Advanced Drug Delivery Reviews 129 (2018) 262–284

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

Nanomedicines and gene therapy for the delivery of growth factors to improve perfusion and oxygenation in wound healing☆

Céline M. Desmet [a], Véronique Préat [b,*], Bernard Gallez [a]

[a] Université catholique de Louvain, Louvain Drug Research Institute, Biomedical Magnetic Resonance Research Group, Brussels, Belgium
[b] Université catholique de Louvain, Louvain Drug Research Institute, Advanced Drug Delivery and Biomaterials, Brussels, Belgium

ARTICLE INFO

Article history:
Received 23 October 2017
Received in revised form 25 January 2018
Accepted 3 February 2018
Available online 12 February 2018

Keywords:
Wound healing
Oxygenation
Perfusion
Diabetes
Growth factors
Nanomedicines
Gene therapy
Skin

ABSTRACT

Oxygen plays a key role in wound healing, and hypoxia is a major cause of wound healing impairment; therefore, treatments to improve hemodynamics and increase wound oxygenation are of particular interest for the treatment of chronic wounds. This article describes the roles of oxygen and angiogenesis in wound healing as well as the tools used to evaluate tissue oxygenation and perfusion and then presents a review of nanomedicines and gene therapies designed to improve perfusion and oxygenation and accelerate wound healing.

Contents

Abbreviations: Ang-1, angiopoietin 1; AP-57, antimicrobial peptide 57; CS, chitosan; Cur, curcumin; ECM, extracellular matrix; EGF, epidermal growth factor; eNOS, endothelial nitric oxide synthase; EPCs, endothelial progenitor cells; EPR, electron paramagnetic resonance; FGF, fibroblast growth factor; FPRL1, formyl peptide receptor-like 1; GFs, growth factors; GM-CSF, granulocyte macrophage-colony stimulating factor; hCAP18, human cationic antimicrobial protein; HGF, hepatocyte growth factor; HIF-1, hypoxia-inducible factor 1; HRE, hypoxia responsive element; IGF, insulin-like growth factor; IL, interleukin; iNOS, inducible nitric oxyde synthase; LDH1, lactate deshydrogenase 1; MMP, matrix metalloproteinase; NFs, nanofibers; NLC, nanostructured lipid carriers; NPs, nanoparticles; ORP150, 150 kDa oxygen regulated protein; PCL, poly(ε-caprolactone); PDGF, platelet-derived growth factor; PHD, prolylhydroxylase; PLA, poly(lactic acid); PLGA, poly(lactic-*co*-glycolic acid); PlGF, placenta growth factor; PLLA, poly(L-lactic acid); pO_2, partial pressure of oxygen; PPADT, poly-(1,4-phenyleneacetone dimethylene thioketal); PRP, platelet-rich plasma; ROS, reactive oxygen species; SDF-1α, stromal cell-derived factor 1α; SLN, solid lipid nanoparticles; $TcPO_2$, transcutaneous partial pressure of oxygen; TGF-β, transforming growth factor β; TNF-α, tumor necrosis factor α; TSP-1, thrombospondin 1; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; vWF, Von Willebrand factor; α-SMA, α-smooth muscle actin.

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Wound healing and scar wars - Part 1".

* Corresponding author.
E-mail address: veronique.preat@uclouvain.be (V. Préat).

https://doi.org/10.1016/j.addr.2018.02.001
0169-409X/

1. Introduction

Wound healing is a complex and dynamic process that is classically described to encompass three interrelated and overlapping phases (the inflammatory, proliferative and remodeling phases) involving the release of many cytokines and growth factors (GFs) by different cell types [1]. The inflammatory phase involves the recruitment of neutrophils and monocytes at the wound site to prevent the wound from becoming infected. The proliferative phase consists of tissue reconstruction via the proliferation and migration of fibroblasts, endothelial cells (angiogenesis), and keratinocytes (re-epithelialization). Finally, remodeling of the extracellular matrix (ECM) occurs during the third phase. After this sequence of events occurs, wound healing is achieved. However, chronic wounds fail to complete this sequence of events, and impaired healing is observed in such cases.

The causes of wound healing impairment are multifactorial [2], but one major cause is wound hypoxia. As we will describe later, wound healing is particularly sensitive to tissue perfusion and oxygenation [3–6] (Fig. 1). Consequently, improving hemodynamics via the delivery of GFs or compounds that increase oxygenation in wound tissue is an

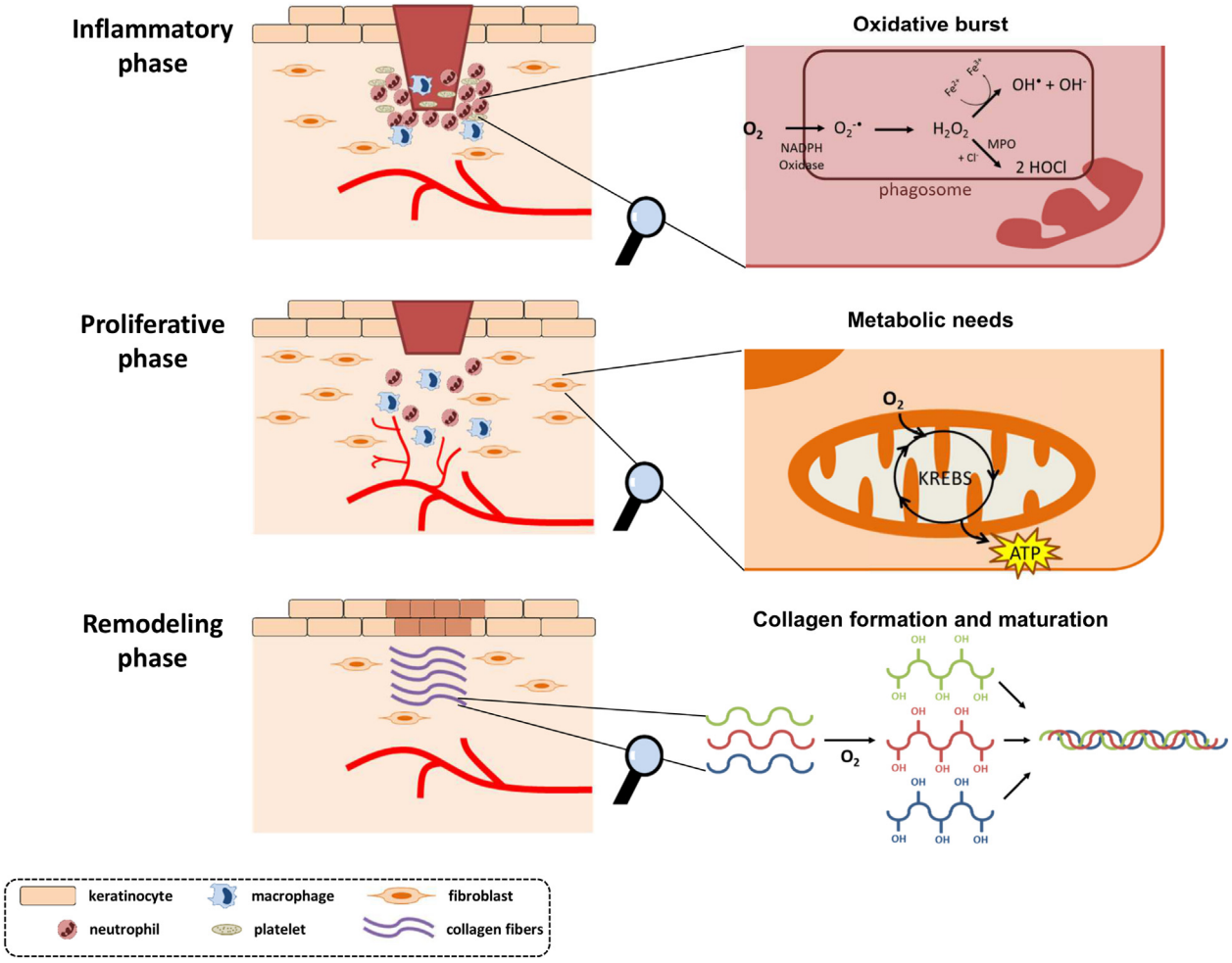

Fig. 1. The pivotal role of oxygen during wound healing. During the inflammatory phase, oxygen is highly consumed within the phagosome (respiratory burst) to produce oxidants, which favor the bactericidal defense against pathogens. During the proliferative phase, oxygen is used as an energy supplier to generate ATP, which supplies energy to promote cell proliferation. During the proliferative and remodeling phases, oxygen is necessary for mature collagen production and deposition by fibroblasts.

emerging strategy to promote the healing of chronic wounds. However, the use of GFs or other molecules/proteins in chronic wound tissue is hampered by their rapid degradation [7–9].

In this review article, we will focus on the potential role of two innovative tools that are nanomedicines and gene therapy GF-based delivery strategies that are designed to improve perfusion, oxygenation and angiogenesis and to accelerate wound healing. These strategies are proposed as they promote a sustained delivery or production of GFs which is particularly important in the proteolytic environment of wounds (and more importantly in chronic wounds).

2. Oxygenation and perfusion in wound healing

The pivotal role of oxygen during the wound healing process is well established [4–6,10] (Fig. 1). We briefly describe its functions in the three steps of the normal healing process as well as the specific problems associated with wound hypoxia in diabetic patients.

2.1. Role of oxygen in wound healing

Due to the disruption of blood vessels in the wound site, acute hypoxia is present during the early stage after wounding. This hypoxia triggers the first steps of the healing process: the activation of platelets and endothelium, the induction of reactive oxygen species (ROS), and cytokine release. However, it is critical that hypoxia remains transient and does not become chronic, as oxygen is a crucial element during subsequent steps of the process and is required to achieve complete healing [5].

During the inflammatory phase, oxygen promotes the bactericidal defense against pathogens. After pathogen phagocytosis, oxygen is used by nicotinamide adenine dinucleotide phosphate (NADPH)-linked oxygenases present in leukocytes to produce superoxide anions. This superoxide anion is then transformed into other ROS, such as hydrogen peroxide or hydroxyl radicals, and is also further used by myeloperoxidase to produce hypochlorous acid [11]. The products of this reaction promote bacterial killing in wounds [4,10,12]. This oxidant production within the phagosome, called the respiratory burst, consumes high amounts of oxygen. It has been reported that approximately 98% of oxygen consumed by neutrophils is used for this respiratory burst [11]. NADPH-linked oxygenases require 45–80 mm Hg to reach 50% of their maximum enzymatic rate (Vmax), and more than 300 mm Hg is required to reach 90% of the Vmax [4,11]. Thus, resistance to infection is critically impaired by wound hypoxia [4]. In addition, ROS produced during the inflammatory phase fulfill other roles in the healing process, such as coagulation, cytokine release, cell proliferation, re-epithelialization, angiogenesis and matrix deposition.

During the proliferative phase, oxygen is used as an energy supplier by cells. To generate ATP, cells utilize oxygen as the final electron acceptor in the aerobic metabolism of glucose during oxidative phosphorylation in mitochondria [10]. Cell proliferation during wound healing consumes a high level of energy due to increased glycolytic metabolism and, to a lesser extent (30%), oxidative metabolism, which increase oxygen consumption in the wound [10,13]. Other roles for ATP in wound healing have also been described, including stimulation of the immune system [14], epidermal GF receptor activation in epithelial cells to promote wound closure [15], and the activation of NADPH-linked oxygenases. Furthermore, ATP is known to induce vasodilation [16] by activating plasma adenosine membrane receptors at the vascular endothelium.

Finally, oxygen is necessary for mature collagen production and deposition by fibroblasts during the proliferative and remodeling phases. The hydroxylation of proline and lysine residues of procollagen chains stabilizes the triple helices of collagen. This reaction is catalyzed by hydroxylases and requires high amounts of oxygen (20 mm Hg to reach 50% of the Vmax and more than 150 mm Hg to reach 90% of the Vmax), iron, ascorbic acid and α-ketoglutarate as cofactors. Additionally, the wound contraction observed during this phase is attributable to the differentiation of fibroblasts into contractile myofibroblasts. This differentiation is activated by transforming growth factor (TGF)-β1, TGF-β2 and platelet-derived growth factor (PDGF) in the presence of oxygen [5].

2.2. Diabetic wounds

Diabetic foot disease is a common complication of diabetes, affecting approximately 1 in 6 diabetic patients [17]. Furthermore, approximately 1 in 4 ulcers will present impaired healing, and it is estimated that 1 million diabetic persons undergo lower limb amputation each year following the impairment of chronic wound healing [18]. Hypoxia is a main cause of diabetic wound impairment and is caused by two factors: a limited oxygen supply and a high oxygen consumption rate inside the wound [4].

In diabetic patients, oxygen supply to the wound is limited due to vascular dysfunction and neuropathy. Diabetic patients often suffer from atherosclerosis in large vessels, resulting in decreased blood flow. At the microvascular level, pathological alterations are also observed. Capillaries exhibit reduced size, basement membrane thickening and hyalinosis [19,20]. Basement membrane thickening is induced by increased hydrostatic pressure and shear forces due to a decrease in the capacity for effective precapillary vasoconstriction [21]. This results in reduced physiological exchanges (nutrients and gases), altered leukocyte migration, and the loss of the elastic properties of capillaries, thus inducing a limited capacity for vasodilation and a decreased hyperhemic response to injury [19–21]. These capillaries suffer from an abnormal auto-regulatory capacity and a reduction in nitric oxide synthase (NOS) [19]. A decrease in NO production induces impaired vasorelaxation that contributes to microvascular dysfunction [22]. Shunts in the microcirculation combined with autonomic neuropathy and denervation induce a maldistribution of blood flow, which is deviated from nutritional capillaries to subpapillary vessels [19,21]. Limited blood flow to the wound is also attributable to inadequate pressure exerted on the foot as a consequence of motor and sympathetic nerve function impairment (neuropathy) [20]. Periwound fibrosis and edema, which are often observed in diabetes, limit the oxygen supply by increasing the distance between the wound and the capillaries [4].

In addition to inadequate oxygen supply, high oxygen consumption by wound cells during inflammation also induces hypoxia in wounds. Inflammatory cells consume large amounts of oxygen during respiratory bursts. If a sustained inflammatory phase is observed, oxygen availability will be a limiting factor for the other steps in wound healing.

The consequences of chronic hypoxia in wounds include an inability to achieve the inflammatory phase, a lack of cellular proliferation and motility, and a lack of mature collagen production. All of these factors lead to impaired wound healing. Lack of oxygen induces a decrease in ATP production.

2.3. Angiogenesis in wounds

Angiogenesis is the process through which new blood vessels form from pre-existing vessels. Angiogenesis is mainly regulated by vascular endothelial growth factor (VEGF) and fibroblast growth factor 2 (FGF-2). FGF-2 is released by fibroblasts, keratinocytes, endothelial cells, smooth muscle cells, and chondrocytes early in the healing process [23,24] and may initiate angiogenesis in wounds. The VEGF family comprises 5 members: VEGF-A (commonly named VEGF), VEGF-B, VEGF-C, VEGF-D, and placental growth factor (PlGF) [25,26]. Different VEGF isoforms exist due to alternative mRNA splicing [25]. VEGF binds three different tyrosine kinase receptors: VEGFR-1 (Flt-1), VEGFR-2 (KDR) and VEGFR-3. While VEGFR-1 and VEGFR-2 play key roles in angiogenesis, VEGFR-3 plays a role in lymphangiogenesis [25–28]. VEGF, which is mainly expressed during granulation tissue formation (from day 4 to

7) [29], is released by platelets [30], macrophages, endothelial cells [29], activated epidermal cells and fibroblasts following tumor necrosis factor (TNF)-α induction [31]. Furthermore, VEGF promotes migration and mitogenic stimulation of endothelial cells as well as smooth muscle cell migration [1,2], which is required to build new blood vessels. Additionally, VEGF enhances the permeability of vessels. VEGF expression in wounds is upregulated by EGF, FGF-2, PDGF (synergistically with hypoxia) and by hypoxia through hypoxia-inducible factor 1 (HIF-1) activation [32]. Under hypoxia, the HIF-1α subunit migrates to the nucleus, where it binds to the HIF-1β subunit. The formed HIF-1 complex binds to hypoxia responsive element (HRE), a DNA sequence in the promoters of target genes implicated in angiogenesis (via VEGF gene expression) as well as in genes involved in cell mobility, metabolic adaptation (anaerobic glycolysis), erythropoiesis, and GF signaling [32]. PlGF is expressed by keratinocytes and endothelial cells [23]. PlGF release permits monocyte chemotaxis and the mobilization of bone marrow-derived endothelial progenitor cells (EPCs) [33,34] as well as granulation tissue formation and maturation. PDGF also plays a role in angiogenesis by promoting the recruitment of pericytes to capillaries to increase the structural integrity of vessels [35]. SDF-1α promotes angiogenesis by recruiting EPCs from the circulation to peripheral tissues [36]. Other molecules, such as angiogenin, angiotropin, angiopoietin 1 (Ang-1), GM-CSF and Thrombospondin 1 released by macrophages and keratinocytes [37], promote angiogenic activity [38–40] as well as the migration and proliferation of endothelial cells.

3. Tools to evaluate oxygenation, perfusion and angiogenesis in wound healing

Oxygen is an important factor in wound healing, and measurement of tissue oxygenation is of great interest to better understand the physiopathology of wound healing and to develop new treatments. In clinical practice, measuring oxygen is useful to evaluate wound outcomes and to evaluate treatment efficacy. Only a few techniques permit the measurement of oxygenation inside wounds. Therefore, other techniques providing indirect information about tissue oxygenation, such as blood perfusion or the formation of new vessels (angiogenesis), are also used. Intensive research is ongoing to overcome existing limitations, such as invasiveness or complexity of use. Below we describe certain reviewed techniques used to evaluate the tissue partial pressure of oxygen (pO_2), angiogenesis and perfusion in wound healing studies and clinical practice.

3.1. Measurement of pO_2 by polarographic electrodes

The use of polarographic electrodes is a direct method to measure tissue oxygenation. It is based on the electrochemical reduction of oxygen molecules at the electrodes. The use of polarographic Clark electrodes [41] is still considered the gold standard method for measuring tissue oxygenation [5,42]. However, the introduction of electrodes is invasive and induces injuries that modify the tissue environment. Electrodes cannot be introduced exactly at the same location on consecutive days, preventing reliable longitudinal studies of tissue oxygenation. Additionally, electrodes by themselves consume oxygen during measurement, modifying the wound environment. Finally, electrodes cannot be calibrated when introduced inside the tissue. Hunt and his group, who were first to study oxygenation in wound healing, improved the polarographic electrodes [4,43]. Measurements were first made in human volunteers [44] and then in trauma patients [45,46] and animal models; all consistently demonstrated the role of hypoxia in impaired healing [4]. However, this old technique is not currently used in clinical practice due to its invasiveness.

