of epidermal determination, differentiation and wound healing. Biol Cell. 2005;97(3):173–83.
68. Hoffman RM. The pluripotency of hair follicle stem cells. Cell Cycle. 2006;5(3):232–3.
69. Amoh Y, Li L, Katsuoka K, Hoffman RM. Multipotent hair follicle stem cells promote repair of spinal cord injury and recovery of walking function. Cell Cycle. 2008;7(12):1865–9.
70. Bickenbach JR, Stern MM, Grinnell KL, Manuel A, Chinnathambi S. Epidermal stem cells have the potential to assist in healing damaged tissues. J Investig Dermatol Symp Proc. 2006;11(1):118–23.
71. Walker MR, Patel KK, Stappenbeck TS. The stem cell niche. J Pathol. 2009;217(2):169–80.
72. Blanpain C, Lowry WE, Geoghegan A, Polak L, Fuchs E. Self-renewal, multipotency, and the existence of two cell populations within an epithelial stem cell niche. Cell. 2004;118(5):635–48.
73. Langton AK, Herrick SE, Headon DJ. An extended epidermal response heals cutaneous wounds in the absence of a hair follicle stem cell contribution. J Invest Dermatol. 2008;128(5):1311–8.
74. Ito M, Cotsarelis G. Is the hair follicle necessary for normal wound healing? J Invest Dermatol. 2008; 128(5):1059–61.
75. Ito M, Liu Y, Yang Z, Nguyen J, Liang F, Morris RJ, Cotsarelis G. Stem cells in the hair follicle bulge contribute to wound repair but not to homeostasis of the epidermis. Nat Med. 2005;11(12):1351–4.
76. Ansell DM, Kloepper JE, Thomason HA, Paus R, Hardman MJ. Exploring the "hair growth-wound healing connection": anagen phase promotes wound re-epithelialization. J Invest Dermatol. 2011;131(2): 518–28.
77. Snippert HJ, Haegebarth A, Kasper M, Jaks V, van Es JH, Barker N, van de Wetering M, van den Born M, Begthel H, Vries RG, Stange DE, Toftgard R, Clevers H. Lgr6 marks stem cells in the hair follicle that generate all cell lineages of the skin. Science. 2010;327(5971):1385–9.
78. Cotsarelis G, Sun TT, Lavker RM. Label-retaining cells reside in the bulge area of pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. Cell. 1990;61(7):1329–37.
79. Ito M, Yang Z, Andl T, Cui C, Kim N, Millar SE, Cotsarelis G. Wnt-dependent de novo hair follicle regeneration in adult mouse skin after wounding. Nature. 2007;447(7142):316–20.
80. Fliniaux I, Viallet JP, Dhouailly D, Jahoda CA. Transformation of amnion epithelium into skin and hair follicles. Differentiation. 2004;72(9–10):558–65.
81. Miki T, Lehmann T, Cai H, Stolz DB, Strom SC. Stem cell characteristics of amniotic epithelial cells. Stem Cells. 2005;23(10):1549–59.
82. Sakuragawa N, Yoshikawa H, Sasaki M. Amniotic tissue transplantation: clinical and biochemical evaluations for some lysosomal storage diseases. Brain Dev. 1992;14(1):7–11.
83. Colocho G, Graham 3rd WP, Greene AE, Matheson DW, Lynch D. Human amniotic membrane as a physiologic wound dressing. Arch Surg. 1974; 109(3):370–3.
84. Gruss JS, Jirsch DW. Human amniotic membrane: a versatile wound dressing. Can Med Assoc J. 1978; 118(10):1237–46.

85. Trelford JD, Trelford-Sauder M. The amnion in surgery, past and present. Am J Obstet Gynecol. 1979; 134(7):833–45.
86. Faulk WP, Matthews R, Stevens PJ, Bennett JP, Burgos H, Hsi BL. Human amnion as an adjunct in wound healing. Lancet. 1980;1(8179):1156–8.
87. Ward DJ, Bennett JP. The long-term results of the use of human amnion in the treatment of leg ulcers. Br J Plast Surg. 1984;37(2):191–3.
88. Ward DJ, Bennett JP, Burgos H, Fabre J. The healing of chronic venous leg ulcers with prepared human amnion. Br J Plast Surg. 1989;42(4):463–7.
89. Subrahmanyam M. Amniotic membrane as a cover for microskin grafts. Br J Plast Surg. 1995;48(7): 477–8.
90. Gomes JA, Romano A, Santos MS, Dua HS. Amniotic membrane use in ophthalmology. Curr Opin Ophthalmol. 2005;16(4):233–40.
91. Parolini O, Alviano F, Bagnara GP, Bilic G, Buhring HJ, Evangelista M, Hennerbichler S, Liu B, Magatti M, Mao N, Miki T, Marongiu F, Nakajima H, Nikaido T, Portmann-Lanz CB, Sankar V, Soncini M, Stadler G, Surbek D, Takahashi TA, Redl H, Sakuragawa N, Wolbank S, Zeisberger S, Zisch A, Strom SC. Concise review: isolation and characterization of cells from human term placenta: outcome of the first international Workshop on Placenta Derived Stem Cells. Stem Cells. 2008;26(2):300–11.
92. Avila M, Espana M, Moreno C, Pena C. Reconstruction of ocular surface with heterologous limbal epithelium and amniotic membrane in a rabbit model. Cornea. 2001;20(4):414–20.
93. Kubo M, Sonoda Y, Muramatsu R, Usui M. Immunogenicity of human amniotic membrane in experimental xenotransplantation. Invest Ophthalmol Vis Sci. 2001;42(7):1539–46.
94. Yuge I, Takumi Y, Koyabu K, Hashimoto S, Takashima S, Fukuyama T, Nikaido T, Usami S. Transplanted human amniotic epithelial cells express connexin 26 and Na-K-adenosine triphosphatase in the inner ear. Transplantation. 2004;77(9):1452–4.