To overcome the invasiveness of this technique, measurement of the transcutaneous partial pressure of oxygen ($TcPO_2$) was developed using heated (42–45 °C) electrodes stitched onto the surface of the skin [47–49]. Heating the electrodes is believe to dilate the

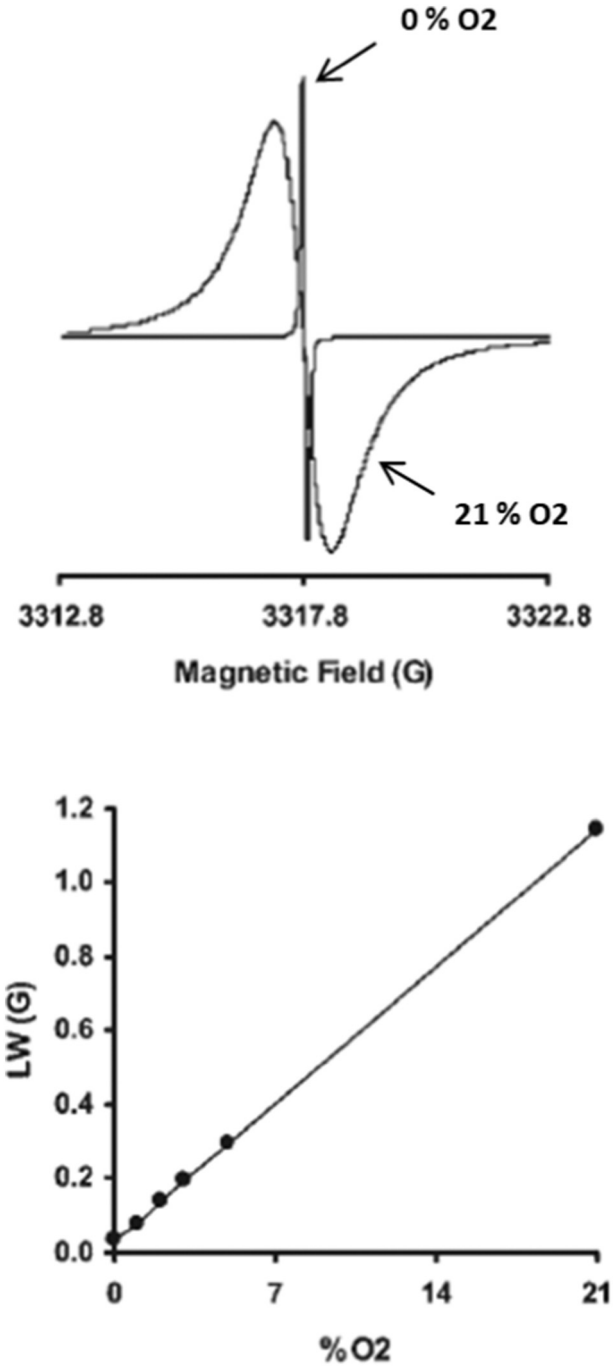

Fig. 2. The effects of oxygen on the line widths of EPR signals and oxygen calibration curves of the particulate paramagnetic probe lithium phthalocyanine (adapted from [55]).

capillaries, open skin pores, decrease O_2 solubility, and shift the oxy-hemoglobin curve to the right for the ready release of oxygen [50]. Changes induced by the heated electrodes cause "arterialization" of the skin capillary blood. The capillary pO_2 reaches arterial levels and then diffuses from the capillaries across the skin, where it is measured. Thus, the recorded O_2 is the excess oxygen that is dispersed through the skin that is not used for cellular metabolism. As the amount of oxygen available for diffusion across the skin depends on oxygen delivery, the release of O_2 greatly exceeds the consumption and the $TcPO_2$ is closer to the arterial pO_2 with a high cutaneous flow. With a low cutaneous flow, the $TcPO_2$ decreases. Consequently, $TcPO_2$ is a measurement of perfusion and vascular reserve rather than a measurement of oxygenation. This technique is widely used

in clinical practice despite some important disadvantages: measurements are indirect, heating may induce physiological changes in the skin, and measurements must be realized near the wound as electrodes cannot be applied inside the wound. Nonetheless, indirect $TcPO_2$ data are predictive of wound healing [49,51,52], and this technique is still commonly used in clinical practice to predict healing or when considering amputation in patients with diabetic foot disease.

3.2. Measurement of pO_2 by electron paramagnetic resonance (EPR) oximetry

EPR is a technique permitting the direct measurement of tissue oxygenation. EPR oximetry is based on the paramagnetic properties of oxygen. As it is not possible to directly measure oxygen dissolved in fluids due to broadening of the EPR signal [53], EPR oximetry is based on the oxygen-dependent signal broadening of a paramagnetic probe. The interaction between unpaired oxygen electrons and certain paramagnetic probes (e.g. lithium derivatives or carbon-centered derivative particulate probes) shortens the T_2 relaxation time, which is related to a broadening of the EPR spectrum recorded from the probe. Line width broadening is directly proportional to the surrounding oxygen concentration, and with appropriate calibration, tissue pO_2 is determined from the line width of the spectrum that is recorded from the paramagnetic oxygen sensor implanted in the tissue [54–56] (Fig. 2).

EPR is minimally invasive: once the oxygen sensor is implanted in the tissue, EPR spectra are recorded non-invasively in vivo using a low frequency (L-band) EPR spectrometer [55,56]. Furthermore, it is possible to record measurements at the same location repeatedly over a long period of time, as long as several years [57], allowing for longitudinal studies. EPR oximetry was used to demonstrate hypoxia in ischemic flaps compared to normal skin [58] and to monitor the influence of Thrombospondin 1, a protein with antiangiogenic activity, on perfusion and oxygenation of ischemic flaps in a mouse model [59]. Additionally, EPR oximetry is applicable for monitoring tissue pO_2 during wound healing in acute and chronic diabetic wounds but is limited to flaps because excisional wound measurements are affected by the diffusion of atmospheric oxygen [60]. The measurement of wound oxygenation by EPR oximetry permitted the assessment of the effects of an LL37 peptide-based treatment designed to modulate hypoxia in wounds [61]. Experimental results obtained in animal studies were sufficiently encouraging to translate the technique from animals to humans, and EPR oxygenation measurements are now feasible in humans [62,63].

3.3. Measurement of perfusion by laser Doppler imaging

Laser Doppler imaging is a technique that measures blood perfusion in tissue. It is based on the measurement of the wavelength change of electromagnetic radiation after reflection on a moving element (Doppler effect), which in this case is moving red blood cells in vessels [64]. A helium neon laser beam [65] is emitted from a source to the skin, where it is backscattered. Differences in wavelengths measured between the emitted and reflected radiation provide information about the speed of the red blood cells and consequently about blood perfusion [64]. With a flowmeter, a laser Doppler imager scans an entire region of interest to generate perfusion maps [65]. Measurement depths are typically between 1 and 1.5 mm, thus limiting measurements in the superficial vascularization, which corresponds in humans to arterioles, capillaries, and postcapillary venules of the upper horizontal plexus [65] but not the deep horizontal plexus. This limited depth of measurement is a limiting factor for deeper wounds. The information provided by the measurements is only semi-quantitative [66], and results are given in relative units. Nonetheless, laser Doppler imaging is commonly used in clinical practice due to the simplicity of use and the non-invasive

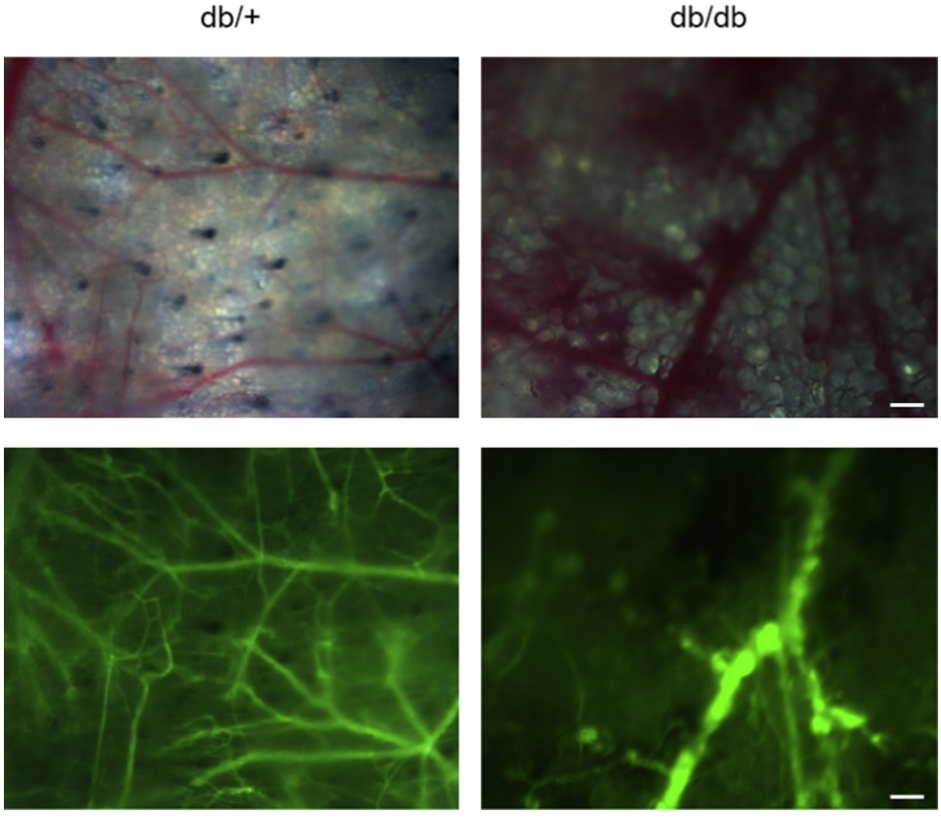

Fig. 3. Skin microvascular network observed by intravital microscopy in db/+ (left) and db/db (right) mice. Top: visible light images; bottom: fluorescence images (FITC-dextran) (scale bar = 200 μm) (adapted from [60]).

nature of the technique for the assessment of burn wounds, diabetic foot ulcers [67], pressure ulcers, venous ulcers and scars [64]. Laser Doppler imaging was also used in experimental studies with animals, primarily to evaluate flap reperfusion [68,69] but also to study perfusion in excisional wounds [70–72] for example, to evaluate the effects of a treatment.

Another recent laser-based technique permitting the study of perfusion is laser speckle imaging. Photons emitted by a laser source are reflected and interfere with each other due to irregularities in the tissue, causing a speckle pattern. With moving red blood cells, dynamic speckle patterns that change with time are created, causing a blurred speckle pattern in recorded images. A study comparing laser Doppler and laser speckle imaging in human burn scars showed that the results obtained with both techniques correlated well [73]. Laser speckle imaging is more advantageous for clinical applications due to the faster scan time, higher resolution and reduction in specular reflection artifacts observed using laser Doppler. The technique was used to evaluate blood flow in free flap surgery in humans [74], to monitor blood flow in full-thickness skin wounds in pigs [75] and to monitor flap revascularization in rats [76,77].

3.4. Measurement of angiogenesis by intravital microscopy

In experimental wound healing studies, intravital microscopy is an interesting technique to observe angiogenesis as a dynamic process during a prolonged period of time (2–3 weeks) in a living animal [78] via the implantation of an observation chamber on the dorsal skinfold. After placement of the chamber, repeated observations of the microcirculation (vessel density, vessel functionality, red blood cell velocity, cell interactions), skin tissue or striated muscle are performed in a non-invasive manner by using intravital microscopy with or without fluorescence, which requires the intravenous administration of a fluorescent dye such as fluorescein isothiocyanate (FITC)-labeled dextran [79]. Chamber implantation and the tissue preparation procedure have been modified to achieve ideal conditions for the in vivo analysis of primary and secondary wound healing as well as for revascularization and blood perfusion of skin grafts, dermal substitutes and myocutaneous flaps [79]. In an incisional wound model, intravital microscopy allowed observers to follow changes in microvessel diameter, red blood cell velocity, and the functional density of microvessels during the healing process via repetitive observations over the course of 12 days. This technique also permitted the visualization of impaired microvascularization in a diabetic model for comparison with a non-diabetic model [60] (Fig. 3).

3.5. Histology/immunohistochemistry and biochemical measures

Histology (hematoxylin and eosin and Masson's trichrome staining) is often used to evaluate wound closure, re-epithelialization, inflammation, fibrosis, infection and angiogenesis. This approach allows the qualitative evaluation of vascularization, e.g. by scoring. Immunohistochemistry is better suited for quantification. The use of specific markers to stain vessels permits the quantification of vessel numbers or an area of stained pixels per random field at one specific moment during the healing process. Commonly used markers are platelet-endothelial cell adhesion molecule-1 (PECAM-1), also known as CD31; CD34; Von Willebrand factor (vWF), also known as factor VIII; isolectin; and α-smooth muscle actin (α-SMA). Additionally, histology/immunohistochemistry does not provide information about blood perfusion or vessel functionality—only the vascular architecture [80]. Immunohistochemistry is also used to detect and/or quantify hypoxic areas in wounds. Hypoxic areas are visualized with the use of nitroimidazoles such as pimonidazole. Pimonidazole binds to peptide thiol groups in cells when pO_2 is less than 10 mm Hg, forming adducts detectable by immunohistochemistry [81]. Pimonidazole staining has been used to observe hypoxia in burn wounds [82], incisional wounds [83,84] and skin flaps [85]. Other markers often associated with tissue hypoxia such as HIF-1 and/or HIF-2 [86,87] or carbonic anhydrase (CA) IX [88–90] were also used to observe hypoxia in wounds in few studies.

Biochemical methods are also used to quantify angiogenesis in wounds. Among them, we cite the quantification of proteins implicated in angiogenesis, such as VEGF [85,91–93], VEGFR [91,94], and HIF-1α [91], by ELISA or Western blot. Quantification of the expression of genes implicated in angiogenesis (such as VEGF, VEGFR, SDF1α, and α-SMA) by RT-qPCR has also been performed to quantify angiogenesis in wounds [91,92,94].

3.6. Measurement of oxygenation, perfusion and angiogenesis in wound healing in animal models

Experimental studies on animals are needed to investigate the complex mechanisms occurring in wound healing and to evaluate the efficacy of treatments. In this context, numerous studies were performed in order to create animal models that can reproduce human wounds.

Animal models the most frequently used in wound healing studies are rodents because of their low cost, high availability, ease to handle. Also, the numerous existing models of transgenic mice, knockout mice and other disease models favor the use of these animals to study the role of some genes in wound healing [95]. But skin morphology of rodents is not similar to human skin morphology. In particular, rodent skin is characterized by the presence of the panniculus carnosus. This muscle layer present under the dermis promotes wound closure preferentially by contraction whereas in humans, the skin is fixed to the underlying tissue and the wound closes by re-epithelialization. However, studies showed that this contraction phenomenon in rodents can be prevented by fixing a silicone ring around the wound [96,97]. In addition, other anatomical and physiological differences between rodent and human skin can influence the wound healing process: rodent skin

Table 1
Growth factors released during the healing process and hemodynamics. (Adapted from [23]).

Growth factors	Cells releasing the growth factor	Function
EGF	Platelets	Re-epithelialization
	Macrophages	
	Fibroblasts	
FGF-2 (=bFGF)	Keratinocytes	Granulation tissue
	Mast cells	formation/angiogenesis
	Fibroblasts	Re-epithelialization
	Endothelial cells	Matrix formation and remodeling
	Smooth muscle cells	
	Chondrocytes	
IL-1	Neutrophils	Inflammation
	Monocytes	Re-epithelialization
	Macrophages	
	Keratinocytes	
IL-6	Neutrophils	Inflammation
	Macrophages	Re-epithelialization
PDGF	Platelets	Inflammation
	Keratinocytes	Granulation tissue
	Macrophages	formation/angiogenesis
	Endothelial cells	Re-epithelialization
	Fibroblasts	Matrix formation and remodeling
TGF-β	Platelets	Inflammation
	Keratinocytes	Granulation tissue formation
	Macrophages	Re-epithelialization
	Lymphocytes	Matrix formation and remodeling
	Fibroblasts	
TNF-α	Neutrophils	Inflammation
	Macrophages	Re-epithelialization
VEGF	Platelets	Granulation tissue
	Neutrophils	formation/angiogenesis
	Macrophages	
	Endothelial cells	
	Smooth muscle cells	
	Fibroblasts	

is thinner than human skin and possesses a different density of hair follicles, sebaceous and sweat glands. Also, until now, rodent models do not perfectly mimic chronic wounds in humans [98]. Most experimental chronic wounds consist in acute wounds which are realized on models reflecting pathological conditions linked to impaired healing, such as hypoxia (ischemic ear model, ischemic flap), diabetes or aging.

Another interesting animal model for wound healing studies is the pig. It was previously shown that wound healing in pigs had more concordance with human wound healing than other animals. Indeed, pig skin presents structure similarities with the human skin (no fur, skin adherence to underlying structures, similar epidermal cell turn-over, similar proteins and lipid composition of the stratum corneum) [99]. The immune system in pig skin is also described to be similar to human skin immune system [100]. But it should be noted that the pig dermis and hair follicles are less vascularized than human skin and the endothelium of cutaneous blood vessels does not produce alkaline phosphatase. Also, pig skin possesses more apocrine sweat glands than eccrine glands whereas human skin possesses more eccrine glands [101].

Concerning more precisely wound oxygenation, perfusion or angiogenesis, all animal models do not have the same relevance. Oxygenation measurement by polarographic electrodes should be more relevant in larger animals, due to the size of the implanted electrodes compared to the skin thickness. Also, EPR oximetry can be applied on small animals but the skin thickness of rodents can be a limiting factor. Indeed, crystals needed for oximetry measurements usually measure hundreds of μm to 1 mm which represent a rather large size compared to the mice skin thickness [60]. That limits the spatial accuracy and prevents accurate measurements of the different parts of the skin (epidermis, dermis, and hypodermis). Pig skin which is thicker should be more appropriate but actually, conventional EPR L-band spectrometers possess a distance between the magnets which allows the placement of rodents but not pigs. Perfusion measurements give satisfactory results in all animal models. Angiogenesis studies by histology and molecular biology methods are relevant in rodents but can also be applied on all animal models whereas intravital microscopy is dedicated for studies on small animals (rodents).

3.7. Concluding remarks on the methods used to assess wound perfusion and oxygenation

Among all techniques cited, $TcPO_2$ to evaluate oxygenation and laser Doppler imaging to evaluate perfusion, sometimes complemented by angiography, are the most common methods used to assess wound status in clinical practice. In experimental animal studies, angiogenesis (primarily measured by histology/immunochemistry) and perfusion (evaluated by laser Doppler imaging) are usually employed. Direct measurement of oxygenation (by EPR oximetry or polarographic electrodes) in experimental models is almost never performed.

4. Nanomedicines for the delivery of growth factor to improve wound oxygenation and perfusion and to hasten wound healing

Because GFs are important mediators in wound healing (Table 1), their administration represents a particularly interesting strategy to treat chronic wounds. Here, we will primarily focus on GFs such as VEGF, bFGF, and PDGF-BB that are known to promote angiogenesis to increase perfusion and wound oxygenation levels.

While theoretically promising, only a few clinical studies investigating GF administration have been performed. To date, only one GF, human recombinant PDGF, was FDA-approved and commercialized as Regranex® (Becaplermin) [102]. Overall, there is scant evidence suggesting the efficacy of GF treatment [103–105] due to the instability of GFs in wounds and the need for sustained exposure. GFs are primarily administered as solutions, creams, gels or ointments [106]. However, repeated high doses of GFs are required to obtain beneficial effects from treatment [106]. Thus, drug delivery systems have been developed to overcome the rapid degradation of GFs by proteases and to facilitate GF release in a sustained and controlled manner. Table 2 summarizes studies in which GFs were included in drug delivery systems to promote wound healing; when available, information on angiogenesis, blood vessels and pO_2 was included. Drug delivery systems used for GF release include hydrogels, matrices, scaffold-like films and sponges. Nanotechnologies have also been employed, including nanoparticles (NPs) and nanofibers (NFs) produced by electrospinning [106]. Different materials are used to create NPs and NFs, including synthetic copolymers such as poly(lactic-*co*-glycolic acid) (PLGA), poly(lactic acid) (PLA), and poly(ε-caprolactone) (PCL) as well as natural polymers such as chitosan, gelatin, alginate, hyaluronan and lipids [106]. Altering the composition, concentration or molecular weight of certain components allows drug release to be modified. These NPs or NFs can be included in a matrix or hydrogel that acts as a scaffold [107].

4.1. GFs encapsulated in PLGA

PLGA is one of the most commonly used polymers to produce NPs and microspheres [106,108]. PLGA is US FDA- and EMA-approved for use in parenteral products in humans. The polymer is biocompatible and completely biodegradable. PLGA-associated properties of NPs that influence drug release can be altered by varying the ratio of lactate and glycol, the molecular weights of polymers and the production method [109]. Drug release in NP PLGA systems involves two phases. During the initial burst phase, the drug is released in a manner dependent on properties such as solubility, hydrophobicity and polymer concentration [110]. During the second phase, polymer hydrolysis into monomers induces the progressive release of the drug via diffusion and erosion until complete polymer solubilization is achieved [110]. Interestingly, PLGA releases lactate during its degradation. Lactate was shown to promote wound healing by inducing collagen synthesis through the activation of collagen prolylhydroxylase (PHD) in fibroblasts [111,112]. Lactate was shown to stimulate endothelial cell migration via VEGF production [113]. In addition, during the oxidation of lactate to pyruvate, lactate dehydrogenase 1 (LDH1) produces NADH and pyruvate. Pyruvate and ROS produced by NADPH oxidase in the presence of NADH inhibit HIF PHD, resulting in the stabilization of HIF, which migrates to the nucleus and promotes the transcription of genes associated with angiogenesis [114]. Porporato et al. showed that the administration of exogenous lactate accelerated wound healing, and PLGA facilitated the sustained delivery of lactate to promote the healing and reperfusion of ischemic wounds [114].