95. Sankar V, Muthusamy R. Role of human amniotic epithelial cell transplantation in spinal cord injury repair research. Neuroscience. 2003;118(1):11–7.
96. Bailo M, Soncini M, Vertua E, Signoroni PB, Sanzone S, Lombardi G, Arienti D, Calamani F, Zatti D, Paul P, Albertini A, Zorzi F, Cavagnini A, Candotti F, Wengler GS, Parolini O. Engraftment potential of human amnion and chorion cells derived from term placenta. Transplantation. 2004;78(10):1439–48.
97. Koyano S, Fukui A, Uchida S, Yamada K, Asashima M, Sakuragawa N. Synthesis and release of activin and noggin by cultured human amniotic epithelial cells. Dev Growth Differ. 2002;44(2):103–12.
98. Blumenstein M, Hansen WR, Deval D, Mitchell MD. Differential regulation in human amnion epithelial and fibroblast cells of prostaglandin E(2) production and prostaglandin H synthase-2 mRNA expression by dexamethasone but not tumour necrosis factor-alpha. Placenta. 2000;21(2–3):210–7.
99. Tahara M, Tasaka K, Masumoto N, Adachi K, Adachi H, Ikebuchi Y, Kurachi H, Miyake A. Expression of messenger ribonucleic acid for epidermal growth factor (EGF), transforming growth factor-alpha (TGF alpha), and EGF receptor in human amnion cells: possible role of TGF alpha in prostaglandin E2 synthesis and cell proliferation. J Clin Endocrinol Metab. 1995;80(1):138–46.
100. Paradowska E, Blach-Olszewska Z, Gejdel E. Constitutive and induced cytokine production by human placenta and amniotic membrane at term. Placenta. 1997;18(5–6):441–6.
101. Denison FC, Kelly RW, Calder AA, Riley SC. Cytokine secretion by human fetal membranes, decidua and placenta at term. Hum Reprod. 1998;13(12):3560–5.
102. Keelan JA, Groome NP, Mitchell MD. Regulation of activin-A production by human amnion, decidua and placenta in vitro by pro-inflammatory cytokines. Placenta. 1998;19(5–6):429–34.
103. Sun K, Ma R, Cui X, Campos B, Webster R, Brockman D, Myatt L. Glucocorticoids induce cytosolic phospholipase A2 and prostaglandin H synthase type 2 but not microsomal prostaglandin E synthase (PGES) and cytosolic PGES expression in cultured primary human amnion cells. J Clin Endocrinol Metab. 2003;88(11):5564–71.
104. Uchida S, Inanaga Y, Kobayashi M, Hurukawa S, Araie M, Sakuragawa N. Neurotrophic function of conditioned medium from human amniotic epithelial cells. J Neurosci Res. 2000;62(4):585–90.
105. Robson MC, Steed DL, Franz MG. Wound healing: biologic features and approaches to maximize healing trajectories. Curr Probl Surg. 2001;38(2):72–140.
106. Longaker MT, Adzick NS. The biology of fetal wound healing: a review. Plast Reconstr Surg. 1991;87(4):788–98.
107. Wilgus TA, Bergdall VK, Tober KL, Hill KJ, Mitra S, Flavahan NA, Oberyszyn TM. The impact of cyclooxygenase-2 mediated inflammation on scarless fetal wound healing. Am J Pathol. 2004;165(3):753–61.
108. Reyes M, Dudek A, Jahagirdar B, Koodie L, Marker PH, Verfaillie CM. Origin of endothelial progenitors in human postnatal bone marrow. J Clin Invest. 2002;109(3):337–46.
109. Nakul-Aquaronne D, Bayle J, Frelin C. Coexpression of endothelial markers and CD14 by cytokine mobilized CD34+ cells under angiogenic stimulation. Cardiovasc Res. 2003;57(3):816–23.
110. Devine SM, Cobbs C, Jennings M, Bartholomew A, Hoffman R. Mesenchymal stem cells distribute to a wide range of tissues following systemic infusion into nonhuman primates. Blood. 2003;101(8):2999–3001.
111. Kawada H, Fujita J, Kinjo K, Matsuzaki Y, Tsuma M, Miyatake H, Muguruma Y, Tsuboi K, Itabashi Y, Ikeda Y, Ogawa S, Okano H, Hotta T, Ando K, Fukuda K. Nonhematopoietic mesenchymal stem cells can be mobilized and differentiate into cardiomyocytes after myocardial infarction. Blood. 2004;104(12):3581–7.

112. Fathke C, Wilson L, Hutter J, Kapoor V, Smith A, Hocking A, Isik F. Contribution of bone marrow-derived cells to skin: collagen deposition and wound repair. Stem Cells. 2004;22(5):812–22.
113. Deng W, Han Q, Liao L, Li C, Ge W, Zhao Z, You S, Deng H, Murad F, Zhao RC. Engrafted bone marrow-derived flk-(1+) mesenchymal stem cells regenerate skin tissue. Tissue Eng. 2005;11(1–2):110–9.
114. Brittan M, Braun KM, Reynolds LE, Conti FJ, Reynolds AR, Poulsom R, Alison MR, Wright NA, Hodivala-Dilke KM. Bone marrow cells engraft within the epidermis and proliferate in vivo with no evidence of cell fusion. J Pathol. 2005;205(1):1–13.
115. Badiavas EV, Abedi M, Butmarc J, Falanga V, Quesenberry P. Participation of bone marrow derived cells in cutaneous wound healing. J Cell Physiol. 2003;196(2):245–50.
116. McNiece I. Delivering cellular therapies: lessons learned from ex vivo culture and clinical applications of hematopoietic cells. Semin Cell Dev Biol. 2007;18(6):839–45.
117. 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(5411):143–7.
118. Pittenger MF, Martin BJ. Mesenchymal stem cells and their potential as cardiac therapeutics. Circ Res. 2004;95(1):9–20.
119. Quevedo HC, Hatzistergos KE, Oskouei BN, Feigenbaum GS, Rodriguez JE, Valdes D, Pattany PM, Zambrano JP, Hu Q, McNiece I, Heldman AW, Hare JM. Allogeneic mesenchymal stem cells restore cardiac function in chronic ischemic cardiomyopathy via trilineage differentiating capacity. Proc Natl Acad Sci USA. 2009;106(33):14022–7.
120. Noel D, Djouad F, Jorgense C. Regenerative medicine through mesenchymal stem cells for bone and cartilage repair. Curr Opin Investig Drugs. 2002;3(7):1000–4.
121. Awad HA, Butler DL, Boivin GP, Smith FN, Malaviya P, Huibregtse B, Caplan AI. Autologous mesenchymal stem cell-mediated repair of tendon. Tissue Eng. 1999;5(3):267–77.
122. Sorrell JM, Caplan AI. Topical delivery of mesenchymal stem cells and their function in wounds. Stem Cell Res Ther. 2010;1(4):30.
123. Badiavas EV, Falanga V. Treatment of chronic wounds with bone marrow-derived cells. Arch Dermatol. 2003;139(4):510–6.
124. 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(6):1299–312.
125. Riekstina U, Cakstina I, Parfejevs V, Hoogduijn M, Jankovskis G, Muiznieks I, Muceniece R, Ancans J. Embryonic stem cell marker expression pattern in human mesenchymal stem cells derived from bone marrow, adipose tissue, heart and dermis. Stem Cell Rev. 2009;5(4):378–86.
126. Shih DT, Lee DC, Chen SC, Tsai RY, Huang CT, Tsai CC, Shen EY, Chiu WT. Isolation and characterization of neurogenic mesenchymal stem cells in human scalp tissue. Stem Cells. 2005;23(7):1012–20.
127. Mosna F, Sensebe L, Krampera M. Human bone marrow and adipose tissue mesenchymal stem cells: a user's guide. Stem Cells Dev. 2010;19(10):1449–70.
128. Byrne JA, Pedersen DA, Clepper LL, Nelson M, Sanger WG, Gokhale S, Wolf DP, Mitalipov SM. Producing primate embryonic stem cells by somatic cell nuclear transfer. Nature. 2007;450(7169): 497–502.
129. 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(1):71–81.
130. Metz CN. Fibrocytes: a unique cell population implicated in wound healing. Cell Mol Life Sci. 2003; 60(7):1342–50.
131. Velazquez OC. Angiogenesis and vasculogenesis: inducing the growth of new blood vessels and wound healing by stimulation of bone marrow-derived progenitor cell mobilization and homing. J Vasc Surg. 2007;45(Suppl A):A39–47.
132. Rafii S, Avecilla S, Shmelkov S, Shido K, Tejada R, Moore MA, Heissig B, Hattori K. Angiogenic factors reconstitute hematopoiesis by recruiting stem cells from bone marrow microenvironment. Ann N Y Acad Sci. 2003;996:49–60.
133. Aicher A, Zeiher AM, Dimmeler S. Mobilizing endothelial progenitor cells. Hypertension. 2005; 45(3):321–5.
134. Janowska-Wieczorek A, Marquez LA, Nabholtz JM, Cabuhat ML, Montano J, Chang H, Rozmus J, Russell JA, Edwards DR, Turner AR. Growth factors and cytokines upregulate gelatinase expression in bone marrow CD34(+) cells and their transmigration through reconstituted basement membrane. Blood. 1999;93(10):3379–90.
135. Cheng T, Rodrigues N, Shen H, Yang Y, Dombkowski D, Sykes M, Scadden DT. Hematopoietic stem cell quiescence maintained by p21cip1/waf1. Science. 2000;287(5459):1804–8.
136. Bompais H, Chagraoui J, Canron X, Crisan M, Liu XH, Anjo A, Tolla-Le Port C, Leboeuf M, Charbord P, Bikfalvi A, Uzan G. Human endothelial cells derived from circulating progenitors display specific functional properties compared with mature vessel wall endothelial cells. Blood. 2004;103(7):2577–84.
137. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G, Isner JM. Isolation of putative progenitor endothelial cells for angiogenesis. Science. 1997;275(5302): 964–7.
138. Gallagher KA, Liu ZJ, Xiao M, Chen H, Goldstein LJ, Buerk DG, Nedeau A, Thom SR, Velazquez OC.

Diabetic impairments in NO-mediated endothelial progenitor cell mobilization and homing are reversed by hyperoxia and SDF-1 alpha. J Clin Invest. 2007;117(5):1249–59.
139. Liu ZJ, Velazquez OC. Hyperoxia, endothelial progenitor cell mobilization, and diabetic wound healing. Antioxid Redox Signal. 2008;10(11):1869–82.
140. Asai J, Takenaka H, Katoh N, Kishimoto S. Dibutyryl cAMP influences endothelial progenitor cell recruitment during wound neovascularization. J Invest Dermatol. 2006;126(5):1159–67.
141. Loomans CJ, de Koning EJ, Staal FJ, Rookmaaker MB, Verseyden C, de Boer HC, Verhaar MC, Braam B, Rabelink TJ, van Zonneveld AJ. Endothelial progenitor cell dysfunction: a novel concept in the pathogenesis of vascular complications of type 1 diabetes. Diabetes. 2004;53(1):195–9.
142. Fadini GP, Miorin M, Facco M, Bonamico S, Baesso I, Grego F, Menegolo M, de Kreutzenberg SV, Tiengo A, Agostini C, Avogaro A. Circulating endothelial progenitor cells are reduced in peripheral vascular complications of type 2 diabetes mellitus. J Am Coll Cardiol. 2005;45(9):1449–57.
143. National Diabetes Information Clearinghouse. National Diabetes Statistics fact sheet. 2007. http://diabetes.niddk.nih.gov/dm/pubs/statistics/index.htm.
144. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care. 2004; 27(5):1047–53.
145. Reiber GE, Vileikyte L, Boyko EJ, del Aguila M, Smith DG, Lavery LA, Boulton AJ. Causal pathways for incident lower-extremity ulcers in patients with diabetes from two settings. Diabetes Care. 1999;22(1):157–62.