The positive effects of PLGA NPs alone on wound healing and angiogenesis were described by Chereddy et al. [94,115,116], who showed that PLGA (50:50) NPs containing VEGF promoted faster wound healing, re-epithelialization, granulation tissue formation, and neovascularization in non-diabetic as well as diabetic wounds, whereas VEGF alone did not exert any beneficial effects on wound healing. Interestingly, the same authors also showed that empty PLGA NPs accelerated wound healing and promoted re-epithelialization, granulation tissue formation and neovascularization, which they attributed to lactate released by hydrolysis of the PLGA NPs (Fig. 4). They concluded that the beneficial effects of these NPs on wound healing were attributable to the combined effects of VEGF sustained release and lactate [94]. In another experimental work, Golub et al. studied the effects of PLGA NPs containing VEGF on hindlimb ischemia in a mouse femoral artery ligation model [117]. Mice receiving encapsulated VEGF experienced increases in total vessel volume and vessel connectivity compared to treatment with non-encapsulated VEGF and saline. The authors concluded that the treatment induced the enlargement of pre-existing vessels and an increase in vessel interconnection. They did not observe any effects on vasculogenesis from PLGA NPs alone. The effects of VEGF on hindlimb ischemia were also tested with a fibrin complex containing heparin-functionalized NPs in a rabbit model [118]. The heparin-functionalized NPs promoted sustained release and enhanced the

Table 2
GFs in NPs/NFs used in experimental wound studies.

GF	Delivery mode	Wound model	Results	References
VEGF	PLGA 50:50 NPs	Excisional wound in NMRI and db/db mice	↗ wound kinetics closure ↗ angiogenesis (CD34) ↗ VEGFR-2 expression	[115]
	PLGA 50:50 NPs	Hindlimb ischemia in 7- to 8- week-old female 129/Sv	↗ angiogenesis (microCT angiography)	[117]
	Pluronic - PLGA 75:25 heparin-conjugated in fibrin gel complex	Hindlimb ischemia in male New Zealand White rabbits	↗ calf blood pressure (Doppler Blood Pressure System) ↗ angiogenesis (angiography, H&E, $_{vWF}$)	[118]
VEGF (in combination with HBOT)	Gelatine microspheres	Modified McFarlane flap model in male Sprague Dawley rats (3×9 cm^2)	↗ skin flap survival ↗ angiogenesis (H&E)	[138]
bFGF	Liposomes	Male and female Sprague Dawley rats with deep second degree burn wounds	↘ inflammation ↗ angiogenesis (H&E) ↗ wound closure kinetics ↗TGFβ ↗ cell proliferation PCNA ↗ collagen content	[136]
PDGF-BB	PLGA microspheres in nanofibrous PLLA scaffold	Mid-sagittal incisions on Sprague Dawley rats	↗ angiogenesis (vWF) Upregulation CXCL1, 2, 5, CCL 21b; Downregulation of CCL22 Upregulation muscle-related or cell-backbone-related genes Upregulation of IL-1 related genes	[120]
	Hyaluronan-based porous NPs/microparticles HYAFF11	Excisional wound (1 cm diameter) in male Wistar rats	↗ wound closure kinetics ↗ fibroblasts, macrophages, collagen, endothelial cells	[137]
PDGF-BB (in combination with chlorhexidine)	PLGA 50:50 and 85:15 microspheres + CHX	Sprague Dawley rats with 8-mm infected with *P. aeruginosa*	↘ infection (for 85:15) ↗ wound closure kinetics ↗ angiogenesis (CD31 and α-SMA)	[119]
SDF-1alpha	ROS-responsive NPs PPADT (poly-(1,4-phenyleneacetone dimethylene thioketal)	Full-thickness skin excisional wound (8 mm diameter) in 6-week-old male nude mice	↗ BMSC chemotaxis ↗ angiogenesis ↗ wound closure kinetics	[125]
EGF (in combination with curcumin)	$_{L}$-lactic acid ($_{L}$-LA) and reverse Pluronic®10R5 (10R5) NPs in hydrogel system	Male SD rats with full-thickness excision skin 2×2 cm^2	↗ wound closure kinetics ↗granulation tissue quality/maturity of dermis and epidermis/collagen deposition (H&E + MT) ↗ angiogenesis (CD31) ↗ TGF-β1 staining	[139]
Combination of GFs				
PDGF-BB-VEGF	PLGA NP(PBGF-BB)-CS/PEO NFs (VEGF)	Full-thickness excisional wound (5 mm diameter) in Sprague Dawley rats	↗ wound closure kinetics ↗ angiogenesis (H&E) ↗ granulation tissue (H&E) ↗ collagen deposition (MT)	[140]
PDGF-A-EGF-IGF-1	Nanocomplex Poloxamer 188 containing the GF conjugated to low-molecular-weight-protamine	Full-thickness excisional wound (6 mm diameter) in female hairless mice	↗ levels and proliferation of dermal fibroblasts ↗ granulation tissue formation ↗collagen content in the wound 9 days after acute wound healing (H&E) ↗ wound closure kinetics	[144]
VEGF-bFGF	PEtU-PDMS fibrin scaffold with PLGA 50:50-Pluronic F-127 NPs	Full-thickness dorsal wound (8 mm diameter) in 12 week-old db/db mice	↗ wound closure kinetics ↗ re-epithelialization ↘ inflammation ↗ granulation tissue + angiogenesis (H&E) ↗ collagen ↗ proliferation (Ki67) No significant changes compared to GFs alone	[142]
bFGF-VEGF-EGF-PDGF	Hyaluronic NFs loaded with bFGF and VEGF-loaded gelatine NPs Collagen NFs loaded with EGF and PDGF-loaded gelatine NPs	Excisional wound (15 mm diameter) in male Sprague Dawley rats STZ induced	↗ wound closure kinetics ↗ re-epithelialization ↗ angiogenesis (H&E, CD31, α-SMA) ↗ collagen	[141]
VEGF-EGF	CS microparticles in dextran-based hydrogel	Burn wound (2 cm diameter) in Wistar rats	↗ wound closure kinetics ↗ re-epithelialization ↗ granulation tissue (H&E)	[143]
PRP	Fragmin-protamine micro-nanoparticles	Split-thickness skin graft donor site model in male Fisher 344 rats ($3 \times 4 \times 0.04$ cm)	↗ re-epithelialization ↗ angiogenesis (H&E)	[148]
	Heparin-conjugated PLGA nanospheres in fibrin gel	2×2 cm excisional skin wound in 4 week-old female Balb/c nude mice	↗ wound closure kinetics ↗ re-epithelialization ↗ angiogenesis (vWF) ↗ PECAM-1 expression (RT-PCR) ↗ cell proliferation (PCNA)	[147]

efficacy of VEGF. Fibrin acted as a matrix for cell migration and as a support for proliferative vessels. Moreover, the inclusion of NPs in a fibrin scaffold (as well as other forms of matrix, described hereafter) permitted the slower release of GFs inside the wound than NPs alone. In vitro free VEGF was completely released from fibrin within 7 days, with 80% released on the first day, whereas 80% of VEGF loaded in NPs in fibrin

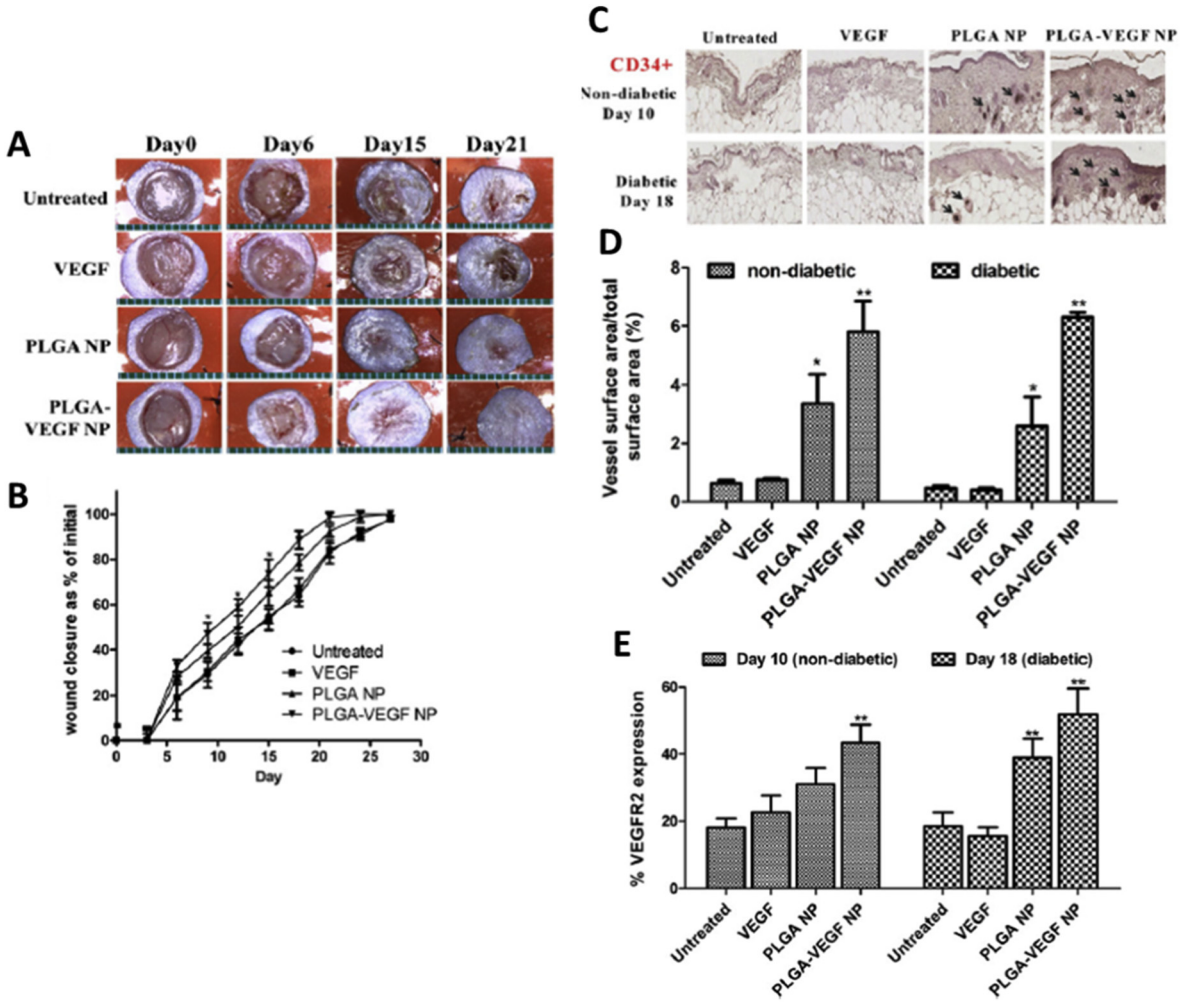

Fig. 4. Effects of PLGA-VEGF NPs on wound closure kinetics and angiogenesis. Effects of VEGF, PLGA NP and PLGA-VEGF NPs on wound healing: representative pictures of wounds (A) and the wound kinetics of closure. (B) Effects of VEGF, PLGA NP, and PLGA-VEGF NPs on angiogenesis: representative images of CD34 staining in the wound at days 10 and 18 (arrows indicate blood vessels). (C) Quantification of vessel surface area and (D) quantification of VEGFR-2 expression in wounds. The results are expressed as the mean ± standard deviation (SD) (adapted from [94]).

was released over more than 4 weeks with a reduced initial burst. This system promoted angiogenesis, unlike free VEGF in fibrin [118].

PLGA was also used to prepare microspheres releasing GFs. PLGA microparticles containing PDGF-BB and chlorhexidine were tested in excisional wounds infected via *P. aeruginosa* inoculation [119]. PLGA 85:15 was more efficient at decreasing infection, accelerating wound healing and stabilizing vascularization, as determined by increased CD31 and α-SMA staining. In this way, PLGA 85:15 demonstrated a better-adapted active drug release profile [119]. PDGF-BB was also encapsulated in PLGA microspheres incorporated in a poly(L-lactic acid) (PLLA) nanofibrous scaffold [120]. In vitro studies showed that PDGF was released in a sustained manner that was controlled by the molecular weight of the PLGA forming the microspheres [121]. Sustained release of PDGF from the microspheres in the NF delivery system also promoted cell migration and angiogenesis in vivo. One week after implantation in a surgical pocket in rats, scaffolds containing PLGA microspheres loaded with PDGF-BB presented notable tissue invasion and neovascularization (quantified by vWF staining) compared to controls. This scaffold also promoted the upregulation of several chemokines, such as CXCL1, 2, 5 and CCL21b, IL-1 [120].

4.2. GFs encapsulated in chitosan

Another material frequently used to produce GF delivery systems is chitosan (CS). In addition to its biocompatibility and biodegradability, an advantage of CS for wound healing lies in its antimicrobial properties [122]. Mechanisms underlying the antimicrobial actions of CS are still not fully understood, but CS, which is positively charged, interacts with the negatively charged microbial cell membrane, leading to alterations in cell permeability [122–125] and/or microbial cell membrane disruption that induces microbial cell death [123,124,126,127]. CS also promotes wound healing by increasing the functions of inflammatory cells [128–130] and fibroblasts [129,131,132] and increasing the tensile strength of a wound [133]. Bertoncelj et al. developed CS/poly(ethylene oxide) (PEO) NFs loaded with platelet-rich plasma (PRP) [134], which is enriched for several GFs, including PDGF, VEGF, and TGF-β. The authors showed that electrospinning enabled the production of CS/PEO NFs without modifying the biological activities of GFs. NFs demonstrated favorable properties in vitro (stimulation of keratinocyte and fibroblast metabolic activities, cell proliferation, and appropriate morphology) and maintained their morphology in a moist environment, making them a suitable support for cell adhesion and growth. CS NPs were also used to obtain hydrophobically modified CS via ionic interactions with either oleic and linoleic acid [135], as unsaturated fatty acids accelerate tissue repair mechanisms and encapsulate clarithromycin [135].

4.3. GFs encapsulated in other materials

In terms other materials that have been used to produce nanomedicines or microparticles releasing GFs in experimental wound

healing studies, illustrative examples include liposomes containing bFGF [136], hyaluronan-based porous NPs loaded with PDGF-BB [137], gelatin microspheres loaded with VEGF [138], and mesoporous silica NPs containing bFGF.

To achieve the sustained and specific release of a chemokine in a wound, an illustrative example used ROS-responsive NPs for the sustained release of SDF-1α, the chemokine that facilitates the mobilization and homing of EPCs to the wound site [125]. The rationale for using ROS-responsive NPs is that higher amounts of ROS are present in wounds than in normal tissues. Thus, this difference provides an efficient opportunity to target wound treatment because the system only dissociates in the wound, which is rich in ROS, and remains intact in normal tissues. The material used for this purpose was poly-(1,4-phenyleneacetone dimethylene thioketal) (PPADT), a polymer containing ROS-reactive thioketals. Thioketal bonds are cleaved in the presence of ROS, leading to depolymerization of the NPs and the release of SDF-1α in the wound. After daily intravenous injection in mice with excisional skin wounds, the wounds were specifically targeted by NPs, which accelerated wound closure, promoted wound vascularization, and promoted bone marrow-derived stem cells chemotaxis into the wound [125]. Importantly, pathologies such as diabetes also induce elevated levels of ROS in some tissues [22], therefore potentially leading to drug release in non-specific tissues, which would provoke side effects.

4.4. Delivery systems loaded with multiple GFs

An increasing number of studies have recently used combined therapies (several GFs or other drugs) rather than only one GF to identify efficient treatments for chronic wounds. As described previously, wound healing is a complex phenomenon involving many GFs and cytokines. Therefore, acting on only one GF may not be sufficient to reverse healing impairments. For example, Li et al. [139] designed a dual drug co-loaded in situ in a gel-forming NP/hydrogel system (EGF-Cur-NP/H), which acted not only as a supportive matrix for the regenerative tissue but also as a sustained drug depot for EGF and curcumin (Cur). This system was shown to accelerate the healing of full-thickness excisional wounds in rats and to increase granulation tissue, collagen deposition and angiogenesis, effects that were enhanced by the combination of EGF and Cur compared to the separate administration of each drug [139]. VEGF was loaded into CS/PEO NFs to promote angiogenesis in the short term (rapid release of VEGF within one day). In addition, PDGF-BB-encapsulated PLGA nanoparticles were embedded inside NFs to generate the sustained release of PDGF-BB (40% released by day 7) for accelerated tissue regeneration and remodeling [140]. In excisional wounds in rats, a scaffold increased capillary formation, collagen deposition, and epithelial regeneration [140]. Using a sequential GF-release system, Lai et al. [141] showed that excisional wounds in diabetic rats exhibited more rapid wound healing and enhanced vessel formation when administered a scaffold composed of hyaluronic acid NFs and collagen NFs loaded with bFGF plus VEGF-loaded gelatin NPs and EGF plus PDGF-loaded gelatin NPs, respectively. Losi et al. [142] used a fibrin-based scaffold incorporating VEGF and bFGF-loaded NPs to stimulate wound healing in full-thickness excisional wounds in db/db mice. This scaffold was composed of a fibrin and poly(ether)urethane-polydimethylsiloxane (PEtU-PDMS) layer, which provided mechanical resistance and improved the ease of handling. The encapsulation of GFs in PLGA 50:50 NPs did not improve treatment because faster wound healing was observed with free GFs in the fibrin scaffold. In addition, collagen deposition and vascularization were improved either with GFs encapsulated in NPs or free GFs. Ribeiro et al. [143] administered a dextran-based hydrogel containing CS microparticles loaded with VEGF and EGF in Wistar rat burn wounds. Hydrogel served as a dressing that mitigated tissue dehydration and bacterial contamination, and slow drug release was observed from the microparticles. This system facilitated faster healing and improved re-epithelialization and angiogenesis in wounds [143]. PDGF-A, EGF and IGF-1 [144] were administered in Poloxamer 188 nanocomplexes after their fusion with a highly positively charged low-molecular-weight protamine and their combination with low-molecular-weight heparin. EGF conjugation to protamine increased skin permeability in vitro and significantly accelerated wound healing, an effect that was attributed to higher absorption in the wound area [144–146]. No single GF promoted wound healing, unlike combined therapy. Encapsulation of the protamine-conjugated GFs in NPs significantly accelerated wound healing by protecting the GFs from proteolytic inactivation and promoting fibroblast proliferation and granulation tissue formation. Native GF complexes did not improve wound healing. Notably, it was necessary to administer the treatment twice per day over 9 days. Additionally, PRPs have been used in some studies because they contain several different GFs [147,148].

4.5. Delivery of other drugs

Molecules other than GFs exert beneficial effects on angiogenesis and wound healing. Similar to the use of GFs, strategies for NP/NF formulations to protect these molecules from degradation and prolong their release in wounds have been described. Among them, we cite NO, Cur, and antibacterial peptides such as LL37 (Table 3).

NO is a gaseous radical species with multiple roles in several physiological conditions and pathologies. Notably, in the case of wound healing, NO is present at each step of the process. NO acts as a vasodilator, has antimicrobial properties, prevents platelet aggregation, and induces vascular permeability during inflammation. NO also promotes angiogenesis through the activation of VEGF, bFGF and TGF-β and stimulates re-epithelialization by attracting IL-1 during the proliferative phase. Finally, NO promotes fibroblast activation and collagen production [149,150]. Decreased NO levels are observed in chronic wounds such as diabetic wounds [149]. Various NP formulations have been investigated to sustainably deliver NO into wounds [151]. Several studies demonstrated the antimicrobial or anti-fungal effects of NO released from NPs [152–154] or NFs [155,156] in wounds as well as beneficial effects on wound vascularization and wound healing [157,158]. Han et al. synthesized a hydrogel-glass composite using a mixture of tetramethylorthosilicate, polyethylene glycol, CS, glucose and sodium nitrite in sodium phosphate buffer. NO was produced via reduction of the nitrite inside the hydrogel. Mice receiving daily topical applications exhibited enhanced wound healing compared to non-treated mice or mice receiving empty NPs. Lower levels of inflammation and increased fibroblast migration and collagen deposition were observed. NPs modified leukocyte migration into the wounds and increased TGF-β production in wounds, promoting angiogenesis [158]. The same group used this system to improve wound healing in mice presenting immune deficiency and diabetes (NOD-SCID mice). NPs releasing NO were administered topically every 2 days, and decreased wound size was observed compared to the group receiving only NPs or a synthetic donor that spontaneously released NO. Beneficial effects were also observed in immunodeficient diabetic mice. Reduced numbers of inflammatory cells and increased fibroblast numbers were observed. Collagen deposition was significantly increased in mice compared to other groups. Neovascularization was also improved as demonstrated by CD34 staining.

Curcumin (Cur), a polyphenol extracted from the rhizome of *Curcuma longa*, has been described to promote wound healing [159]. Cur possesses antioxidant, anti-inflammatory and antimicrobial properties and also promotes granulation tissue formation, re-epithelialization, fibroblast proliferation, angiogenesis, collagen deposition and tissue remodeling [159]. Cur is highly hydrophobic and has poor stability, but its encapsulation in NPs or NFs improves wound healing. Many studies have demonstrated the antibacterial, anti-inflammatory and antioxidant activities of Cur encapsulated in NPs or NFs to favor wound healing [115,160–169]. Some studies also showed that Cur enhanced angiogenesis during wound healing [139,165,168,170]. The mechanisms

Table 3
Other wound therapies in NPs/NFs.