146. Reiber GE, Boyko EJ, Smith DG. Lower extremity foot ulcers and amputations in diabetes. In: Harris MI, Cowie CC, Stern MP, Boyko EJ, Reiber GE, Bennett PH, editors. Diabetes in America. Bethesda, MD: U.S. Government Printing Office; 1995. p. 409–28.
147. Acosta JB, del Barco DG, Vera DC, Savigne W, Lopez-Saura P, Guillen Nieto G, Schultz GS. The pro-inflammatory environment in recalcitrant diabetic foot wounds. Int Wound J. 2008;5(4):530–9.
148. Maruyama K, Asai J, Ii M, Thorne T, Losordo DW, D'Amore PA. Decreased macrophage number and activation lead to reduced lymphatic vessel formation and contribute to impaired diabetic wound healing. Am J Pathol. 2007;170(4):1178–91.
149. Khanna S, Biswas S, Shang Y, Collard E, Azad A, Kauh C, Bhasker V, Gordillo GM, Sen CK, Roy S. Macrophage dysfunction impairs resolution of inflammation in the wounds of diabetic mice. PLoS One. 2010;5(3):e9539.
150. Siqueira MF, Li J, Chehab L, Desta T, Chino T, Krothpali N, Behl Y, Alikhani M, Yang J, Braasch C, Graves DT. Impaired wound healing in mouse models of diabetes is mediated by TNF-alpha dysregulation and associated with enhanced activation of forkhead box O1 (FOXO1). Diabetologia. 2009;53(2):378–88.
151. Al-Mashat HA, Kandru S, Liu R, Behl Y, Desta T, Graves DT. Diabetes enhances mRNA levels of proapoptotic genes and caspase activity, which contribute to impaired healing. Diabetes. 2006;55(2): 487–95.
152. Liu R, Desta T, He H, Graves DT. Diabetes alters the response to bacteria by enhancing fibroblast apoptosis. Endocrinology. 2004;145(6):2997–3003.
153. Loots MA, Lamme EN, Mekkes JR, Bos JD, Middelkoop E. Cultured fibroblasts from chronic diabetic wounds on the lower extremity (non-insulin-dependent diabetes mellitus) show disturbed proliferation. Arch Dermatol Res. 1999;291(2–3):93–9.
154. Brem H, Golinko MS, Stojadinovic O, Kodra A, Diegelmann RF, Vukelic S, Entero H, Coppock DL, Tomic-Canic M. Primary cultured fibroblasts derived from patients with chronic wounds: a methodology to produce human cell lines and test putative growth factor therapy such as GMCSF. J Transl Med. 2008;6:75.
155. Vaalamo M, Leivo T, Saarialho-Kere U. Differential expression of tissue inhibitors of metalloproteinases (TIMP-1, -2, -3, and -4) in normal and aberrant wound healing. Hum Pathol. 1999;30(7):795–802.
156. Pirila E, Korpi JT, Korkiamaki T, Jahkola T, Gutierrez-Fernandez A, Lopez-Otin C, Saarialho-Kere U, Salo T, Sorsa T. Collagenase-2 (MMP-8) and matrilysin-2 (MMP-26) expression in human wounds of different etiologies. Wound Repair Regen. 2007;15(1):47–57.
157. Wysocki AB, Staiano-Coico L, Grinnell F. Wound fluid from chronic leg ulcers contains elevated levels of metalloproteinases MMP-2 and MMP-9. J Invest Dermatol. 1993;101(1):64–8.
158. Liu Y, Min D, Bolton T, Nube V, Twigg SM, Yue DK, McLennan SV. Increased matrix metalloproteinase-9 predicts poor wound healing in diabetic foot ulcers. Diabetes Care. 2009;32(1):117–9.
159. Muller M, Trocme C, Lardy B, Morel F, Halimi S, Benhamou PY. Matrix metalloproteinases and diabetic foot ulcers: the ratio of MMP-1 to TIMP-1 is a predictor of wound healing. Diabet Med. 2008; 25(4):419–26.
160. Lobmann R, Ambrosch A, Schultz G, Waldmann K, Schiweck S, Lehnert H. Expression of matrix-metalloproteinases and their inhibitors in the wounds of diabetic and non-diabetic patients. Diabetologia. 2002;45(7):1011–6.
161. Galkowska H, Wojewodzka U, Olszewski WL. Chemokines, cytokines, and growth factors in keratinocytes and dermal endothelial cells in the margin of chronic diabetic foot ulcers. Wound Repair Regen. 2006;14(5):558–65.
162. Bennett NT, Schultz GS. Growth factors and wound healing: Part II. Role in normal and chronic wound healing. Am J Surg. 1993;166(1):74–81.
163. McLennan SV, Fisher E, Martell SY, Death AK, Williams PF, Lyons JG, Yue DK. Effects of glucose

on matrix metalloproteinase and plasmin activities in mesangial cells: possible role in diabetic nephropathy. Kidney Int Suppl. 2000;77:S81–7.
164. Pastar I, Stojadinovic O, Krzyzanowska A, Barrientos S, Stuelten C, Zimmerman K, Blumenberg M, Brem H, Tomic-Canic M. Attenuation of the transforming growth factor beta-signaling pathway in chronic venous ulcers. Mol Med. 2010;16(3–4):92–101.
165. Vodovotz Y, Bogdan C, Paik J, Xie QW, Nathan C. Mechanisms of suppression of macrophage nitric oxide release by transforming growth factor beta. J Exp Med. 1993;178(2):605–13.
166. Vodovotz Y, Letterio JJ, Geiser AG, Chesler L, Roberts AB, Sparrow J. Control of nitric oxide production by endogenous TGF-beta1 and systemic nitric oxide in retinal pigment epithelial cells and peritoneal macrophages. J Leukoc Biol. 1996;60(2):261–70.