Molecules	Delivery mode	Wound model	Results	References
NO	Hydrogel-glass composite NPs (composed by tetramethylorthosilicate, polyethylene glycol, chitosan, glucose)	Full-thickness excisional skin wounds (5 mm diameter) in female 6–8 week-old Balb/c mice	↗ wound closure kinetics ↗ inflammation ↗ neutrophils infiltration ↗ macrophages infiltration ↗ fibroblasts migration ↗ collagen deposition ↗ TGF β ↗ angiogenesis (CD34)	[158]
		Full-thickness excisional skin wounds (5 mm diameter) in female 6–8 week-old NOD.SCID/NCr mice	↗ wound closure kinetics ↗ granulation tissue ↘ inflammatory cells ↗ fibroblast-like cells ↗ collagen ↗ angiogenesis (CD34)	[157]
Curcumin	Tetramethyl orthosilicate NPs	Burn injuries (5 mm diameter) in female 6–8 week-old Balb/c mice infected or not with MRSA	↘ bacterial count in infected wounds ↗ wound closure kinetics ↗ granulation tissue ↗ collagen deposition ↗ angiogenesis (CD34)	[165]
	Gum tragacanth/poly(ε-caprolactone) electrospun nanofibers	Full-thickness excisional skin wounds (10 mm diameter) in male adult Sprague Dawley rats STZ induced diabetic	↗ wound closure kinetics ↗ epithelial gap closure ↗ granulation tissue ↗ collagen deposition ↗ angiogenesis (H&E)	[168]
	Methoxy poly(ethylene glycol)-b-poly (ε-caprolactone) copolymer NPs loaded in CCS-OA hydrogel	Full-thickness excisional skin wounds (2 × 1.5 cm) in male adult Sprague Dawley rats STZ induced diabetic	↗ wound closure kinetics ↗ re-epithelialization ↗ collagen deposition ↗ granulation tissue ↗ angiogenesis (α-SMA) ↗ DNA and protein content	[170]
LL37	PLGA 50:50 NPs	Full-thickness excisional skin wounds (8 mm) in 6–7 week-old male NMRI mice	↗ wound closure kinetics ↗ re-epithelialization ↗ granulation tissue formation ↗ collagen deposition ↗ IL-6 and VEGFa expression ↗ angiogenesis (CD31) ↘ myeloperoxidase activity	[116]
	Nanostructured lipid carriers	Full-thickness excisional skin wounds (8 mm) in 8 week-old male db/db mice	↗ wound closure kinetics ↗ re-epithelialization Accelerated inflammation recovery ↗ granulation tissue ↗ angiogenesis (CD31) but not significantly ↗ collagen deposition but not significantly	[177]
LL37 in combination with Serpin A1	Solid lipid NPs (glyceryl monostearate and α-$_L$-phosphatidylcholine)	In vitro wound healing assay and BJ fibroblast cells and keratinocytes	↗ in vitro wound closure ↗ proinflammatory cytokines production (IL-6, IL-1β, TNF-α) ↗ collagen deposition ↗ antibacterial activity against *S. aureus* and *E. coli*	[178]
AP-57	Pluronic® L35 NPs in hydrogel	Full-thickness excisional skin wounds (2 × 2 cm) in male Sprague-Dawley rats	↗ wound closure kinetics ↗ re-epithelialization ↗ granulation tissue formation ↗ dermal remodeling ↗ angiogenesis (CD31) ↗ collagen deposition	[179]

underlying the enhancement of angiogenesis are still unknown, but Krautz et al. suggested that the effects of Cur on angiogenesis are mediated by NO synthase [165].

Human cationic antimicrobial protein (hCAP18)/LL37 is a 37-amino acid host defense peptide that belongs to the cathelicidin family [171]. In addition to its well-described role in innate and adaptive immunology [171,172], proangiogenic activity has been reported for this peptide [172–174]; this activity may be mediated by the formyl peptide receptor-like 1 (FPRL1) receptor without implicating VEGF [173] or by VEGF itself [116,175,176]. Its antimicrobial and angiogenic activities make LL37 a potential candidate for wound healing treatments. While topical administration of free LL37 did not improve wound healing in mice, the encapsulation of LL37 in PLGA NPs allowed the sustained release of LL37 [116]. LL37 in PLGA NPs induced faster wound closure, increased collagen production, upregulated IL-6 expression, increased VEGF expression, and increased angiogenesis [116]. The beneficial effects of the treatment were attributed to the sustained release of LL37 and to the intrinsic activity of lactate released from the NPs after degradation. LL37 was also encapsulated in nanostructured lipid carriers (NLCs) [177]. When administered in excisional wounds in db/db mice, LL37 NLCs promoted faster wound closure, increased re-epithelialization, and accelerated resolution of inflammation compared to untreated

Table 4
Gene therapies with GFs used in experimental wound studies.

GF	Delivery mode	Wound model	Results	References
VEGF	Adenoviral vector	Full-thickness excisional skin wounds (3.5 mm diameter) in CD1 diabetic (STZ) mice	↗ wound closure kinetics ↗ granulation tissue ↗ angiogenesis (CD31)	[180]
		Full-thickness excisional skin wounds (1.4 cm diameter) and 3-cm linear incisional wound in 8 week-old db/db mice and 24–27 week-old NOD mice	↗ wound closure kinetics ↗ re-epithelialization ↗ tensile stiffness ↗ wound contraction (through collagen deposition) ↗ granulation tissue deposition ↗ angiogenesis (H&E) Thicker epidermal layer	[181]
		Quadrangular flap (2 × 8 cm) on abdominal wall of male Sprague-Dawley rats	↗ flap survival (if applied 3 or 7 days prior surgery) ↗ perfusion (if applied 7 days prior surgery) ↗ angiogenesis (CD31)	[182]
		Full-thickness excisional skin wounds (25 × 25 mm) in female Yucatan minipigs 4–6 years-old (old) and Yorkshire pigs	No effect on vascularization No effect on re-epithelialization	[187]
	VEGF-C Adenoviral vector	Full-thickness excisional skin wounds (3–5 mm diameter) in 10 week old db/db mice	↗ wound closure kinetics ↗ hematopoietic cells (CD45) and macrophages (MOMA2) recruitment ↗ angiogenesis (CD31) ↗ lymphangiogenesis (LYVE)	[188]
	Recombinant adeno-associated virus (rAAV) gene transfer	Full-thickness excisional skin wounds (8 mm diameter) in male Wistar rats	↗ wound closure kinetics ↗ angiogenesis (α-SMA)	[189]
		Two full thickness longitudinal incisions (4 cm) in 14 week-old db/db mice	↗ re-epithelialization ↗ synthesis/maturation of ECM ↗ breaking strength ↗ angiogenesis (histological score on H&E)	[190]
		Right-sided axial pattern skin flaps (6 cm × 3 cm) in female Sprague-Dawley rats	↗ flap survival ↗ angiogenesis (H&E)	[191]
	Plasmid injection (VEGF-A_{165}, VEGF-B_{167} or VEGF-B_{186})	Ischaemic skin square flaps of 8 cm length in Sprague-Dawley rats	↗ flap survival (but not significant for VEGF-B_{186}) ↗ angiogenesis (H&E) (but not significant) No changes in the calibre of vessels (angiography)	[197]
	Plasmid in polycation complex and human fibrin sealant CROSSEAL	Fasciocutaneous flap (3 × 6 cm) in Sprague-Dawley rats	↗ flap survival	[199]
	Plasmid in porous hyaluronic acid hydrogel	Full-thickness excisional skin wounds (6 mm diameter) in female 10–12 week-old db/db mice and Balb/c mice.	↗ wound closure kinetics with empty porous hydrogel but no enhancement of wound closure with incorporation of VEGF plasmid ↗ angiogenesis with empty porous hydrogel but no enhancement of wound closure with incorporation of VEGF plasmid	[200]
		Random pattern skin flaps (8 × 3 cm) in male Sprague-Dawley rats	↗ skin flap survival ↗ perfusion (Doppler) ↗ expression VEGF ↗ expression eNOS ↘ inflammation	[69]
	Plasmid injection and electroporation using a non-invasive multi-electrode array	Random pattern skin flaps (8 × 3 cm) in male Sprague-Dawley rats	↗ skin flap survival ↗ perfusion (Doppler)	[68]
	Sonoporation with minicircle DNA	Full-thickness excisional skin wounds (6 mm diameter) in C57BL/6J mice injected with STZ (1 week diabetes)	↗ wound kinetics closure ↗ perfusion (Doppler) ↗ angiogenesis (CD31, H&E)	[72]
		Full-thickness excisional skin wounds (6 mm diameter) in C57BL/6J mice injected with STZ (1 week diabetes)	↗ wound closure kinetics ↗ neoangiogenesis (Doppler) ↗ histological findings	[202]
	$_{L}$-arginine-grafted polyamidoamine (PAM-RG4)/mini-circles complexes	Full-thickness excisional skin wounds (6 mm diameter) in C57BL/6J mice injected with STZ (1 week diabetes)	↗ wound closure kinetics ↗ re-epithelialization ↗ collagen deposition ↗ angiogenesis (CD31)	[203]
TGF-β	Plasmid electroporation with a syringe electrode	Full-thickness excisional skin wounds (7 × 7 mm) in 7–9 week-old db/db mice	↗ wound closure kinetics ↗ re-epithelialization ↗ cell density in granulation tissue ↗ angiogenesis (vWF)	[205]
PDGF-B	Adenoviral vector	Full-thickness excisional skin wounds (8 mm diameter) in db/db, NOD and C57BLKS/J STZ-induced diabetic mice	↗ wound closure kinetics ↗ re-epithelialization ↗ granulation tissue formation ↗ angiogenesis (CD31) ↗ EPCs recruitment	[194]
aFGF	Plasmid topically applied once a day during 3 days	Full-thickness excisional skin wounds (6 mm diameter) and insicional wound (2 cm) in 8-week-old female db/db mice	↗ wound closure kinetics ↗ breaking strength (incisional wound)	[240]

(continued on next page)

Table 4 (*continued*)

GF	Delivery mode	Wound model	Results	References
HGF	Plasmid injection and electroporation	Dorsal skin flap (McFarlane) in male Wistar rats	↗ flap survival ↗ flap perfusion (laser Doppler) ↗ angiogenesis	[212]
HIF-1α	Plasmid injection and (custom-designed pin electrode)	Full-thickness excisional skin wounds (5 mm diameter) in young (1.5–2 months) vs old (4–6 months) db/db mice	↗ wound closure kinetics ↗ mRNA expression VEFG, PLGF, PDGF-B, ANGPT1, ANGPT2 ↗ circulating angiogenic cells ↗ angiogenesis (CD31)	[206]
	Plasmid + PLL_{20}-g_8-PEG_5-TG or PLL_{20}-g_3-PEG_5-polyR to form a nanocondensates contained in a 3D fibrin matrix	Full-thickness excisional skin wounds (10 mm diameter) in Sprague-Dawley rats (healthy or STZ-induced diabetic)	↗ VEGF, Pecam1 (CD31) and Acta2 (α-SMA) expression (in normal rats and also STZ rats but in a lesser extent) ↗ angiogenesis (CD31 and α-SMA) in normal, but not significant, and no effect in STZ rats	[207]
	CMV-Hif-$\alpha^{\Delta ODD}$ plasmid pellets placed directly in the wound	Full-thickness excisional skin wounds (2 cm diameter) in 8 to 14-week-old db/db mice	↗ wound closure kinetics ↗ overall wound histological score ↗ angiogenesis (CD31) ↗ expression VEGF, HMOX1, NOS2	[208]
Combination of GFs				
IGF-I-KGF	Liposomes	Full-thickness scald burn on back of Sprague-Dawley rats	↗ VEGF, IGF-I, IGFBP-3, KGF production (↗↗ combination) ↗ re-epithelialization (↗↗ combination) ↗ cell proliferation (↗↗ combination) ↘ apoptosis (↘↘ combination) ↗ collagen deposition (↗↗ combination) ↗ angiogenesis (H&E) (↗↗ combination)	[215]
VEGF-bFGF-PDGF	Plasmid	Random pattern McFarlane flaps (3 × 10 cm) in female Sprague-Dawley rats	↗ skin flap survival for VEGF, bFGF, VEGF + bFGF, VEGF + PDGF but ↘ skin flap survival for VEGF + bFGF + PDGF ↗ angiogenesis (↗↗ combination)	[214]
VEGF-FGF4	AAV vector	Full-thickness excisional skin wounds (4 mm diameter) in 14-week-old db/db mice	↗ wound closure kinetics (VEGF and ↗↗ combination) ↗ epithelium thickness ↗ cell density ↗ granulation tissue formation ↗ collagen deposition ↗ angiogenesis (isolectin staining) ↗ proliferation of fibroblasts	[213]

wounds and wounds treated with free LL37 [177]. Wound healing was also enhanced after the administration of solid lipid nanoparticles (SLNs) containing LL37 and Serpin A1, an elastase inhibitor with wound healing activity. This treatment demonstrated in vitro antibacterial and anti-inflammatory activity, but its effects on angiogenesis were not investigated [178]. Another antimicrobial peptide, antimicrobial peptide 57 (AP-57), was tested following encapsulation in poly($_L$-lactic acid)-pluronic L35-poly($_L$-lactic acid) NPs, which were further included in a thermosensitive hydrogel (AP-57-NPs-H) [179] to allow the slow release of AP-57. AP-57-NPs-H induced more rapid wound closure of full-thickness excisional wounds in rats and increased granulation tissue formation, collagen deposition and angiogenesis compared to untreated, empty NPs or AP-57-NPs.

5. Gene therapy in wound healing

In the context of wound healing, gene therapy may be used to counteract the underexpression of certain genes induced by pathologies (e.g., diabetes) or the rapid degradation of proteins and GFs in chronic wounds. Gene therapy is easily applicable to the skin and wounds due to the ease of tissue access. Additionally, cells in the skin have rapid turnover, leading to transient gene expression. Notwithstanding, gene therapy has been mainly investigated in preclinical studies.

5.1. Viral gene transfer to enhance GF expression

Viral gene transfers have been used to enhance the expression of GFs during wound healing, notably GFs involved in angiogenesis (Table 4). Adenoviral vector AdCMV·$VEGF_{165}$, carrying the $VEGF_{165}$ gene, was successfully administered in CD1 streptozotocin-induced diabetic mice in which faster wound closure, increased granulation tissue formation and angiogenesis were observed compared to non-treated mice [180]. Brem et al. [181] constructed an adenoviral vector by cloning human $VEGF_{165}$ into the multiple cloning site of an adenovirus shuttle vector (pXC1) containing adenovirus type 5 sequences (bp 22–5790) and a Rous sarcoma virus promoter. In diabetic mouse models (db/db and NOD), the administration of this adenoviral vector promoted wound healing, re-epithelialization, wound contraction, granulation tissue deposition and angiogenesis compared to the control group. In addition, the treatment increased skin tensile stiffness in db/db and NOD mice. Adenoviral vectors carrying the VEGF gene, administered before surgery, were also shown to improve skin flap survival and perfusion [182–186]. Vranckx administered a VEGF-expressing adenoviral vector via microseeding in aged pig wounds [187]. While increased expression of VEGF was observed during the 5 days of monitoring, the administration of high doses of VEGF (10^{11} particles per wound) impaired healing. At adenovirus doses that were able to increase VEGF expression in wounds (10^9 particles per wound), no effects on wound healing and vascularization were observed. This could be because the time frame for VEGF expression was not adapted to wound healing, as the major peak of VEGF expression occurred two days after treatment, whereas VEGF acts in the later phase of wound healing [187]. Saaristo et al. [188] used adenoviruses to transfer a VEGF-C gene that promoted angiogenesis, lymphangiogenesis and wound healing in diabetic mice. The generation of new lymphatic vessels was described to be important in wound healing because it facilitates the removal of excess fluid and leukocytes and helps reduce the edema associated with the inflammatory response [188]. Deodato [189] and Galeano [190] used recombinant adeno-associated virus for VEGF gene transfer. Deodato et al. administered the treatment in excisional wounds in Wistar rats, while

Galeano et al. administered it in incisional wounds realized in db/+ and db/db mice. Deodato et al. observed increased angiogenesis (via α-SMA staining), increased granulation tissue formation, increased re-epithelialization as estimated by histological scoring and faster wound closure [189]. Galeano et al. also observed that the treatment significantly improved histological scores of angiogenesis, re-epithelialization, granulation tissue formation, and wound breaking strength in db/+ mice as well as correction of the poor results obtained in db/db mice [190]. A VEGF-expressing adeno-associated virus vector in liposomes was administered by intra-arterial infusion in Sprague-Dawley rats with skin flaps [191]. This treatment promoted flap survival and angiogenesis, as increased vascularity and smaller-size vessels were observed compared to saline or vector treatments.

PDGF-B is another GF implicated in angiogenesis. Liechty et al. [192] showed that the administration of an adenoviral vector mediated overexpression PDGF-B promoted healing in an ischemic rabbit ear model of impaired excisional wound healing, significantly improved re-epithelialization compared to non-treated wounds or wounds receiving PDGF-B protein, and increased granulation tissue formation. In this study, vessel formation was not evaluated [193]. The beneficial effects of an adenoviral vector expressing PDFG-B on wound healing and granulation tissue formation were also described by Keswani et al. [194] in three models of diabetic mice (streptozotocin-induced, db/db mice and NOD mice). The treatment promoted angiogenesis and the recruitment of EPCs to wounds, which was correlated with wound healing and angiogenesis [194].

Hepatocyte growth factor (HGF) has also been administered via gene therapy in wounds because this GF improves wound healing and promotes dermal tissue regeneration and angiogenesis [195]. An adenoviral vector expressing HGF was shown to improve wound healing and reduce scarring [196].

5.2. Non-viral gene transfer to enhance GF expression in wounds

Although non-viral gene therapy may lower the risk of triggering immune responses and carcinogenesis compared to viral vectors, a safe and efficient method such electroporation or sonoporation is required to enhance plasmid delivery in targeted cells (Table 4). O'Toole et al. [197] tested a plasmid encoding different VEGF isoforms. Injection of the plasmid, which expressed VEGF-A_{165} and VEGF-B_{167}, in flaps in Sprague-Dawley rats significantly improved flap survival compared to flaps receiving saline. However, no differences in vessel number or size were observed by angiography and histology, suggesting that the protective effects of microvessel repair and the prevention of microvessel regression by VEGF were sufficient to improve flap survival [197].

As plasmid injection is not very efficient [198], a VEGF-expressing plasmid was included in a polymer matrix carrier, specifically a polycation complex mixed with the human fibrin sealant Crosseal, to stabilize it and prolong its release, which resulted in increased flap survival at day 5 post-surgery compared to controls receiving the matrix alone [199]. This effect was attributed to increased angiogenesis. No differences in flap survival were observed between the group of Sprague-Dawley rats receiving VEGF protein (the control group) in the matrix and the animals receiving VEGF-expressing plasmid in the matrix at this time point [199]. Porous hyaluronic acid-matrix metalloproteinase hydrogel was also tested as a scaffold for DNA delivery. Porous hydrogel containing a VEGF-expressing plasmid was applied in mice wounds. The

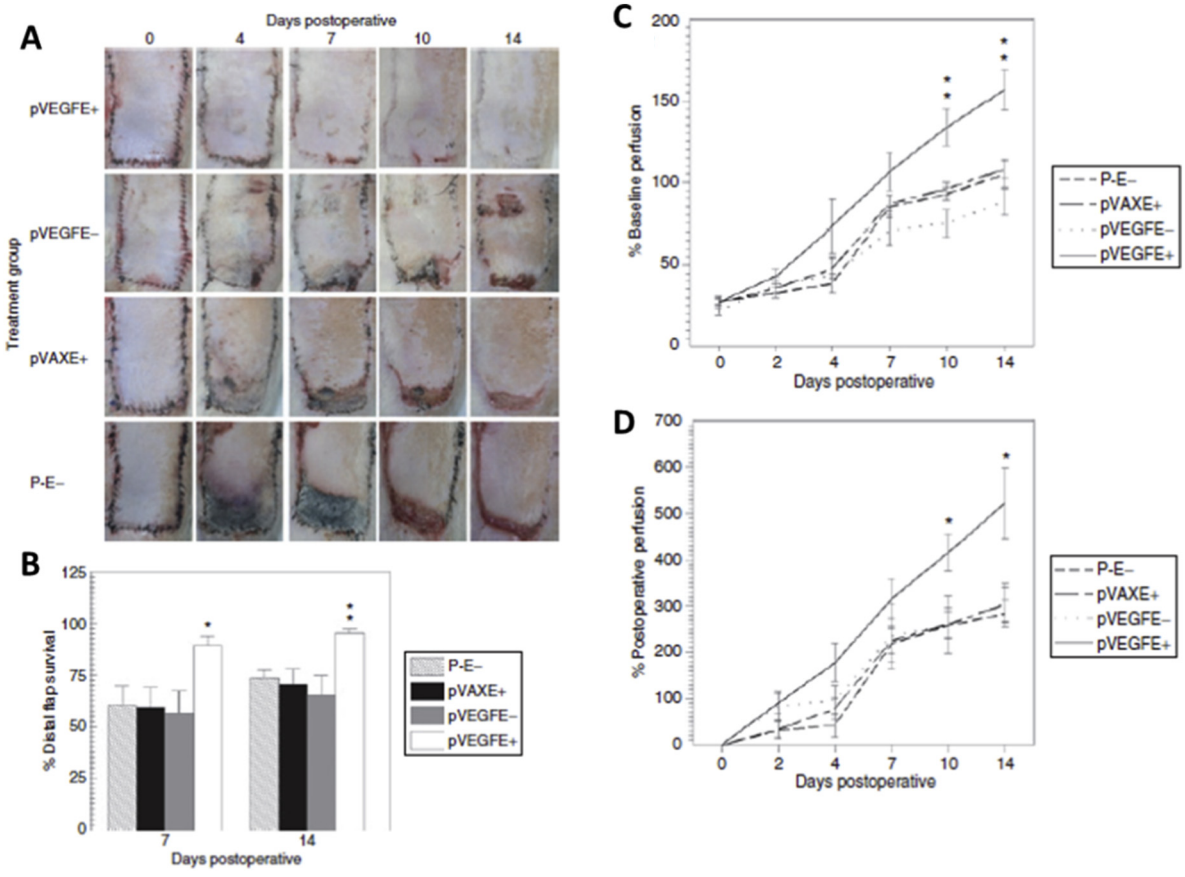

Fig. 5. Effects of VEGF plasmid electroporation in flaps in Sprague-Dawley rats. Representative pictures of a flap (A) and quantification of the percentage of flap survival (B). Effects of treatment on perfusion evaluated by laser Doppler imaging: the percentage of perfusion relative to baseline perfusion (C) and the percentage of perfusion relative to postoperative perfusion (D). P-E-: no treatment (no plasmid, no electroporation), pVAXE+: electroporation of the pVAX plasmid (control), pVEGFE-: administration of the VEGF plasmid without electroporation, pVEGFE+: electroporation of the VEGF plasmid (adapted from [69]).

Table 5
Other gene therapies used in experimental wound studies.

Molecules	Delivery mode	Wound model	Results	References
Ang-1	rAAV	Incisional skin wound in 14 week-old db/db	↗ wound closure kinetics ↗ re-epithelialization, collagen maturation ↗ breaking strength ↗ angiogenesis (CD31) ↗expression eNOS and VEGFR-2 No change in VEGF expression	[218]
LL37	Plasmid followed by electroporation	Full-thickness excisional skin wounds (4 mm diameter) in 7-week-old C57BL/6 and db/db mice and ischemic hindlimb model in 7-week-old C57BL/6 mice	↗ wound closure kinetics ↗ VEGFa expression in wounds Upregulation of IL-6 ↗ perfusion in hindlimb muscle (Laser Doppler) ↘ muscular atrophy in hindlimb ischemia model ↗ angiogenic cytokines (VEGFa, VEGFR-1, SDF-1a and CXCR-4) expression in ischemic hindlimb muscles	[176]
	Adenoviral vector	Full-thickness excisional skin wounds (4 mm diameter) and incisional wound (2 cm) in 8–10-week-old female ob/ob mice	↗ wound closure kinetics ↗ re-epithelialization ↗ granulation tissue formation	[219]
iNOS	Adenoviral vector	Full-thickness excisional skin wounds (1.5 × 1.5 cm) in mice with targeted disruption of the iNOS gene	↗ wound closure kinetics	[221]
eNOS	Adenoviral vector in a fibrin scaffold	Full-thickness excisional skin wounds (6 mm diameter) on the ear of New Zealand white rabbits	↗ eNOS expression ↗ re-epithelialization ↘ inflammation ↗ angiogenesis (surface and length) (CD31)	[223]
ORP150	Adenoviral vector	Full-thickness excisional skin wounds (6 or 12 mm diameter) in 8-week-old C57BL/6 and db/db	↗ wound closure kinetics ↗ angiogenesis ↗ tissue levels of extracellular VEGF	[224]
IL10	Plasmid injection and electroporation		↘ flap necrosis ↗ cutaneous perfusion ↗ vessel density	[225]

Table 6
Gene therapies with GFs used in clinical studies.

GF	Delivery mode	Patients/wounds	Results	References
VEGF	Arterial gene transfer of a phVEGF$_{165}$ plasmid applied to hydrogel polymer coating of an angioplasty balloon	1 patient with ischemic limb	↗ collateral vessels ↗ distal flow ↗ angiogenesis Presence of oedema	[227]
	Intra-arterial or intramuscular phVEGF$_{165}$ gene transfer	Patients with critical limb ischemia or claudication	↗ vascular permeability and oedema ↘ rest pain ↗ collateral vessels ↗ hemodynamics (ankle/toe-brachial index)	[228]
	Catheter mediated delivery of VEGF-adenovirus or VEGF-plasmid/liposome	Patients with critical limb ischemia	↗ vascularity No change in major amputation No effect on ulcer healing No effect on rest pain	[229]
	Intramuscular injection of pCK-VEGF$_{165}$ plasmid	Patients with peripheral arterial disease	↘ rest pain ↗ ulcers healing ↗ ankle-brachial index ↗ collateral vessels Transient oedema	[230]
	Intramuscular injection of phVEGF$_{165}$ plasmid	Patients with critical limb ischemia	↗ ankle-brachial index ↗ distal flow ↗ ulcers healing ↘ rest pain	[231]
	Intramuscular injection of a phVEGF$_{165}$ plasmid	Diabetic patients with critical limb ischemia	↗ hemodynamics (ankle/toe-brachial index) ↗ ulcers healing ↘ pain No significant reduction in amputation rates	[232]
PDGF	Adenoviral vector H5.020CMV.PDGF-β administered subcutaneously around the wound edge	Chronic venous leg ulcers	↘ wound size ↗ recruitment immature bone marrow derived endothelial progenitor cells ↗ angiogenesis (CD31) but not significantly	[233]
	Topical application of the adenoviral vector Ad-5PDGF-B incorporated into a gene-activated matrix (GAM501)	Chronic diabetic ulcers	↘ wound size	[234]
HGF	Intramuscular injection of AMG0001 plasmid	Patients with lower extremity ischemic tissue loss	↗ toe-brachial index ↗ $TcPO_2$ ↘ rest pain No significant different rate of wound healing No difference in major amputation rate	[235]
	Intramuscular injection of a pVAX1-HGF plasmid	Patients with critical limb ischemia	↗ ankle brachial index ↗ toe brachial index No changes in$TcPO_2$ ↘ rest pain ↘ wound size ↗ maximum walking distance	[236]
	Intramuscular injection of a pUDK-HGF plasmid	Patients with critical limb ischemia	↘ pain ↗ healing ↗ $TcPO_2$ but not significantly ↗ ankle brachial index but not significantly	[237]
	Intramuscular injection of a VM202 plasmid (expressing 2 isoforms of HGF)	Patients with critical limb ischemia	↘ ulcer size ↗ $TcPO_2$ (high dose) No effect on ankle or toe-brachial index, rest pain, amputation rate	[238]

hydrogel by itself was shown to improve wound healing; however, the presence of the VEGF-expressing plasmid did not significantly increase angiogenesis compared to hydrogel containing a control plasmid expressing reporter genes [200].

Electroporation has also been used to transfer genes encoding VEGF expression with good results, notably in terms of flap survival and flap perfusion. Ferraro et al. administered a plasmid expressing VEGF by electroporation 2 days after flap surgery in rats. Peak expression of VEGF occurred at the onset of the proliferative phase. Compared to flaps receiving VEGF-expressing plasmid without electroporation, electroporation induced significantly higher levels of transgene expression. VEGF and eNOS expression was increased. Additionally, skin flap survival and flap perfusion were improved [69] (Fig. 5). The same group developed a non-invasive microelectrode array to efficiently transfer genes with significantly less muscle twitching and discomfort compared to the traditionally used electrodes. Different plasmid dosages and electroporation protocols were tested to optimize the treatment: 50–100 μg of plasmid delivered by electroporation at two sites on the flap during the first two days after flap surgery [201].

Sonoporation was used to deliver minicircles expressing VEGF in wounds. Minicircle DNA delivery demonstrated more robust and prolonged transgene expression than plasmids and improved wound healing and angiogenesis were observed in streptozotocin-induced diabetic mice [72,202]. Minicircle VEGF DNA was also administered in the form of complexes after its combination with arginine-grafted cationic dendrimers (PAM-RG4) into the wounds of streptozotocin-induced diabetic mice, leading to effective wound healing and the promotion of angiogenesis [203].

Ex vivo transfection was performed to promote VEGF expression in wounds. Rinsch et al. [204] introduced vectors expressing VEGF or FGF-2 in vitro in myofibroblasts to produce high levels of proteins. These cells were then encapsulated in a microporous polymer membrane to form an implant that would potentially be removed from the wound after healing and facilitate the isolation of the cells from host immune reactions. Cells expressing FGF-2 improved flap survival, while cells expressing VEGF did not increase angiogenesis compared to cells expressing FGF-2. This absence of an effect exerted by cells expressing VEGF was explained by the potentially inappropriate dosing of VEGF, the use of an inappropriate implantation site, and the inappropriate timing of administration, inducing angiogenesis too late to promote wound healing.

Electroporation with a syringe electrode was also successfully used to treat wounds with a plasmid expressing the TGF-β gene: TGF-β expression promoted re-epithelialization, granulation tissue formation, angiogenesis, and wound closure [205]. A HIF-1α-expressing plasmid was administered by electroporation and improved wound healing and angiogenesis [206]. Additionally, a HIF-1-encoding plasmid was encapsulated in nanocondensates in a 3D fibrin matrix [207] and directly administered in the form of pellets into the wound [208]. All of these treatments promoted wound healing and angiogenesis.

Injection of a plasmid encoding HGF induced angiogenesis in a rabbit hind limb ischemia model, a rat hind limb ischemia model [209] and a rat diabetic ischemia model [210]. This technique also improved angiogenesis in a hind limb ischemic model in transgenic mice that presented impaired collateral vessel formation [211]. Furthermore, electroporation of HGF decreased flap necrosis and significantly improved angiogenesis and perfusion of the skin flaps, as observed by CD31 staining in histological sections and laser Doppler imaging [212].

5.3. Gene transfer to improve the expression of several GFs

Some groups have combined gene therapy with several GFs to act at different levels throughout the complex process of wound healing. Jazwa et al. [213] demonstrated improved wound healing using a combination of VEGF and FGF4 expressed from adeno-associated viral vectors. Liu et al. showed improved flap survival and angiogenesis with combined therapy using the plasmid $VEGF_{165}$ in combination with bFGF, whereas combined therapy with $VEGF_{165}$, bFGF and PDGF improved angiogenesis but impaired flap survival under experimental conditions [214]. Jeschke et al. showed the beneficial effects of IGF-I combined with KGF liposomal gene transfer on wound healing, angiogenesis, and re-epithelialization [215]. Kunugiza et al. reported improved wound healing, increased blood flow, and increased neovascularization after the administration of HGF and a prostacyclin synthase-expressing plasmid by Shima Jet, a technique first developed for the non-needle administration of insulin in diabetic patients [216].

5.4. Gene transfer of other peptides and proteins

Other factors have also been administered via gene therapy to promote wound healing. These include angiogenesis-promoting treatments (Table 5). For example, Ang-1, known to stimulate angiogenesis, promoted angiogenesis in in vitro studies and in an in vivo matrigel assay when administered using a recombinant adeno-associated viral vector [217]. When administered in diabetic mice, Ang-1 improved wound healing and increased wound breaking strength, eNOS expression, wound NO content, angiogenesis, and VEGFR2 levels. As VEGF levels were not restored by this treatment, it was proposed that Ang-1 promoted angiogenesis without implicating VEGF [218].

The LL37 antimicrobial peptide also favors wound healing and angiogenesis when administered in obese and diabetic mice either by electroporation or by using adenoviral vectors [176,219]. Additionally, an adenoviral vector expressing LL37 improved burn wound healing and decreased bacterial growth [220].

iNOS gene therapy is another promising strategy to treat chronic wounds. Indeed, iNOS produces NO, which is notably involved in cell proliferation and angiogenesis during wound healing. Low levels of iNOS have also been described in diabetic wounds with impaired healing. Using iNOS-deficient mice, Yamasaki et al. demonstrated the important role of iNOS in wound healing. By treating iNOS-deficient mice with an adenovirus promoting iNOS expression, wound healing was restored to the normal levels observed in healthy mice [221]. eNOS gene therapy was shown to improve wound healing in type I diabetic mice. Administration of eNOS by an adenoviral vector improved healing by decreasing the superoxide anion content present in the wounds. Additionally, the same effect was observed with the administration of manganese superoxide dismutase [222]. Furthermore, Breen et al. showed that adenoviral vectors expressing eNOS encapsulated in a fibrin scaffold promoted wound healing, demonstrating increased epithelial coverage, decreased inflammation, and the promotion of angiogenesis compared to non-treated fibrin alone or adenoviral vector alone [223].

Ozawa et al. administered a 150-kDa oxygen-regulated protein (ORP150) with an adenoviral vector to promote wound healing in C57BL6/J and db/db mice [224]. ORP150 is an inducible endoplasmic reticulum chaperone expressed in a range of pathologic situations and is proposed to contribute to the cellular response to environmental stress. ORP150 overexpression with an adenoviral vector accelerated wound closure and correlated with angiogenesis and the overexpression of VEGF [224].

Electroporation-mediated IL-10 gene transfer decreased percentage of flap necrosis and increased cutaneous perfusion compared to the control group: significantly higher mean $CD31^+$ vessel density was detected and semi-quantitative image analysis showed lower inflammatory cell count. In vivo electroporation-mediated IL-10 gene transfer reduced necrosis, enhanced survival and vascularity in the ischemic skin flap [225].

6. Conclusions and clinical perspectives

Perfusion and oxygenation play key roles in the different steps of wound healing. Hence, enhancing hemodynamics and pO_2 in wounds is crucial to accelerate the healing of acute or chronic wounds. Numerous preclinical studies have shown that the delivery of angiogenic GFs promotes wound healing in normal and diabetic rodents. However, unless high doses and repeated administrations are achieved, advanced drug delivery systems such as (nano)carriers or gene therapies are required to avoid GF degradation by proteases and to induce their sustained release during the healing process. Moreover, the carrier can be included in a matrix or hydrogel that can act as a scaffold, moister the wound, act as a dressing, further sustain and control drug release or act as a dual delivery system [107]. While the efficacy of such systems has been experimentally demonstrated (see Sections 4 and 5), only a few clinical studies have been performed to translate this approach from bench to bedside.

Wound cleaning, wound debridement and the selection of wound dressings favoring a moist environment remain the first choices for the treatment of diabetic foot ulcers. The use of hyperbaric oxygen therapy (topical or systemic) is still controversial and requires further clinical study to evaluate the benefits of this treatment [226]. This is also true for negative pressure wound therapy [226]. The use of GFs to treat diabetic ulcers is limited due to a lack of evidence regarding the treatment efficacy. Certain clinical studies described below gave encouraging results that must be confirmed in larger blinded and randomized trials (Table 6).

Whereas VEGF is a potent proangiogenic factor showing interesting results in experimental wound healing studies, clinical studies have demonstrated the variable efficacy of gene therapy in lower limb ischemia [227–231]. Another clinical study showed that the intramuscular injection of a VEGF-expressing plasmid ($phVEGF_{165}$) improved hemodynamics (determined by the ankle and toe brachial index) and healing but did not significantly reduce amputation rates [232].

The feasibility of PDGF administration via gene therapy to improve wound healing and angiogenesis has been investigated in 2 clinical studies [233,234]. A phase 1 study applied the adenoviral vector H5.020CMV·PDGF-β in chronic venous leg ulcers [233]. The plasmid demonstrated safety and efficacy accompanied by a reduction in wound size. Histological studies performed on biopsies showed the recruitment of increased numbers of immature bone marrow-derived endothelial progenitor cells in wounds as well as increased angiogenesis [233]. A phase 1/2 trial was carried out with an adenoviral vector expressing PDGF-B (Ad-5PDGF-B) incorporated into a gene-activated matrix (GAM501) [234]. This collagen-containing matrix stabilized the vector and acted as a support for proliferating cells during the proliferative phase. The application of a GAM501-containing PDGF adenoviral vector was shown to be safe and to promote wound healing with a more rapid decrease in wound size, but the effects of the treatment on hemodynamics were not investigated [234]. These 2 small studies provided encouraging results, but their findings must be confirmed in larger studies.

As an alternative to VEGF and PDGF, other clinical studies have investigated HGF administered by gene therapy to promote ulcer healing. Four groups showed the safety and efficacy of intravascular HGF plasmid gene injection in wound healing in patients with critical limb ischemia. In the HGF-0205 trial, one group demonstrated that limb perfusion and $TcPO_2$ were increased and resting pain was decreased after administration of the plasmid AMG0001 [235]. However, no differences in major amputation were observed in this study. The second group showed that intramuscular HGF gene therapy was safe, improved the ankle brachial index and decreased ulcer size during 6-month follow-up [236]. Using a pUDK plasmid, a third group demonstrated decreased pain and improved healing after treatment. This group also showed improved hemodynamics (evaluated by $TcPO_2$ and the ankle brachial index), although this was not statistically significant [237]. A fourth group showed the safety of VM202 use and demonstrated that a low dose of an HGF-expressing plasmid was sufficient to decrease ulcer size, whereas a high dose significantly improved complete healing and the $TcPO_2$ [238]. Again, the results obtained with HGF gene therapy must be confirmed by larger studies.

In conclusion, the results of these small studies are encouraging, but there is insufficient evidence to suggest that the use of GFs administered by gene therapy improves hemodynamics and wound healing in patients. Also, concerning NPs, even if their use in the clinical practice might present several advantages upon gene therapy, no clinical trial using this type of material in wound healing studies is described. However, experimental studies showed the interest of NPs that could be incorporated in several scaffolds already approved by regulatory agencies (such as hydrogels or carboxymethylcellulose dressing) in the clinical practice. Such “nanocomposite smart materials” are promising and should promote clinical research on wound treatment [107,239]. Based on the preclinical studies providing proof of concept and on the selection of systems that can easily be translated to humans (e.g. PLGA particles), larger clinical trials are required to determine the efficacy of GF therapies for wound oxygenation and wound healing.