167. Blakytny R, Jude E. The molecular biology of chronic wounds and delayed healing in diabetes. Diabet Med. 2006;23(6):594–608.
168. Moilanen E, Vapaatalo H. Nitric oxide in inflammation and immune response. Ann Med. 1995;27(3): 359–67.
169. Babaei S, Teichert-Kuliszewska K, Monge JC, Mohamed F, Bendeck MP, Stewart DJ. Role of nitric oxide in the angiogenic response in vitro to basic fibroblast growth factor. Circ Res. 1998;82(9): 1007–15.
170. Jude EB, Tentolouris N, Appleton I, Anderson S, Boulton AJ. Role of neuropathy and plasma nitric oxide in recurrent neuropathic and neuroischemic diabetic foot ulcers. Wound Repair Regen. 2001; 9(5):353–9.
171. Hehenberger K, Kratz G, Hansson A, Brismar K. Fibroblasts derived from human chronic diabetic wounds have a decreased proliferation rate, which is recovered by the addition of heparin. J Dermatol Sci. 1998;16(2):144–51.
172. 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(3):153–60.
173. Brem H, Kodra A, Golinko MS, Entero H, Stojadinovic O, Wang VM, Sheahan CM, Weinberg AD, Woo SL, Ehrlich HP, Tomic-Canic M. Mechanism of sustained release of vascular endothelial growth factor in accelerating experimental diabetic healing. J Invest Dermatol. 2009;129(9):2275–87.
174. Stojadinovic O, Brem H, Vouthounis C, Lee B, Fallon J, Stallcup M, Merchant A, Galiano RD, Tomic-Canic M. Molecular pathogenesis of chronic wounds: the role of beta-catenin and c-myc in the inhibition of epithelialization and wound healing. Am J Pathol. 2005;167(1):59–69.
175. Brem H, Stojadinovic O, Diegelmann RF, Entero H, Lee B, Pastar I, Golinko M, Rosenberg H, Tomic-Canic M. Molecular markers in patients with chronic wounds to guide surgical debridement. Mol Med. 2007;13(1–2):30–9.
176. 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(7):687–96.
177. Galkowska H, Olszewsk WL, Wojewodzka U, Mijal J, Filipiuk E. Expression of apoptosis- and cell cycle-related proteins in epidermis of venous leg and diabetic foot ulcers. Surgery. 2003;134(2):213–20.
178. Galkowska H, Olszewski WL, Wojewodzka U. Expression of natural antimicrobial peptide beta-defensin-2 and Langerhans cell accumulation in epidermis from human non-healing leg ulcers. Folia Histochem Cytobiol. 2005;43(3):133–6.
179. Ekmektzoglou KA, Zografos GC. A concomitant review of the effects of diabetes mellitus and hypothyroidism in wound healing. World J Gastroenterol. 2006;12(17):2721–9.
180. Hunt TK, Pai MP. The effect of varying ambient oxygen tensions on wound metabolism and collagen synthesis. Surg Gynecol Obstet. 1972;135(4):561–7.
181. Jonsson K, Jensen JA, Goodson 3rd WH, Scheuenstuhl H, West J, Hopf HW, Hunt TK. Tissue oxygenation, anemia, and perfusion in relation to wound healing in surgical patients. Ann Surg. 1991;214(5):605–13.
182. Shandall A, Lowndes R, Young HL. Colonic anastomotic healing and oxygen tension. Br J Surg. 1985;72(8):606–9.
183. Chavakis E, Hain A, Vinci M, Carmona G, Bianchi ME, Vajkoczy P, Zeiher AM, Chavakis T, Dimmeler S. High-mobility group box 1 activates integrin-dependent homing of endothelial progenitor cells. Circ Res. 2007;100(2):204–12.
184. Hohn DC, MacKay RD, Halliday B, Hunt TK. Effect of O2 tension on microbicidal function of leukocytes in wounds and in vitro. Surg Forum. 1976;27(62):18–20.
185. Fitzgerald RH, Mills JL, Joseph W, Armstrong DG. The diabetic rapid response acute foot team: 7 essential skills for targeted limb salvage. Eplasty. 2009;9:e15.
186. Boulton AJ, Armstrong DG, Albert SF, Frykberg RG, Hellman R, Kirkman MS, Lavery LA, LeMaster JW, Mills Sr JL, Mueller MJ, Sheehan P, Wukich DK. Comprehensive foot examination and risk assessment. A report of the Task Force of the Foot Care Interest Group of the American Diabetes Association, with endorsement by the American Association of Clinical Endocrinologists. Phys Ther. 2008;88(11):1436–43.
187. Boyko EJ, Lipsky B. Infection and diabetes mellitus. In: Harris MI, editor. Diabetes in America. 2nd ed. Washington, DC: National Institutes of Health; 1995. p. 485–96.
188. Gontcharova V, Youn E, Sun Y, Wolcott RD, Dowd SE. A comparison of bacterial composition in diabetic ulcers and contralateral intact skin. Open Microbiol J. 2010;4:8–19.
189. Bjarnsholt T, Kirketerp-Moller K, Jensen PO, Madsen KG, Phipps R, Krogfelt K, Hoiby N, Givskov M. Why chronic wounds will not heal: a novel hypothesis. Wound Repair Regen. 2008;16(1):2–10.

190. Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scott HM. Microbial biofilms. Annu Rev Microbiol. 1995;49:711–45.
191. Wolcott RD, Rhoads DD. A study of biofilm-based wound management in subjects with critical limb ischaemia. J Wound Care. 2008;17(4):145–8, 150–2, 154–5.
192. James GA, Swogger E, Wolcott R, Pulcini E, Secor P, Sestrich J, Costerton JW, Stewart PS. Biofilms in chronic wounds. Wound Repair Regen. 2008;16(1): 37–44.
193. Carmeliet P, Storkebaum E. Vascular and neuronal effects of VEGF in the nervous system: implications for neurological disorders. Semin Cell Dev Biol. 2002;13(1):39–53.