References

[1] G.C. Gurtner, S. Werner, Y. Barrandon, M.T. Longaker, Wound repair and regeneration, Nature 453 (2008) 314–321.
[2] L.M. Morton, T.J. Phillips, Wound healing and treating wounds: differential diagnosis and evaluation of chronic wounds, J. Am. Acad. Dermatol. 74 (2016) 589–605 (quiz 605-586).
[3] G.M. Gordillo, C.K. Sen, Revisiting the essential role of oxygen in wound healing, Am. J. Surg. 186 (2003) 259–263.
[4] H.W. Hopf, M.D. Rollins, Wounds: an overview of the role of oxygen, Antioxid. Redox Signal. 9 (2007) 1183–1192.
[5] S. Schreml, R.M. Szeimies, L. Prantl, S. Karrer, M. Landthaler, P. Babilas, Oxygen in acute and chronic wound healing, Br. J. Dermatol. 163 (2010) 257–268.
[6] A.A. Tandara, T.A. Mustoe, Oxygen in wound healing–more than a nutrient, World J. Surg. 28 (2004) 294–300.
[7] S.M. McCarty, S.L. Percival, Proteases and delayed wound healing, Adv. Wound Care (New Rochelle) 2 (2013) 438–447.
[8] M. Muller, C. Trocme, B. Lardy, F. Morel, S. Halimi, P.Y. Benhamou, Matrix metalloproteinases and diabetic foot ulcers: the ratio of MMP-1 to TIMP-1 is a predictor of wound healing, Diabet. Med. 25 (2008) 419–426.
[9] G.S. Schultz, A. Wysocki, Interactions between extracellular matrix and growth factors in wound healing, Wound Repair Regen. 17 (2009) 153–162.
[10] C.K. Sen, Wound healing essentials: let there be oxygen, Wound Repair Regen. 17 (2009) 1–18.
[11] D.B. Allen, J.J. Maguire, M. Mahdavian, C. Wicke, L. Marcocci, H. Scheuenstuhl, M. Chang, A.X. Le, H.W. Hopf, T.K. Hunt, Wound hypoxia and acidosis limit neutrophil bacterial killing mechanisms, Arch. Surg. 132 (1997) 991–996.
[12] N. Bryan, H. Ahswin, N. Smart, Y. Bayon, S. Wohlert, J.A. Hunt, Reactive oxygen species (ROS)–a family of fate deciding molecules pivotal in constructive inflammation and wound healing, Eur. Cell. Mater. 24 (2012) 249–265.
[13] M.J. Im, J.E. Hoopes, Energy metabolism in healing skin wounds, J. Surg. Res. 10 (1970) 459–464.
[14] S. Gorini, L. Gatta, L. Pontecorvo, L. Vitiello, A. la Sala, Regulation of innate immunity by extracellular nucleotides, Am. J. Blood Res. 3 (2013) 14–28.
[15] J. Yin, K. Xu, J. Zhang, A. Kumar, F.S. Yu, Wound-induced ATP release and EGF receptor activation in epithelial cells, J. Cell Sci. 120 (2007) 815–825.
[16] L.S. Harrington, R.J. Evans, J. Wray, L. Norling, K.E. Swales, C. Vial, F. Ali, M.J. Carrier, J.A. Mitchell, Purinergic 2X1 receptors mediate endothelial dependent vasodilation to ATP, Mol. Pharmacol. 72 (2007) 1132–1136.
[17] K. Bakker, J. Apelqvist, B.A. Lipsky, J.J. Van Netten, F. International Working Group on the Diabetic, The 2015 IWGDF guidance documents on prevention and management of foot problems in diabetes: development of an evidence-based global consensus, Diabetes Metab. Res. Rev. 32 (Suppl. 1) (2016) 2–6.
[18] A.J.M. Boulton, L. Vileikyte, G. Ragnarson-Tennvall, J. Apelqvist, The global burden of diabetic foot disease, Lancet 366 (2005) 1719–1724.
[19] V. Falanga, Wound healing and its impairment in the diabetic foot, Lancet 366 (2005) 1736–1743.
[20] D.G. Greenhalgh, Wound healing and diabetes mellitus, Clin. Plast. Surg. 30 (2003) 37–45.
[21] C.Y. Chao, G.L. Cheing, Microvascular dysfunction in diabetic foot disease and ulceration, Diabetes Metab. Res. Rev. 25 (2009) 604–614.
[22] G.K. Kolluru, S.C. Bir, C.G. Kevil, Endothelial dysfunction and diabetes: effects on angiogenesis, vascular remodeling, and wound healing, Int. J. Vasc. Med. 2012 (2012) 918267.
[23] S. Barrientos, O. Stojadinovic, M.S. Golinko, H. Brem, M. Tomic-Canic, Growth factors and cytokines in wound healing, Wound Repair Regen. 16 (2008) 585–601.
[24] S.P. Bennett, G.D. Griffiths, A.M. Schor, G.P. Leese, S.L. Schor, Growth factors in the treatment of diabetic foot ulcers, Br. J. Surg. 90 (2003) 133–146.

[25] D.I. Holmes, I. Zachary, The vascular endothelial growth factor (VEGF) family: angiogenic factors in health and disease, Genome Biol. 6 (2005) 209.
[26] K.E. Johnson, T.A. Wilgus, Vascular endothelial growth factor and angiogenesis in the regulation of cutaneous wound repair, Adv. Wound Care (New Rochelle) 3 (2014) 647–661.
[27] P. Bao, A. Kodra, M. Tomic-Canic, M.S. Golinko, H.P. Ehrlich, H. Brem, The role of vascular endothelial growth factor in wound healing, J. Surg. Res. 153 (2009) 347–358.
[28] T.D. Crafts, A.R. Jensen, E.C. Blocher-Smith, T.A. Markel, Vascular endothelial growth factor: therapeutic possibilities and challenges for the treatment of ischemia, Cytokine 71 (2015) 385–393.
[29] N.N. Nissen, P.J. Polverini, A.E. Koch, M.V. Volin, R.L. Gamelli, L.A. DiPietro, Vascular endothelial growth factor mediates angiogenic activity during the proliferative phase of wound healing, Am. J. Pathol. 152 (1998) 1445–1452.
[30] R. Mohle, D. Green, M.A. Moore, R.L. Nachman, S. Rafii, Constitutive production and thrombin-induced release of vascular endothelial growth factor by human megakaryocytes and platelets, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 663–668.
[31] M. Jinnin, H. Ihn, Y. Mimura, Y. Asano, K. Yamane, K. Tamaki, Regulation of fibrogenic/fibrolytic genes by platelet-derived growth factor C, a novel growth factor, in human dermal fibroblasts, J. Cell. Physiol. 202 (2005) 510–517.
[32] R.J. Ruthenborg, J.J. Ban, A. Wazir, N. Takeda, J.W. Kim, Regulation of wound healing and fibrosis by hypoxia and hypoxia-inducible factor-1, Mol. Cells 37 (2014) 637–643.
[33] K. Hattori, B. Heissig, Y. Wu, S. Dias, R. Tejada, B. Ferris, D.J. Hicklin, Z. Zhu, P. Bohlen, L. Witte, J. Hendrikx, N.R. Hackett, R.G. Crystal, M.A. Moore, Z. Werb, D. Lyden, S. Rafii, Placental growth factor reconstitutes hematopoiesis by recruiting VEGFR1(+) stem cells from bone-marrow microenvironment, Nat. Med. 8 (2002) 841–849.
[34] F. Pipp, M. Heil, K. Issbrucker, T. Ziegelhoeffer, S. Martin, J. van den Heuvel, H. Weich, B. Fernandez, G. Golomb, P. Carmeliet, W. Schaper, M. Clauss, VEGFR-1-selective VEGF homologue PlGF is arteriogenic: evidence for a monocyte-mediated mechanism, Circ. Res. 92 (2003) 378–385.
[35] P. Lindahl, B.R. Johansson, P. Leveen, C. Betsholtz, Pericyte loss and microaneurysm formation in PDGF-B-deficient mice, Science 277 (1997) 242–245.
[36] M. Grunewald, I. Avraham, Y. Dor, E. Bachar-Lustig, A. Itin, S. Jung, S. Chimenti, L. Landsman, R. Abramovitch, E. Keshet, VEGF-induced adult neovascularization: recruitment, retention, and role of accessory cells, Cell 124 (2006) 175–189.
[37] N.S. Greaves, K.J. Ashcroft, M. Baguneid, A. Bayat, Current understanding of molecular and cellular mechanisms in fibroplasia and angiogenesis during acute wound healing, J. Dermatol. Sci. 72 (2013) 206–217.
[38] J. Folkman, P.A. D'Amore, Blood vessel formation: what is its molecular basis? Cell 87 (1996) 1153–1155.
[39] M.L. Iruela-Arispe, H.F. Dvorak, Angiogenesis: a dynamic balance of stimulators and inhibitors, Thromb. Haemost. 78 (1997) 672–677.
[40] W. Risau, Mechanisms of angiogenesis, Nature 386 (1997) 671–674.
[41] L.C. Clark Jr., R. Wolf, D. Granger, Z. Taylor, Continuous recording of blood oxygen tensions by polarography, J. Appl. Physiol. 6 (1953) 189–193.
[42] E. Roussakis, Z. Li, A.J. Nichols, C.L. Evans, Oxygen-sensing methods in biomedicine from the macroscale to the microscale, Angew. Chem. Int. Ed. Engl. 54 (2015) 8340–8362.
[43] H.W. Hopf, Development of subcutaneous wound oxygen measurement in humans: contributions of Thomas K Hunt, MD, Wound Repair Regen. 11 (2003) 424–430.
[44] J. Ninikoski, C. Heughan, T.K. Hunt, Oxygen tensions in human wounds, J. Surg. Res. 12 (1972) 77–82.
[45] R.B. Heppenstall, F.N. Littooy, R. Fuchs, G.F. Sheldon, T.K. Hunt, Gas tensions in healing tissues of traumatized patients, Surgery 75 (1974) 874–880.
[46] G.F. Sheldon, J. Holcroft, R.B. Heppenstall, R. Fuchs, T.K. Hunt, Massive transfusion: a metabolic and hemodynamic lesion, Surg. Forum 24 (1973) 17–18.
[47] D. Mathieu, R. Mani, A review of the clinical significance of tissue hypoxia measurements in lower extremity wound management, Int. J. Low. Extrem. Wounds 6 (2007) 273–283.
[48] P.J. Sheffield, Measuring tissue oxygen tension: a review, Undersea Hyperb. Med. 25 (1998) 179–188.
[49] W.L. Yip, Evaluation of the clinimetrics of transcutaneous oxygen measurement and its application in wound care, Int. Wound J. 12 (2015) 625–629.
[50] D.W. Lubbers, Theoretical basis of the transcutaneous blood gas measurements, Crit. Care Med. 9 (1981) 721–733.
[51] R.E. Pecoraro, J.H. Ahroni, E.J. Boyko, V.L. Stensel, Chronology and determinants of tissue repair in diabetic lower-extremity ulcers, Diabetes 40 (1991) 1305–1313.
[52] Z. Wang, R. Hasan, B. Firwana, T. Elraiyah, A. Tsapas, L. Prokop, J.L. Mills Sr., M.H. Murad, A systematic review and meta-analysis of tests to predict wound healing in diabetic foot, J. Vasc. Surg. 63 (29S-36S) (2016) e21–22.
[53] J.S. Hyde, W.K. Subczynski, Spin-label oximetry, in: L.J. Berliner, J. Reuben (Eds.), Spin Labeling: Theory and Applications, Springer US, Boston, MA 1989, pp. 399–425.
[54] R. Ahmad, P. Kuppusamy, Theory, instrumentation, and applications of electron paramagnetic resonance oximetry, Chem. Rev. 110 (2010) 3212–3236.
[55] B. Gallez, C. Baudelet, B.F. Jordan, Assessment of tumor oxygenation by electron paramagnetic resonance: principles and applications, NMR Biomed. 17 (2004) 240–262.
[56] N. Khan, B.B. Williams, H. Hou, H. Li, H.M. Swartz, Repetitive tissue pO2 measurements by electron paramagnetic resonance oximetry: current status and future potential for experimental and clinical studies, Antioxid. Redox Signal. 9 (2007) 1169–1182.
[57] H.M. Swartz, B.B. Williams, B.I. Zaki, A.C. Hartford, L.A. Jarvis, E.Y. Chen, R.J. Comi, M.S. Ernstoff, H. Hou, N. Khan, S.G. Swarts, A.B. Flood, P. Kuppusamy, Clinical EPR: unique opportunities and some challenges, Acad. Radiol. 21 (2014) 197–206.
[58] S. Biswas, S. Roy, J. Banerjee, S.R. Hussain, S. Khanna, G. Meenakshisundaram, P. Kuppusamy, A. Friedman, C.K. Sen, Hypoxia inducible microRNA 210 attenuates keratinocyte proliferation and impairs closure in a murine model of ischemic wounds, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 6976–6981.
[59] J.S. Isenberg, F. Hyodo, K. Matsumoto, M.J. Romeo, M. Abu-Asab, M. Tsokos, P. Kuppusamy, D.A. Wink, M.C. Krishna, D.D. Roberts, Thrombospondin-1 limits ischemic tissue survival by inhibiting nitric oxide-mediated vascular smooth muscle relaxation, Blood 109 (2007) 1945–1952.
[60] C.M. Desmet, A. Lafosse, S. Veriter, P.E. Porporato, P. Sonveaux, D. Dufrane, P. Leveque, B. Gallez, Application of electron paramagnetic resonance (EPR) oximetry to monitor oxygen in wounds in diabetic models, PLoS One 10 (2015), e0144914. .
[61] C.M. Desmet, G. Vandermeulen, C. Bouzin, M.C. Lam, V. Preat, P. Leveque, B. Gallez, EPR monitoring of wound oxygenation as a biomarker of response to gene therapy encoding hCAP-18/LL37 peptide, Magn. Reson. Med. (2017)https://doi.org/10.1002/mrm.26956.
[62] H.M. Swartz, B.B. Williams, H. Hou, N. Khan, L.A. Jarvis, E.Y. Chen, P.E. Schaner, A. Ali, B. Gallez, P. Kuppusamy, A.B. Flood, Direct and repeated clinical measurements of pO2 for enhancing cancer therapy and other applications, Adv. Exp. Med. Biol. 923 (2016) 95–104.
[63] B.B. Williams, N. Khan, B. Zaki, A. Hartford, M.S. Ernstoff, H.M. Swartz, Clinical electron paramagnetic resonance (EPR) oximetry using India ink, Adv. Exp. Med. Biol. 662 (2010) 149–156.
[64] D.W. Paul, P. Ghassemi, J.C. Ramella-Roman, N.J. Prindeze, L.T. Moffatt, A. Alkhalil, J.W. Shupp, Noninvasive imaging technologies for cutaneous wound assessment: a review, Wound Repair Regen. 23 (2015) 149–162.
[65] C.M. Choi, R.G. Bennett, Laser Dopplers to determine cutaneous blood flow, Dermatol. Surg. 29 (2003) 272–280.
[66] M.L. Iabichella, E. Melillo, G. Mosti, A review of microvascular measurements in wound healing, Int. J. Low. Extrem. Wounds 5 (2006) 181–199.
[67] J.R. Brownrigg, R.J. Hinchliffe, J. Apelqvist, E.J. Boyko, R. Fitridge, J.L. Mills, J. Reekers, C.P. Shearman, R.E. Zierler, N.C. Schaper, F. International Working Group on the Diabetic, Performance of prognostic markers in the prediction of wound healing or amputation among patients with foot ulcers in diabetes: a systematic review, Diabetes Metab. Res. Rev. 32 (Suppl. 1) (2016) 128–135.
[68] G. Basu, H. Downey, S. Guo, A. Israel, A. Asmar, B. Hargrave, R. Heller, Prevention of distal flap necrosis in a rat random skin flap model by gene electrotransfer delivering VEGF165plasmid, J. Gene Med. 16 (2014) 55–65.
[69] B. Ferraro, Y.L. Cruz, D. Coppola, R. Heller, Intradermal delivery of plasmid VEGF165 by electroporation promotes wound healing, Mol. Ther. 17 (2009) 651–657.
[70] A.Y. Sheikh, M.D. Rollins, H.W. Hopf, T.K. Hunt, Hyperoxia improves microvascular perfusion in a murine wound model, Wound Repair Regen. 13 (2005) 303–308.
[71] E. Uhl, F. Rosken, A. Sirsjo, K. Messmer, Influence of platelet-derived growth factor on microcirculation during normal and impaired wound healing, Wound Repair Regen. 11 (2003) 361–367.
[72] C.S. Yoon, H.S. Jung, M.J. Kwon, S.H. Lee, C.W. Kim, M.K. Kim, M. Lee, J.H. Park, Sonoporation of the minicircle-VEGF(165) for wound healing of diabetic mice, Pharm. Res. 26 (2009) 794–801.
[73] C.J. Stewart, R. Frank, K.R. Forrester, J. Tulip, R. Lindsay, R.C. Bray, A comparison of two laser-based methods for determination of burn scar perfusion: laser Doppler versus laser speckle imaging, Burns 31 (2005) 744–752.
[74] T. Furuta, M. Sone, Y. Fujimoto, S. Yagi, M. Sugiura, Y. Kamei, H. Fujii, T. Nakashima, Free flap blood flow evaluated using two-dimensional laser speckle flowgraphy, Int. J. Otolaryngol. 2011 (2011) 297251.
[75] C.J. Stewart, C.L. Gallant-Behm, K. Forrester, J. Tulip, D.A. Hart, R.C. Bray, Kinetics of blood flow during healing of excisional full-thickness skin wounds in pigs as monitored by laser speckle perfusion imaging, Skin Res. Technol. 12 (2006) 247–253.
[76] T.R. Howdieshell, L. McGuire, J. Maestas, P.G. McGuire, Pattern recognition receptor gene expression in ischemia-induced flap revascularization, Surgery 150 (2011) 418–428.
[77] P.G. McGuire, T.R. Howdieshell, The importance of engraftment in flap revascularization: confirmation by laser speckle perfusion imaging, J. Surg. Res. 164 (2010) e201–212.
[78] M.D. Menger, M.W. Laschke, B. Vollmar, Viewing the microcirculation through the window: some twenty years experience with the hamster dorsal skinfold chamber, Eur. Surg. Res. 34 (2002) 83–91.
[79] M.W. Laschke, M.D. Menger, The dorsal skinfold chamber: a versatile tool for preclinical research in tissue engineering and regenerative medicine, Eur. Cell. Mater. 32 (2016) 202–215.
[80] J.E. Bluff, S. O'Ceallaigh, S. O'Kane, M.W. Ferguson, G. Ireland, The microcirculation in acute murine cutaneous incisional wounds shows a spatial and temporal variation in the functionality of vessels, Wound Repair Regen. 14 (2006) 434–442.
[81] M.A. Varia, D.P. Calkins-Adams, L.H. Rinker, A.S. Kennedy, D.B. Novotny, W.C. Fowler Jr., J.A. Raleigh, Pimonidazole: a novel hypoxia marker for complementary study of tumor hypoxia and cell proliferation in cervical carcinoma, Gynecol. Oncol. 71 (1998) 270–277.
[82] D. Xing, L. Liu, G.P. Marti, X. Zhang, M. Reinblatt, S.M. Milner, J.W. Harmon, Hypoxia and hypoxia-inducible factor in the burn wound, Wound Repair Regen. 19 (2011) 205–213.
[83] S. Kang, D. Lee, B.E. Theusch, C.J. Arpey, T.J. Brennan, Wound hypoxia in deep tissue after incision in rats, Wound Repair Regen. 21 (2013) 730–739.
[84] Z. Lokmic, I.A. Darby, E.W. Thompson, G.M. Mitchell, Time course analysis of hypoxia, granulation tissue and blood vessel growth, and remodeling in healing rat cutaneous incisional primary intention wounds, Wound Repair Regen. 14 (2006) 277–288.
[85] M. Frangoulis, P. Georgiou, C. Chrisostomidis, D. Perrea, I. Dontas, N. Kavantzas, A. Kostakis, O. Papadopoulos, Rat epigastric flap survival and VEGF expression after local copper application, Plast. Reconstr. Surg. 119 (2007) 837–843.