194. Young MJ, Boulton AJ, MacLeod AF, Williams DR, Sonksen PH. A multicentre study of the prevalence of diabetic peripheral neuropathy in the United Kingdom hospital clinic population. Diabetologia. 1993;36(2):150–4.
195. Young MJ, Breddy JL, Veves A, Boulton AJ. The prediction of diabetic neuropathic foot ulceration using vibration perception thresholds. A prospective study. Diabetes Care. 1994;17(6):557–60.
196. Pham H, Armstrong DG, Harvey C, Harkless LB, Giurini JM, Veves A. Screening techniques to identify people at high risk for diabetic foot ulceration: a prospective multicenter trial. Diabetes Care. 2000; 23(5):606–11.
197. Urbancic-Rovan V. Causes of diabetic foot lesions. Lancet. 2005;366(9498):1675–6.
198. Lavery LA, Armstrong DG, Vela SA, Quebedeaux TL, Fleischli JG. Practical criteria for screening patients at high risk for diabetic foot ulceration. Arch Intern Med. 1998;158(2):157–62.
199. Veves A, Murray HJ, Young MJ, Boulton AJ. The risk of foot ulceration in diabetic patients with high foot pressure: a prospective study. Diabetologia. 1992;35(7):660–3.
200. Delbridge L, Perry P, Marr S, Arnold N, Yue DK, Turtle JR, Reeve TS. Limited joint mobility in the diabetic foot: relationship to neuropathic ulceration. Diabet Med. 1988;5(4):333–7.
201. Harry LE, Paleolog EM. From the cradle to the clinic: VEGF in developmental, physiological, and pathological angiogenesis. Birth Defects Res C Embryo Today. 2003;69(4):363–74.
202. Parker TA, le Cras TD, Kinsella JP, Abman SH. Developmental changes in endothelial nitric oxide synthase expression and activity in ovine fetal lung. Am J Physiol Lung Cell Mol Physiol. 2000;278(1):L202–8.
203. Duckworth WC, Fawcett J, Reddy S, Page JC. Insulin-degrading activity in wound fluid. J Clin Endocrinol Metab. 2004;89(2):847–51.
204. 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(1):303–12.
205. 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(5):850–7.
206. Knighton DR, Hunt TK, Scheuenstuhl H, Halliday BJ, Werb Z, Banda MJ. Oxygen tension regulates the expression of angiogenesis factor by macrophages. Science. 1983;221(4617):1283–5.
207. Knighton DR, Silver IA, Hunt TK. Regulation of wound-healing angiogenesis-effect of oxygen gradients and inspired oxygen concentration. Surgery. 1981;90(2):262–70.
208. Mieno S, Boodhwani M, Robich MP, Clements RT, Sodha NR, Sellke FW. Effects of diabetes mellitus on VEGF-induced proliferation response in bone marrow derived endothelial progenitor cells. J Card Surg. 2010;25(5):618–25.
209. Yanagihori H, Oyama N, Nakamura K, Mizuki N, Oguma K, Kaneko F. Role of IL-12B promoter polymorphism in Adamantiades-Behcet's disease susceptibility: An involvement of Th1 immunoreactivity against Streptococcus sanguinis antigen. J Invest Dermatol. 2006;126(7):1534–40.
210. Kopp HG, Ramos CA, Rafii S. Contribution of endothelial progenitors and proangiogenic hematopoietic cells to vascularization of tumor and ischemic tissue. Curr Opin Hematol. 2006;13(3):175–81.
211. Djonov V, Baum O, Burri PH. Vascular remodeling by intussusceptive angiogenesis. Cell Tissue Res. 2003;314(1):107–17.
212. Patan S. Vasculogenesis and angiogenesis. Cancer Treat Res. 2004;117:3–32.
213. Tremolada G, Lattanzio R, Mazzolari G, Zerbini G. The therapeutic potential of VEGF inhibition in diabetic microvascular complications. Am J Cardiovasc Drugs. 2007;7(6):393–8.
214. Carmeliet P. Angiogenesis in health and disease. Nat Med. 2003;9(6):653–60.
215. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. Nat Med. 2003;9(6): 669–76.
216. Congdon NG, Friedman DS, Lietman T. Important causes of visual impairment in the world today. JAMA. 2003;290(15):2057–60.
217. Edwards MS, Wilson DB, Craven TE, Stafford J, Fried LF, Wong TY, Klein R, Burke GL, Hansen KJ. Associations between retinal microvascular abnormalities and declining renal function in the elderly population: the Cardiovascular Health Study. Am J Kidney Dis. 2005;46(2):214–24.
218. Falanga V. Wound healing and its impairment in the diabetic foot. Lancet. 2005;366(9498):1736–43.
219. Grosso A, Veglio F, Porta M, Grignolo FM, Wong TY. Hypertensive retinopathy revisited: some answers, more questions. Br J Ophthalmol. 2005; 89(12):1646–54.
220. Wong TY, Coresh J, Klein R, Muntner P, Couper DJ, Sharrett AR, Klein BE, Heiss G, Hubbard LD, Duncan BB. Retinal microvascular abnormalities and renal dysfunction: the atherosclerosis risk in communities study. J Am Soc Nephrol. 2004;15(9): 2469–76.

221. Cheung N, Wong TY. Diabetic retinopathy and systemic vascular complications. Prog Retin Eye Res. 2008;27(2):161–76.
222. Krentz AJ, Clough G, Byrne CD. Interactions between microvascular and macrovascular disease in diabetes: pathophysiology and therapeutic implications. Diabetes Obes Metab. 2007;9(6):781–91.
223. Stokes KY, Granger DN. The microcirculation: a motor for the systemic inflammatory response and large vessel disease induced by hypercholesterolaemia? J Physiol. 2005;562(Pt 3):647–53.
224. Wirostko B, Wong TY, Simo R. Vascular endothelial growth factor and diabetic complications. Prog Retin Eye Res. 2008;27(6):608–21.
225. Simo R, Carrasco E, Garcia-Ramirez M, Hernandez C. Angiogenic and antiangiogenic factors in proliferative diabetic retinopathy. Curr Diabetes Rev. 2006;2(1):71–98.
226. Eremina V, Sood M, Haigh J, Nagy A, Lajoie G, Ferrara N, Gerber HP, Kikkawa Y, Miner JH, Quaggin SE. Glomerular-specific alterations of VEGF-A expression lead to distinct congenital and acquired renal diseases. J Clin Invest. 2003;111(5):707–16.
227. Raab S, Plate KH. Different networks, common growth factors: shared growth factors and receptors of the vascular and the nervous system. Acta Neuropathol. 2007;113(6):607–26.
228. Fong DS, Aiello L, Gardner TW, King GL, Blankenship G, Cavallerano JD, Ferris 3rd FL, Klein R. Retinopathy in diabetes. Diabetes Care. 2004;27 Suppl 1:S84–7.
229. Cury Jr CE, Rodrigues EB, Meyer CH, Farah ME. VEGF inhibitors and vitrectomy for diabetic vitreoretinopathy. Dev Ophthalmol. 2009;44:69–81.
230. Aiello LP, Gardner TW, King GL, Blankenship G, Cavallerano JD, Ferris 3rd FL, Klein R. Diabetic retinopathy. Diabetes Care. 1998;21(1):143–56.
231. Paulus YM, Gariano RF. Diabetic retinopathy: a growing concern in an aging population. Geriatrics. 2009;64(2):16–20.
232. Tolentino MJ, McLeod DS, Taomoto M, Otsuji T, Adamis AP, Lutty GA. Pathologic features of vascular endothelial growth factor-induced retinopathy in the nonhuman primate. Am J Ophthalmol. 2002;133(3):373–85.
233. Aiello LP, Avery RL, Arrigg PG, Keyt BA, Jampel HD, Shah ST, Pasquale LR, Thieme H, Iwamoto MA, Park JE, et al. Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. N Engl J Med. 1994;331(22):1480–7.
234. Nakagawa T, Kosugi T, Haneda M, Rivard CJ, Long DA. Abnormal angiogenesis in diabetic nephropathy. Diabetes. 2009;58(7):1471–8.
235. Hohenstein B, Hausknecht B, Boehmer K, Riess R, Brekken RA, Hugo CP. Local VEGF activity but not VEGF expression is tightly regulated during diabetic nephropathy in man. Kidney Int. 2006;69(9):1654–61.
236. Flyvbjerg A, Bennett WF, Rasch R, Kopchick JJ, Scarlett JA. Inhibitory effect of a growth hormone receptor antagonist (G120K-PEG) on renal enlargement, glomerular hypertrophy, and urinary albumin excretion in experimental diabetes in mice. Diabetes. 1999;48(2):377–82.
237. de Vriese AS, Tilton RG, Elger M, Stephan CC, Kriz W, Lameire NH. Antibodies against vascular endothelial growth factor improve early renal dysfunction in experimental diabetes. J Am Soc Nephrol. 2001;12(5):993–1000.
238. Waltenberger J. Impaired collateral vessel development in diabetes: potential cellular mechanisms and therapeutic implications. Cardiovasc Res. 2001;49(3):554–60.
239. Abaci A, Oguzhan A, Kahraman S, Eryol NK, Unal S, Arinc H, Ergin A. Effect of diabetes mellitus on formation of coronary collateral vessels. Circulation. 1999;99(17):2239–42.
240. Waltenberger J. VEGF resistance as a molecular basis to explain the angiogenesis paradox in diabetes mellitus. Biochem Soc Trans. 2009;37(Pt 6):1167–70.
241. Kirsner RS, Warriner R, Michela M, Stasik L, Freeman K. Advanced biological therapies for diabetic foot ulcers. Arch Dermatol. 2010;146(8):857–62.
242. Lebrun E, Tomic-Canic M, Kirsner RS. The role of surgical debridement in healing of diabetic foot ulcers. Wound Repair Regen. 2010;18(5):433–8.
243. Li B, Ogasawara AK, Yang R, Wei W, He GW, Zioncheck TF, Bunting S, de Vos AM, Jin H. KDR (VEGF receptor 2) is the major mediator for the hypotensive effect of VEGF. Hypertension. 2002;39(6):1095–100.
244. Kawachi Y, Watanabe A, Uchida T, Yoshizawa K, Kurooka N, Setsu K. Acute arterial thrombosis due to platelet aggregation in a patient receiving granulocyte colony-stimulating factor. Br J Haematol. 1996;94(2):413–6.
245. Tolcher AW, Giusti RM, O'Shaughnessy JA, Cowan KH. Arterial thrombosis associated with granulocyte-macrophage colony-stimulating factor (GM-CSF) administration in breast cancer patients treated with dose-intensive chemotherapy: a report of two cases. Cancer Invest. 1995;13(2):188–92.
246. Fukumoto Y, Miyamoto T, Okamura T, Gondo H, Iwasaki H, Horiuchi T, Yoshizawa S, Inaba S, Harada M, Niho Y. Angina pectoris occurring during granulocyte colony-stimulating factor-combined preparatory regimen for autologous peripheral blood stem cell transplantation in a patient with acute myelogenous leukaemia. Br J Haematol. 1997;97(3):666–8.
247. Lindemann A, Rumberger B. Vascular complications in patients treated with granulocyte colony-stimulating factor (G-CSF). Eur J Cancer. 1993;29A(16):2338–9.
248. To LB, Haylock DN, Simmons PJ, Juttner CA. The biology and clinical uses of blood stem cells. Blood. 1997;89(7):2233–58.
249. Gough A, Clapperton M, Rolando N, Foster AV, Philpott-Howard J, Edmonds ME. Randomised

placebo-controlled trial of granulocyte-colony stimulating factor in diabetic foot infection. Lancet. 1997; 350(9081):855–9.
250. Ferrara N, Henzel WJ. Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. Biochem Biophys Res Commun. 1989;161(2):851–8.