[86] A.S. Cowburn, L.E.C. Alexander, M. Southwood, V. Nizet, E.R. Chilvers, R.S. Johnson, Epidermal deletion of HIF-2alpha stimulates wound closure, J. Invest. Dermatol. 134 (2014) 801–808.
[87] L. Chen, A. Endler, K. Uchida, S. Horiguchi, Y. Morizane, O. Iijima, M. Toi, F. Shibasaki, Int6/eIF3e silencing promotes functional blood vessel outgrowth and enhances wound healing by upregulating hypoxia-induced factor 2alpha expression, Circulation 122 (2010) 910–919.
[88] Z.N. Rabbani, J. Mi, Y. Zhang, M. Delong, I.L. Jackson, K. Fleckenstein, F.K. Salahuddin, X. Zhang, B. Clary, M.S. Anscher, Z. Vujaskovic, Hypoxia inducible factor 1alpha signaling in fractionated radiation-induced lung injury: role of oxidative stress and tissue hypoxia, Radiat. Res. 173 (2010) 165–174.
[89] J.C. Zampell, A. Yan, T. Avraham, S. Daluvoy, E.S. Weitman, B.J. Mehrara, HIF-1alpha coordinates lymphangiogenesis during wound healing and in response to inflammation, FASEB J. 26 (2012) 1027–1039.
[90] H. Barker, M. Aaltonen, P. Pan, M. Vahatupa, P. Kaipiainen, U. May, S. Prince, H. Uusitalo-Jarvinen, A. Waheed, S. Pastorekova, W.S. Sly, S. Parkkila, T.A. Jarvinen, Role of carbonic anhydrases in skin wound healing, Exp. Mol. Med. 49 (2017), e334. .
[91] I.R. Botusan, V.G. Sunkari, O. Savu, A.I. Catrina, J. Grunler, S. Lindberg, T. Pereira, S. Yla-Herttuala, L. Poellinger, K. Brismar, S.B. Catrina, Stabilization of HIF-1alpha is critical to improve wound healing in diabetic mice, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 19426–19431.
[92] H. Huang, Q. Zhang, J. Liu, H. Hao, C. Jiang, W. Han, Granulocyte-colony stimulating factor (G-CSF) accelerates wound healing in hemorrhagic shock rats by enhancing angiogenesis and attenuating apoptosis, Med. Sci. Monit. 23 (2017) 2644–2653.
[93] R.E. Mirza, T.J. Koh, Contributions of cell subsets to cytokine production during normal and impaired wound healing, Cytokine 71 (2015) 409–412.
[94] K.K. Chereddy, A. Lopes, S. Koussoroplis, V. Payen, C. Moia, H. Zhu, P. Sonveaux, P. Carmeliet, A. Des Rieux, G. Vandermeulen, V. Preat, Combined effects of PLGA and vascular endothelial growth factor promote the healing of non-diabetic and diabetic wounds, Nanomedicine 11 (2015) 1975–1984.
[95] S.A. Eming, P. Martin, M. Tomic-Canic, Wound repair and regeneration: mechanisms, signaling, and translation, Sci. Transl. Med. 6 (2014) 265sr266.
[96] R.D. Galiano, J.T. Michaels, M. Dobryansky, J.P. Levine, G.C. Gurtner, Quantitative and reproducible murine model of excisional wound healing, Wound Repair Regen. 12 (2004) 485–492.
[97] X. Wang, J. Ge, E.E. Tredget, Y. Wu, The mouse excisional wound splinting model, including applications for stem cell transplantation, Nat. Protoc. 8 (2013) 302–309.
[98] R. Nunan, K.G. Harding, P. Martin, Clinical challenges of chronic wounds: searching for an optimal animal model to recapitulate their complexity, Dis. Model. Mech. 7 (2014) 1205–1213.
[99] T.P. Sullivan, W.H. Eaglstein, S.C. Davis, P. Mertz, The pig as a model for human wound healing, Wound Repair Regen. 9 (2001) 66–76.
[100] A. Summerfield, F. Meurens, M.E. Ricklin, The immunology of the porcine skin and its value as a model for human skin, Mol. Immunol. 66 (2015) 14–21.
[101] M. Seaton, A. Hocking, N.S. Gibran, Porcine models of cutaneous wound healing, ILAR J. 56 (2015) 127–138.
[102] T.J. Wieman, J.M. Smiell, Y. Su, Efficacy and safety of a topical gel formulation of recombinant human platelet-derived growth factor-BB (becaplermin) in patients with chronic neuropathic diabetic ulcers. A phase III randomized placebo-controlled double-blind study, Diabetes Care 21 (1998) 822–827.
[103] S. Barrientos, H. Brem, O. Stojadinovic, M. Tomic-Canic, Clinical application of growth factors and cytokines in wound healing, Wound Repair Regen. 22 (2014) 569–578.
[104] F.L. Game, C. Attinger, A. Hartemann, R.J. Hinchliffe, M. Londahl, P.E. Price, W.J. Jeffcoate, F. International Working Group on the Diabetic, IWGDF guidance on use of interventions to enhance the healing of chronic ulcers of the foot in diabetes, Diabetes Metab. Res. Rev. 32 (Suppl. 1) (2016) 75–83.
[105] K. Markakis, F.L. Bowling, A.J. Boulton, The diabetic foot in 2015: an overview, Diabetes Metab. Res. Rev. 32 (Suppl. 1) (2016) 169–178.
[106] G. Gainza, S. Villullas, J.L. Pedraz, R.M. Hernandez, M. Igartua, Advances in drug delivery systems (DDSs) to release growth factors for wound healing and skin regeneration, Nanomedicine 11 (2015) 1551–1573.
[107] M. Berthet, Y. Gauthier, C. Lacroix, B. Verrier, C. Monge, Nanoparticle-based dressing: the future of wound treatment? Trends Biotechnol. 35 (2017) 770–784.
[108] F. Danhier, E. Ansorena, J.M. Silva, R. Coco, A. Le Breton, V. Preat, PLGA-based nanoparticles: an overview of biomedical applications, J. Control. Release 161 (2012) 505–522.
[109] C. Rodrigues de Azevedo, M. von Stosch, M.S. Costa, A.M. Ramos, M.M. Cardoso, F. Danhier, V. Preat, R. Oliveira, Modeling of the burst release from PLGA micro- and nanoparticles as function of physicochemical parameters and formulation characteristics, Int. J. Pharm. 532 (2017) 229–240.
[110] H.K. Makadia, S.J. Siegel, Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier, Polymers (Basel) 3 (2011) 1377–1397.
[111] T.K. Hunt, W.B. Conolly, S.B. Aronson, P. Goldstein, Anaerobic metabolism and wound healing: an hypothesis for the initiation and cessation of collagen synthesis in wounds, Am. J. Surg. 135 (1978) 328–332.
[112] O. Trabold, S. Wagner, C. Wicke, H. Scheuenstuhl, M.Z. Hussain, N. Rosen, A. Seremetiev, H.D. Becker, T.K. Hunt, Lactate and oxygen constitute a fundamental regulatory mechanism in wound healing, Wound Repair Regen. 11 (2003) 504–509.
[113] S. Beckert, F. Farrahi, R.S. Aslam, H. Scheuenstuhl, A. Konigsrainer, M.Z. Hussain, T.K. Hunt, Lactate stimulates endothelial cell migration, Wound Repair Regen. 14 (2006) 321–324.
[114] P.E. Porporato, V.L. Payen, C.J. De Saedeleer, V. Preat, J.P. Thissen, O. Feron, P. Sonveaux, Lactate stimulates angiogenesis and accelerates the healing of superficial and ischemic wounds in mice, Angiogenesis 15 (2012) 581–592.
[115] K.K. Chereddy, R. Coco, P.B. Memvanga, B. Ucakar, A. des Rieux, G. Vandermeulen, V. Preat, Combined effect of PLGA and curcumin on wound healing activity, J. Control. Release 171 (2013) 208–215.
[116] K.K. Chereddy, C.H. Her, M. Comune, C. Moia, A. Lopes, P.E. Porporato, J. Vanacker, M.C. Lam, L. Steinstraesser, P. Sonveaux, H. Zhu, L.S. Ferreira, G. Vandermeulen, V. Preat, PLGA nanoparticles loaded with host defense peptide LL37 promote wound healing, J. Control. Release 194 (2014) 138–147.
[117] J.S. Golub, Y.T. Kim, C.L. Duvall, R.V. Bellamkonda, D. Gupta, A.S. Lin, D. Weiss, W. Robert Taylor, R.E. Guldberg, Sustained VEGF delivery via PLGA nanoparticles promotes vascular growth, Am. J. Physiol. Heart Circ. Physiol. 298 (2010) H1959–1965.
[118] Y.I. Chung, S.K. Kim, Y.K. Lee, S.J. Park, K.O. Cho, S.H. Yuk, G. Tae, Y.H. Kim, Efficient revascularization by VEGF administration via heparin-functionalized nanoparticle-fibrin complex, J. Control. Release 143 (2010) 282–289.
[119] B. Jiang, G. Zhang, E.M. Brey, Dual delivery of chlorhexidine and platelet-derived growth factor-BB for enhanced wound healing and infection control, Acta Biomater. 9 (2013) 4976–4984.
[120] Q. Jin, G. Wei, Z. Lin, J.V. Sugai, S.E. Lynch, P.X. Ma, W.V. Giannobile, Nanofibrous scaffolds incorporating PDGF-BB microspheres induce chemokine expression and tissue neogenesis in vivo, PLoS One 3 (2008), e1729. .
[121] G. Wei, Q. Jin, W.V. Giannobile, P.X. Ma, Nano-fibrous scaffold for controlled delivery of recombinant human PDGF-BB, J. Control. Release 112 (2006) 103–110.
[122] T. Dai, M. Tanaka, Y.Y. Huang, M.R. Hamblin, Chitosan preparations for wounds and burns: antimicrobial and wound-healing effects, Expert Rev. Anti-Infect. Ther. 9 (2011) 857–879.
[123] D. Raafat, K. von Bargen, A. Haas, H.G. Sahl, Insights into the mode of action of chitosan as an antibacterial compound, Appl. Environ. Microbiol. 74 (2008) 3764–3773.
[124] E.I. Rabea, M.E. Badawy, C.V. Stevens, G. Smagghe, W. Steurbaut, Chitosan as antimicrobial agent: applications and mode of action, Biomacromolecules 4 (2003) 1457–1465.
[125] T. Tang, H. Jiang, Y. Yu, F. He, S.Z. Ji, Y.Y. Liu, Z.S. Wang, S.C. Xiao, C. Tang, G.Y. Wang, Z.F. Xia, A new method of wound treatment: targeted therapy of skin wounds with reactive oxygen species-responsive nanoparticles containing SDF-1alpha, Int. J. Nanomedicine 10 (2015) 6571–6585.
[126] M. Kong, X.G. Chen, K. Xing, H.J. Park, Antimicrobial properties of chitosan and mode of action: a state of the art review, Int. J. Food Microbiol. 144 (2010) 51–63.
[127] P. Li, Y.F. Poon, W. Li, H.Y. Zhu, S.H. Yeap, Y. Cao, X. Qi, C. Zhou, M. Lamrani, R.W. Beuerman, E.T. Kang, Y. Mu, C.M. Li, M.W. Chang, S.S. Leong, M.B. Chan-Park, A polycationic antimicrobial and biocompatible hydrogel with microbe membrane suctioning ability, Nat. Mater. 10 (2011) 149–156.
[128] K. Kojima, Y. Okamoto, K. Kojima, K. Miyatake, H. Fujise, Y. Shigemasa, S. Minami, Effects of chitin and chitosan on collagen synthesis in wound healing, J. Vet. Med. Sci. 66 (2004) 1595–1598.
[129] H. Ueno, T. Mori, T. Fujinaga, Topical formulations and wound healing applications of chitosan, Adv. Drug Deliv. Rev. 52 (2001) 105–115.
[130] H. Ueno, H. Yamada, I. Tanaka, N. Kaba, M. Matsuura, M. Okumura, T. Kadosawa, T. Fujinaga, Accelerating effects of chitosan for healing at early phase of experimental open wound in dogs, Biomaterials 20 (1999) 1407–1414.
[131] G.I. Howling, P.W. Dettmar, P.A. Goddard, F.C. Hampson, M. Dornish, E.J. Wood, The effect of chitin and chitosan on the proliferation of human skin fibroblasts and keratinocytes in vitro, Biomaterials 22 (2001) 2959–2966.
[132] E.G. Nascimento, T.B. Sampaio, A.C. Medeiros, E.P. Azevedo, Evaluation of chitosan gel with 1% silver sulfadiazine as an alternative for burn wound treatment in rats, Acta Cir. Bras. 24 (2009) 460–465.
[133] Z. Degim, N. Celebi, H. Sayan, A. Babul, D. Erdogan, G. Take, An investigation on skin wound healing in mice with a taurine-chitosan gel formulation, Amino Acids 22 (2002) 187–198.
[134] V. Bertoncelj, J. Pelipenko, J. Kristl, M. Jeras, M. Cukjati, P. Kocbek, Development and bioevaluation of nanofibers with blood-derived growth factors for dermal wound healing, Eur. J. Pharm. Biopharm. 88 (2014) 64–74.
[135] M.C. Bonferoni, G. Sandri, E. Dellera, S. Rossi, F. Ferrari, M. Mori, C. Caramella, Ionic polymeric micelles based on chitosan and fatty acids and intended for wound healing. Comparison of linoleic and oleic acid, Eur. J. Pharm. Biopharm. 87 (2014) 101–106.
[136] Q. Xiang, J. Xiao, H. Zhang, X. Zhang, M. Lu, H. Zhang, Z. Su, W. Zhao, C. Lin, Y. Huang, X. Li, Preparation and characterisation of bFGF-encapsulated liposomes and evaluation of wound-healing activities in the rat, Burns 37 (2011) 886–895.
[137] B. Zavan, V. Vindigni, K. Vezzu, G. Zorzato, C. Luni, G. Abatangelo, N. Elvassore, R. Cortivo, Hyaluronan based porous nano-particles enriched with growth factors for the treatment of ulcers: a placebo-controlled study, J. Mater. Sci. Mater. Med. 20 (2009) 235–247.
[138] X.G. Xie, M. Zhang, Y.K. Dai, M.S. Ding, S.D. Meng, Combination of vascular endothelial growth factor-loaded microspheres and hyperbaric oxygen on random skin flap survival in rats, Exp. Ther. Med. 10 (2015) 954–958.
[139] X. Li, X. Ye, J. Qi, R. Fan, X. Gao, Y. Wu, L. Zhou, A. Tong, G. Guo, EGF and curcumin co-encapsulated nanoparticle/hydrogel system as potent skin regeneration agent, Int. J. Nanomedicine 11 (2016) 3993–4009.
[140] Z. Xie, C.B. Paras, H. Weng, P. Punnakitikashem, L.C. Su, K. Vu, L. Tang, J. Yang, K.T. Nguyen, Dual growth factor releasing multi-functional nanofibers for wound healing, Acta Biomater. 9 (2013) 9351–9359.
[141] H.J. Lai, C.H. Kuan, H.C. Wu, J.C. Tsai, T.M. Chen, D.J. Hsieh, T.W. Wang, Tailored design of electrospun composite nanofibers with staged release of multiple angiogenic growth factors for chronic wound healing, Acta Biomater. 10 (2014) 4156–4166.
[142] P. Losi, E. Briganti, C. Errico, A. Lisella, E. Sanguinetti, F. Chiellini, G. Soldani, Fibrin-based scaffold incorporating VEGF- and bFGF-loaded nanoparticles stimulates wound healing in diabetic mice, Acta Biomater. 9 (2013) 7814–7821.