251. Plouet J, Schilling J, Gospodarowicz D. Isolation and characterization of a newly identified endothelial cell mitogen produced by AtT-20 cells. EMBO J. 1989;8(12):3801–6.
252. Gospodarowicz D, Abraham JA, Schilling J. Isolation and characterization of a vascular endothelial cell mitogen produced by pituitary-derived folliculo stellate cells. Proc Natl Acad Sci USA. 1989;86(19):7311–5.
253. Leung DW, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N. Vascular endothelial growth factor is a secreted angiogenic mitogen. Science. 1989; 246(4935):1306–9.
254. Keck PJ, Hauser SD, Krivi G, Sanzo K, Warren T, Feder J, Connolly DT. Vascular permeability factor, an endothelial cell mitogen related to PDGF. Science. 1989;246(4935):1309–12.
255. Connolly DT, Heuvelman DM, Nelson R, Olander JV, Eppley BL, Delfino JJ, Siegel NR, Leimgruber RM, Feder J. Tumor vascular permeability factor stimulates endothelial cell growth and angiogenesis. J Clin Invest. 1989;84(5):1470–8.
256. Yoshida A, Anand-Apte B, Zetter BR. Differential endothelial migration and proliferation to basic fibroblast growth factor and vascular endothelial growth factor. Growth Factors. 1996;13(1–2):57–64.
257. Noiri E, Lee E, Testa J, Quigley J, Colflesh D, Keese CR, Giaever I, Goligorsky MS. Podokinesis in endothelial cell migration: role of nitric oxide. Am J Physiol. 1998;274(1 Pt 1):C236–44.
258. Senger DR, Galli SJ, Dvorak AM, Perruzzi CA, Harvey VS, Dvorak HF. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science. 1983;219(4587):983–5.
259. Senger DR, Connolly DT, Van de Water L, Feder J, Dvorak HF. Purification and NH2-terminal amino acid sequence of guinea pig tumor-secreted vascular permeability factor. Cancer Res. 1990;50(6): 1774–8.
260. Brkovic A, Sirois MG. Vascular permeability induced by VEGF family members in vivo: role of endogenous PAF and NO synthesis. J Cell Biochem. 2007;100(3):727–37.
261. Ferrara N. Molecular and biological properties of vascular endothelial growth factor. J Mol Med. 1999;77(7):527–43.
262. Gavard J, Gutkind JS. VEGF controls endothelial-cell permeability by promoting the beta-arrestin-dependent endocytosis of VE-cadherin. Nat Cell Biol. 2006;8(11):1223–34.
263. Yamagishi S, Yonekura H, Yamamoto Y, Fujimori H, Sakurai S, Tanaka N, Yamamoto H. Vascular endothelial growth factor acts as a pericyte mitogen under hypoxic conditions. Lab Invest. 1999;79(4):501–9.
264. Abidia A, Laden G, Kuhan G, Johnson BF, Wilkinson AR, Renwick PM, Masson EA, McCollum PT. The role of hyperbaric oxygen therapy in ischaemic diabetic lower extremity ulcers: a double-blind randomised-controlled trial. Eur J Vasc Endovasc Surg. 2003;25(6):513–8.
265. Baroni G, Porro T, Faglia E, Pizzi G, Mastropasqua A, Oriani G, Pedesini G, Favales F. Hyperbaric oxygen in diabetic gangrene treatment. Diabetes Care. 1987;10(1):81–6.
266. Doctor N, Pandya S, Supe A. Hyperbaric oxygen therapy in diabetic foot. J Postgrad Med. 1992; 38(3):111–4.
267. Faglia E, Favales F, Aldeghi A, Calia P, Quarantiello A, Oriani G, Michael M, Campagnoli P, Morabito A. Adjunctive systemic hyperbaric oxygen therapy in treatment of severe prevalently ischemic diabetic foot ulcer. A randomized study. Diabetes Care. 1996;19(12):1338–43.
268. Kalani M, Jorneskog G, Naderi N, Lind F, Brismar K. Hyperbaric oxygen (HBO) therapy in treatment of diabetic foot ulcers. Long-term follow-up. J Diabetes Complications. 2002;16(2):153–8.
269. Zamboni WA, Wong HP, Stephenson LL, Pfeifer MA. Evaluation of hyperbaric oxygen for diabetic wounds: a prospective study. Undersea Hyperb Med. 1997;24(3):175–9.
270. Rivard A, Silver M, Chen D, Kearney M, Magner M, Annex B, Peters K, Isner JM. Rescue of diabetes-related impairment of angiogenesis by intramuscular gene therapy with adeno-VEGF. Am J Pathol. 1999;154(2):355–63.
271. Falanga V. Apligraf treatment of venous ulcers and other chronic wounds. J Dermatol. 1998;25(12): 812–7.
272. Falanga VJ. Tissue engineering in wound repair. Adv Skin Wound Care. 2000;13(2 Suppl):15–9.
273. Ehrlich HP. Understanding experimental biology of skin equivalent: from laboratory to clinical use in patients with burns and chronic wounds. Am J Surg. 2004;187(5A):29S–33.
274. van Schie CH, Rawat F, Boulton AJ. Reduction of plantar pressure using a prototype pressure-relieving dressing. Diabetes Care. 2005;28(9):2236–7.
275. Jeffcoate WJ, Price PE, Phillips CJ, Game FL, Mudge E, Davies S, Amery CM, Edmonds ME, Gibby OM, Johnson AB, Jones GR, Masson E, Patmore JE, Price D, Rayman G, Harding KG. Randomised controlled trial of the use of three dressing preparations in the management of chronic ulceration of the foot in diabetes. Health Technol Assess. 2009;13(54):1–86, iii–iv.
276. Donaghue VM, Chrzan JS, Rosenblum BI, Giurini JM, Habershaw GM, Veves A. Evaluation of a collagen-alginate wound dressing in the management of diabetic foot ulcers. Adv Wound Care. 1998;11(3):114–9.