[143] M.P. Ribeiro, P.I. Morgado, S.P. Miguel, P. Coutinho, I.J. Correia, Dextran-based hydrogel containing chitosan microparticles loaded with growth factors to be used in wound healing, Mater Sci Eng C Mater Biol Appl 33 (2013) 2958–2966.
[144] I.H. Bae, J.W. Park, D.Y. Kim, Enhanced regenerative healing efficacy of a highly skin-permeable growth factor nanocomplex in a full-thickness excisional mouse wound model, Int. J. Nanomedicine 9 (2014) 4551–4567.
[145] J.K. Choi, J.H. Jang, W.H. Jang, J. Kim, I.H. Bae, J. Bae, Y.H. Park, B.J. Kim, K.M. Lim, J.W. Park, The effect of epidermal growth factor (EGF) conjugated with low-molecular-weight protamine (LMWP) on wound healing of the skin, Biomaterials 33 (2012) 8579–8590.
[146] J.H. Lee, I.H. Bae, J.K. Choi, J.W. Park, Evaluation of a highly skin permeable low-molecular-weight protamine conjugated epidermal growth factor for novel burn wound healing therapy, J. Pharm. Sci. 102 (2013) 4109–4120.
[147] W.G. La, H.S. Yang, Heparin-conjugated poly(lactic-co-glycolic acid) nanospheres enhance large-wound healing by delivering growth factors in platelet-rich plasma, Artif. Organs 39 (2015) 388–394.
[148] Y. Takabayashi, M. Ishihara, Y. Sumi, M. Takikawa, S. Nakamura, T. Kiyosawa, Platelet-rich plasma-containing fragmin-protamine micro-nanoparticles promote epithelialization and angiogenesis in split-thickness skin graft donor sites, J. Surg. Res. 193 (2015) 483–491.
[149] J.S. Isenberg, L.A. Ridnour, M.G. Espey, D.A. Wink, D.D. Roberts, Nitric oxide in wound-healing, Microsurgery 25 (2005) 442–451.
[150] A. Soneja, M. Drews, T. Malinski, Role of nitric oxide, nitroxidative and oxidative stress in wound healing, Pharmacol. Rep. 57 (Suppl) (2005) 108–119.
[151] J.F. Quinn, M.R. Whittaker, T.P. Davis, Delivering nitric oxide with nanoparticles, J. Control. Release 205 (2015) 190–205.
[152] J. Chouake, D. Schairer, A. Kutner, D.A. Sanchez, J. Makdisi, K. Blecher-Paz, P. Nacharaju, C. Tuckman-Vernon, P. Gialanella, J.M. Friedman, J.D. Nosanchuk, A.J. Friedman, Nitrosoglutathione generating nitric oxide nanoparticles as an improved strategy for combating *Pseudomonas aeruginosa*-infected wounds, J. Drugs Dermatol. 11 (2012) 1471–1477.
[153] M.R. Mihu, U. Sandkovsky, G. Han, J.M. Friedman, J.D. Nosanchuk, L.R. Martinez, The use of nitric oxide releasing nanoparticles as a treatment against *Acinetobacter baumannii* in wound infections, Virulence 1 (2010) 62–67.
[154] H. Nurhasni, J. Cao, M. Choi, I. Kim, B.L. Lee, Y. Jung, J.W. Yoo, Nitric oxide-releasing poly(lactic-co-glycolic acid)-polyethylenimine nanoparticles for prolonged nitric oxide release, antibacterial efficacy, and in vivo wound healing activity, Int. J. Nanomedicine 10 (2015) 3065–3080.
[155] C. Vogt, Q. Xing, W. He, B. Li, M.C. Frost, F. Zhao, Fabrication and characterization of a nitric oxide-releasing nanofibrous gelatin matrix, Biomacromolecules 14 (2013) 2521–2530.
[156] K.A. Wold, V.B. Damodaran, L.A. Suazo, R.A. Bowen, M.M. Reynolds, Fabrication of biodegradable polymeric nanofibers with covalently attached NO donors, ACS Appl. Mater. Interfaces 4 (2012) 3022–3030.
[157] K. Blecher, L.R. Martinez, C. Tuckman-Vernon, P. Nacharaju, D. Schairer, J. Chouake, J.M. Friedman, A. Alfieri, C. Guha, J.D. Nosanchuk, A.J. Friedman, Nitric oxide-releasing nanoparticles accelerate wound healing in NOD-SCID mice, Nanomedicine 8 (2012) 1364–1371.
[158] G. Han, L.N. Nguyen, C. Macherla, Y. Chi, J.M. Friedman, J.D. Nosanchuk, L.R. Martinez, Nitric oxide-releasing nanoparticles accelerate wound healing by promoting fibroblast migration and collagen deposition, Am. J. Pathol. 180 (2012) 1465–1473.
[159] D. Akbik, M. Ghadiri, W. Chrzanowski, R. Rohanizadeh, Curcumin as a wound healing agent, Life Sci. 116 (2014) 1–7.
[160] I. Castangia, A. Nacher, C. Caddeo, D. Valenti, A.M. Fadda, O. Diez-Sales, A. Ruiz-Sauri, M. Manconi, Fabrication of quercetin and curcumin bionanovesicles for the prevention and rapid regeneration of full-thickness skin defects on mice, Acta Biomater. 10 (2014) 1292–1300.
[161] X. Dai, J. Liu, H. Zheng, J. Wichmann, U. Hopfner, S. Sudhop, C. Prein, Y. Shen, H.-G. Machens, A.F. Schilling, Nano-formulated curcumin accelerates acute wound healing through Dkk-1-mediated fibroblast mobilization and MCP-1-mediated anti-inflammation, NPG Asia Mater. 9 (2017), e368. .
[162] C. Gong, Q. Wu, Y. Wang, D. Zhang, F. Luo, X. Zhao, Y. Wei, Z. Qian, A biodegradable hydrogel system containing curcumin encapsulated in micelles for cutaneous wound healing, Biomaterials 34 (2013) 6377–6387.
[163] A. Hasan, G. Waibhaw, S. Tiwari, K. Dharmalingam, I. Shukla, L.M. Pandey, Fabrication and characterization of chitosan, polyvinylpyrrolidone, and cellulose nanowhiskers nanocomposite films for wound healing drug delivery application, J. Biomed. Mater. Res. A 105 (2017) 2391–2404.
[164] V.V. Karri, G. Kuppusamy, S.V. Talluri, S.S. Mannemala, R. Kollipara, A.D. Wadhwani, S. Mulukutla, K.R. Raju, R. Malayandi, Curcumin loaded chitosan nanoparticles impregnated into collagen-alginate scaffolds for diabetic wound healing, Int. J. Biol. Macromol. 93 (2016) 1519–1529.
[165] A.E. Krausz, B.L. Adler, V. Cabral, M. Navati, J. Doerner, R.A. Charafeddine, D. Chandra, H. Liang, L. Gunther, A. Clendaniel, S. Harper, J.M. Friedman, J.D. Nosanchuk, A.J. Friedman, Curcumin-encapsulated nanoparticles as innovative antimicrobial and wound healing agent, Nanomedicine 11 (2015) 195–206.
[166] J.G. Merrell, S.W. McLaughlin, L. Tie, C.T. Laurencin, A.F. Chen, L.S. Nair, Curcumin-loaded poly(epsilon-caprolactone) nanofibres: diabetic wound dressing with anti-oxidant and anti-inflammatory properties, Clin. Exp. Pharmacol. Physiol. 36 (2009) 1149–1156.
[167] N. Ramalingam, T.S. Natarajan, S. Rajiv, Preparation and characterization of electrospun curcumin loaded poly(2-hydroxyethyl methacrylate) nanofiber–a biomaterial for multidrug resistant organisms, J. Biomed. Mater. Res. A 103 (2015) 16–24.
[168] M. Ranjbar-Mohammadi, S. Rabbani, S.H. Bahrami, M.T. Joghataei, F. Moayer, Antibacterial performance and in vivo diabetic wound healing of curcumin loaded gum tragacanth/poly(epsilon-caprolactone) electrospun nanofibers, Mater Sci Eng C Mater Biol Appl 69 (2016) 1183–1191.
[169] G.D. Venkatasubbu, T. Anusuya, Investigation on curcumin nanocomposite for wound dressing, Int. J. Biol. Macromol. 98 (2017) 366–378.
[170] X. Li, S. Chen, B. Zhang, M. Li, K. Diao, Z. Zhang, J. Li, Y. Xu, X. Wang, H. Chen, In situ injectable nano-composite hydrogel composed of curcumin, N,O-carboxymethyl chitosan and oxidized alginate for wound healing application, Int. J. Pharm. 437 (2012) 110–119.
[171] D. Xhindoli, S. Pacor, M. Benincasa, M. Scocchi, R. Gennaro, A. Tossi, The human cathelicidin LL-37–a pore-forming antibacterial peptide and host-cell modulator, Biochim. Biophys. Acta 1858 (2016) 546–566.
[172] D. Vandamme, B. Landuyt, W. Luyten, L. Schoofs, A comprehensive summary of LL-37, the factotum human cathelicidin peptide, Cell. Immunol. 280 (2012) 22–35.
[173] R. Koczulla, G. von Degenfeld, C. Kupatt, F. Krötz, S. Zahler, T. Gloe, K. Issbrücker, P. Unterberger, M. Zaiou, C. Lebherz, A. Karl, P. Raake, A. Pfosser, P. Boekstegers, U. Welsch, P.S. Hiemstra, C. Vogelmeier, R.L. Gallo, M. Clauss, R. Bals, An angiogenic role for the human peptide antibiotic LL-37/hCAP-18, J. Clin. Invest. 111 (2003) 1665–1672.
[174] L. Steinstraesser, A. Ring, R. Bals, H.-U. Steinau, S. Langer, The human host defense peptide LL37/hCAP accelerates angiogenesis in PEGT/PBT biopolymers, Ann. Plast. Surg. 56 (2006) 93–98.
[175] S. Rodriguez-Martinez, J.C. Cancino-Diaz, L.M. Vargas-Zuniga, M.E. Cancino-Diaz, LL-37 regulates the overexpression of vascular endothelial growth factor (VEGF) and c-IAP-2 in human keratinocytes, Int. J. Dermatol. 47 (2008) 457–462.
[176] L. Steinstraesser, M.C. Lam, F. Jacobsen, P.E. Porporato, K.K. Chereddy, M. Becerikli, I. Stricker, R.E. Hancock, M. Lehnhardt, P. Sonveaux, V. Preat, G. Vandermeulen, Skin electroporation of a plasmid encoding hCAP-18/LL-37 host defense peptide promotes wound healing, Mol. Ther. 22 (2014) 734–742.
[177] I. Garcia-Orue, G. Gainza, C. Girbau, R. Alonso, J.J. Aguirre, J.L. Pedraz, M. Igartua, R.M. Hernandez, LL37 loaded nanostructured lipid carriers (NLC): a new strategy for the topical treatment of chronic wounds, Eur. J. Pharm. Biopharm. 108 (2016) 310–316.
[178] M. Fumakia, E.A. Ho, Nanoparticles encapsulated with LL37 and serpin A1 promotes wound healing and synergistically enhances antibacterial activity, Mol. Pharm. 13 (2016) 2318–2331.
[179] X. Li, R. Fan, A. Tong, M. Yang, J. Deng, L. Zhou, X. Zhang, G. Guo, In situ gel-forming AP-57 peptide delivery system for cutaneous wound healing, Int. J. Pharm. 495 (2015) 560–571.
[180] S. Romano Di Peppe, A. Mangoni, G. Zambruno, G. Spinetti, G. Melillo, M. Napolitano, M.C. Capogrossi, Adenovirus-mediated VEGF(165) gene transfer enhances wound healing by promoting angiogenesis in CD1 diabetic mice, Gene Ther. 9 (2002) 1271–1277.
[181] H. Brem, A. Kodra, M.S. Golinko, H. Entero, O. Stojadinovic, V.M. Wang, C.M. Sheahan, A.D. Weinberg, S.L. Woo, H.P. Ehrlich, M. Tomic-Canic, Mechanism of sustained release of vascular endothelial growth factor in accelerating experimental diabetic healing, J. Invest. Dermatol. 129 (2009) 2275–2287.
[182] R.E. Giunta, T. Holzbach, C. Taskov, P.S. Holm, M.A. Konerding, D. Schams, E. Biemer, B. Gansbacher, AdVEGF165 gene transfer increases survival in overdimensioned skin flaps, J. Gene Med. 7 (2005) 297–306.
[183] R. Gurunluoglu, K. Ozer, B. Skugor, P. Lubiatowski, K. Carnevale, M. Siemionow, Effect of transfection time on the survival of epigastric skin flaps pretreated with adenovirus encoding the VEGF gene, Ann. Plast. Surg. 49 (2002) 161–169.
[184] P. Lubiatowski, C.K. Goldman, R. Gurunluoglu, K. Carnevale, M. Siemionow, Enhancement of epigastric skin flap survival by adenovirus-mediated VEGF gene therapy, Plast. Reconstr. Surg. 109 (2002) 1986–1993.
[185] P. Lubiatowski, R. Gurunluoglu, C.K. Goldman, B. Skugor, K. Carnevale, M. Siemionow, Gene therapy by adenovirus-mediated vascular endothelial growth factor and angiopoietin-1 promotes perfusion of muscle flaps, Plast. Reconstr. Surg. 110 (2002) 149–159.
[186] L. Cui, F.C. Li, Q. Zhang, Y.L. Qian, W.X. Guan, Effect of adenovirus-mediated gene transfection of vascular endothelial growth factor on survival of random flaps in rats, Chin. J. Traumatol. 6 (2003) 199–204.
[187] J.J. Vranckx, F. Yao, N. Petrie, H. Augustinova, D. Hoeller, S. Visovatti, J. Slama, E. Eriksson, In vivo gene delivery of Ad-VEGF121 to full-thickness wounds in aged pigs results in high levels of VEGF expression but not in accelerated healing, Wound Repair Regen. 13 (2005) 51–60.
[188] A. Saaristo, T. Tammela, A. Farkkila, M. Karkkainen, E. Suominen, S. Yla-Herttuala, K. Alitalo, Vascular endothelial growth factor-C accelerates diabetic wound healing, Am. J. Pathol. 169 (2006) 1080–1087.
[189] B. Deodato, N. Arsic, L. Zentilin, M. Galeano, D. Santoro, V. Torre, D. Altavilla, D. Valdembri, F. Bussolino, F. Squadrito, M. Giacca, Recombinant AAV vector encoding human VEGF165 enhances wound healing, Gene Ther. 9 (2002) 777–785.
[190] M. Galeano, B. Deodato, D. Altavilla, D. Cucinotta, N. Arsic, H. Marini, V. Torre, M. Giacca, F. Squadrito, Adeno-associated viral vector-mediated human vascular endothelial growth factor gene transfer stimulates angiogenesis and wound healing in the genetically diabetic mouse, Diabetologia 46 (2003) 546–555.
[191] P.J. Taub, J.D. Marmur, W.X. Zhang, D. Senderoff, P.D. Nhat, R. Phelps, M.L. Urken, L. Silver, H. Weinberg, Locally administered vascular endothelial growth factor cDNA increases survival of ischemic experimental skin flaps, Plast. Reconstr. Surg. 102 (1998) 2033–2039.
[192] K.W. Liechty, M. Nesbit, M. Herlyn, A. Radu, N.S. Adzick, T.M. Crombleholme, Adenoviral-mediated overexpression of platelet-derived growth factor-B corrects ischemic impaired wound healing, J. Invest. Dermatol. 113 (1999) 375–383.

[193] G.T. Stavri, Y. Hong, I.C. Zachary, G. Breier, P.A. Baskerville, S. Yla-Herttuala, W. Risau, J.F. Martin, J.D. Erusalimsky, Hypoxia and platelet-derived growth factor-BB synergistically upregulate the expression of vascular endothelial growth factor in vascular smooth muscle cells, FEBS Lett. 358 (1995) 311–315.
[194] S.G. Keswani, A.B. Katz, F.Y. Lim, P. Zoltick, A. Radu, D. Alaee, M. Herlyn, T.M. Crombleholme, Adenoviral mediated gene transfer of PDGF-B enhances wound healing in type I and type II diabetic wounds, Wound Repair Regen. 12 (2004) 497–504.
[195] I. Ono, T. Yamashita, T. Hida, H.Y. Jin, Y. Ito, H. Hamada, Y. Akasaka, T. Ishii, K. Jimbow, Local administration of hepatocyte growth factor gene enhances the regeneration of dermis in acute incisional wounds, J. Surg. Res. 120 (2004) 47–55.
[196] X. Ha, Y. Li, M. Lao, B. Yuan, C.T. Wu, Effect of human hepatocyte growth factor on promoting wound healing and preventing scar formation by adenovirus-mediated gene transfer, Chin. Med. J. 116 (2003) 1029–1033.
[197] G. O'Toole, D. MacKenzie, R. Lindeman, M.F. Buckley, D. Marucci, N. McCarthy, M. Poole, Vascular endothelial growth factor gene therapy in ischaemic rat skin flaps, Br. J. Plast. Surg. 55 (2002) 55–58.
[198] C.K. Byrnes, R.W. Malone, N. Akhter, P.H. Nass, A. Wetterwald, M.G. Cecchini, M.D. Duncan, J.W. Harmon, Electroporation enhances transfection efficiency in murine cutaneous wounds, Wound Repair Regen. 12 (2004) 397–403.
[199] C.D. McKnight, S.R. Winn, X. Gong, J.E. Hansen, M.K. Wax, Revascularization of rat fasciocutaneous flap using CROSSEAL with VEGF protein or plasmid DNA expressing VEGF, Otolaryngol. Head Neck Surg. 139 (2008) 245–249.
[200] T. Tokatlian, C. Cam, T. Segura, Porous hyaluronic acid hydrogels for localized non-viral DNA delivery in a diabetic wound healing model, Adv. Healthc. Mater. 4 (2015) 1084–1091.
[201] B. Ferraro, L.C. Heller, Y.L. Cruz, S. Guo, A. Donate, R. Heller, Evaluation of delivery conditions for cutaneous plasmid electrotransfer using a multielectrode array, Gene Ther. 18 (2011) 496–500.
[202] J. Ko, H. Jun, H. Chung, C. Yoon, T. Kim, M. Kwon, S. Lee, S. Jung, M. Kim, J.H. Park, Comparison of EGF with VEGF non-viral gene therapy for cutaneous wound healing of streptozotocin diabetic mice, Diabetes Metab. J. 35 (2011) 226–235.
[203] M.J. Kwon, S. An, S. Choi, K. Nam, H.S. Jung, C.S. Yoon, J.H. Ko, H.J. Jun, T.K. Kim, S.J. Jung, J.H. Park, Y. Lee, J.S. Park, Effective healing of diabetic skin wounds by using nonviral gene therapy based on minicircle vascular endothelial growth factor DNA and a cationic dendrimer, J. Gene Med. 14 (2012) 272–278.
[204] C. Rinsch, P. Quinodoz, B. Pittet, N. Alizadeh, D. Baetens, D. Montandon, P. Aebischer, M.S. Pepper, Delivery of FGF-2 but not VEGF by encapsulated genetically engineered myoblasts improves survival and vascularization in a model of acute skin flap ischemia, Gene Ther. 8 (2001) 523–533.
[205] P.Y. Lee, S. Chesnoy, L. Huang, Electroporatic delivery of TGF-beta1 gene works synergistically with electric therapy to enhance diabetic wound healing in db/db mice, J. Invest. Dermatol. 123 (2004) 791–798.
[206] L. Liu, G.P. Marti, X. Wei, X. Zhang, H. Zhang, Y.V. Liu, M. Nastai, G.L. Semenza, J.W. Harmon, Age-dependent impairment of HIF-1alpha expression in diabetic mice: correction with electroporation-facilitated gene therapy increases wound healing, angiogenesis, and circulating angiogenic cells, J. Cell. Physiol. 217 (2008) 319–327.
[207] M. Thiersch, M. Rimann, V. Panagiotopoulou, E. Ozturk, T. Biedermann, M. Textor, T.C. Luhmann, H. Hall, The angiogenic response to PLL-g-PEG-mediated HIF-1alpha plasmid DNA delivery in healthy and diabetic rats, Biomaterials 34 (2013) 4173–4182.
[208] K.A. Mace, D.H. Yu, K.Z. Paydar, N. Boudreau, D.M. Young, Sustained expression of Hif-1alpha in the diabetic environment promotes angiogenesis and cutaneous wound repair, Wound Repair Regen. 15 (2007) 636–645.
[209] Y. Taniyama, R. Morishita, M. Aoki, H. Nakagami, K. Yamamoto, K. Yamazaki, K. Matsumoto, T. Nakamura, Y. Kaneda, T. Ogihara, Therapeutic angiogenesis induced by human hepatocyte growth factor gene in rat and rabbit hindlimb ischemia models: preclinical study for treatment of peripheral arterial disease, Gene Ther. 8 (2001) 181–189.
[210] Y. Taniyama, R. Morishita, K. Hiraoka, M. Aoki, H. Nakagami, K. Yamasaki, K. Matsumoto, T. Nakamura, Y. Kaneda, T. Ogihara, Therapeutic angiogenesis induced by human hepatocyte growth factor gene in rat diabetic hind limb ischemia model: molecular mechanisms of delayed angiogenesis in diabetes, Circulation 104 (2001) 2344–2350.
[211] R. Morishita, M. Sakaki, K. Yamamoto, S. Iguchi, M. Aoki, K. Yamasaki, K. Matsumoto, T. Nakamura, R. Lawn, T. Ogihara, Y. Kaneda, Impairment of collateral formation in lipoprotein(a) transgenic mice: therapeutic angiogenesis induced by human hepatocyte growth factor gene, Circulation 105 (2002) 1491–1496.
[212] S.M. Seyed Jafari, M. Shafighi, H. Beltraminelli, T. Geiser, R.E. Hunger, A. Gazdhar, Improvement of flap necrosis in a rat random skin flap model by in vivo electroporation-mediated HGF gene transfer, Plast. Reconstr. Surg. 139 (2017) 1116e–1127e.
[213] A. Jazwa, P. Kucharzewska, J. Leja, A. Zagorska, A. Sierpniowska, J. Stepniewski, M. Kozakowska, H. Taha, T. Ochiya, R. Derlacz, E. Vahakangas, S. Yla-Herttuala, A. Jozkowicz, J. Dulak, Combined vascular endothelial growth factor-A and fibroblast growth factor 4 gene transfer improves wound healing in diabetic mice, Genet. Vaccines Ther. 8 (2010) 6.
[214] P.Y. Liu, K. Liu, X.T. Wang, E. Badiavas, K.M. Rieger-Christ, J.B. Tang, I.C. Summerhayes, Efficacy of combination gene therapy with multiple growth factor cDNAs to enhance skin flap survival in a rat model, DNA Cell Biol. 24 (2005) 751–757.
[215] M.G. Jeschke, D. Klein, Liposomal gene transfer of multiple genes is more effective than gene transfer of a single gene, Gene Ther. 11 (2004) 847–855.
[216] Y. Kunugiza, N. Tomita, Y. Taniyama, T. Tomita, M.K. Osako, K. Tamai, T. Tanabe, Y. Kaneda, H. Yoshikawa, R. Morishita, Acceleration of wound healing by combined gene transfer of hepatocyte growth factor and prostacyclin synthase with Shima Jet, Gene Ther. 13 (2006) 1143–1152.
[217] Y.H. Cho, H. Park, E.S. Cho, W.J. Kim, B.S. Kang, B.Y. Park, Y.J. Kim, Y.I. Lee, S.I. Chang, K. Park, A novel way of therapeutic angiogenesis using an adeno-associated virus-mediated angiogenin gene transfer, Exp. Mol. Med. 39 (2007) 412–418.
[218] A. Bitto, L. Minutoli, M.R. Galeano, D. Altavilla, F. Polito, T. Fiumara, M. Calo, P. Lo Cascio, L. Zentilin, M. Giacca, F. Squadrito, Angiopoietin-1 gene transfer improves impaired wound healing in genetically diabetic mice without increasing VEGF expression, Clin. Sci. (Lond.) 114 (2008) 707–718.
[219] M. Carretero, M.J. Escamez, M. Garcia, B. Duarte, A. Holguin, L. Retamosa, J.L. Jorcano, M.D. Rio, F. Larcher, In vitro and in vivo wound healing-promoting activities of human cathelicidin LL-37, J. Invest. Dermatol. 128 (2008) 223–236.
[220] F. Jacobsen, D. Mittler, T. Hirsch, A. Gerhards, M. Lehnhardt, B. Voss, H.U. Steinau, L. Steinstraesser, Transient cutaneous adenoviral gene therapy with human host defense peptide hCAP-18/LL-37 is effective for the treatment of burn wound infections, Gene Ther. 12 (2005) 1494–1502.
[221] K. Yamasaki, H.D. Edington, C. McClosky, E. Tzeng, A. Lizonova, I. Kovesdi, D.L. Steed, T.R. Billiar, Reversal of impaired wound repair in iNOS-deficient mice by topical adenoviral-mediated iNOS gene transfer, J. Clin. Invest. 101 (1998) 967–971.
[222] J.D. Luo, Y.Y. Wang, W.L. Fu, J. Wu, A.F. Chen, Gene therapy of endothelial nitric oxide synthase and manganese superoxide dismutase restores delayed wound healing in type 1 diabetic mice, Circulation 110 (2004) 2484–2493.
[223] A.M. Breen, P. Dockery, T. O'Brien, A.S. Pandit, The use of therapeutic gene eNOS delivered via a fibrin scaffold enhances wound healing in a compromised wound model, Biomaterials 29 (2008) 3143–3151.
[224] K. Ozawa, T. Kondo, O. Hori, Y. Kitao, D.M. Stern, W. Eisenmenger, S. Ogawa, T. Ohshima, Expression of the oxygen-regulated protein ORP150 accelerates wound healing by modulating intracellular VEGF transport, J. Clin. Invest. 108 (2001) 41–50.
[225] S.M. Seyed Jafari, M. Shafighi, H. Beltraminelli, B. Weber, R.A. Schmid, T. Geiser, A. Gazdhar, R.E. Hunger, Efficacy of in vivo electroporation-mediated IL-10 gene delivery on survival of skin flaps, J. Membr. Biol. (2017)https://doi.org/10.1007/s00232-017-9974-x.
[226] F.L. Game, J. Apelqvist, C. Attinger, A. Hartemann, R.J. Hinchliffe, M. Londahl, P.E. Price, W.J. Jeffcoate, F. International Working Group on the Diabetic, Effectiveness of interventions to enhance healing of chronic ulcers of the foot in diabetes: a systematic review, Diabetes Metab. Res. Rev. 32 (Suppl. 1) (2016) 154–168.
[227] J.M. Isner, A. Pieczek, R. Schainfeld, R. Blair, L. Haley, T. Asahara, K. Rosenfield, S. Razvi, K. Walsh, J.F. Symes, Clinical evidence of angiogenesis after arterial gene transfer of phVEGF165 in patient with ischaemic limb, Lancet 348 (1996) 370–374.
[228] I. Baumgartner, G. Rauh, A. Pieczek, D. Wuensch, M. Magner, M. Kearney, R. Schainfeld, J.M. Isner, Lower-extremity edema associated with gene transfer of naked DNA encoding vascular endothelial growth factor, Ann. Intern. Med. 132 (2000) 880–884.
[229] K. Makinen, H. Manninen, M. Hedman, P. Matsi, H. Mussalo, E. Alhava, S. Yla-Herttuala, Increased vascularity detected by digital subtraction angiography after VEGF gene transfer to human lower limb artery: a randomized, placebo-controlled, double-blinded phase II study, Mol. Ther. 6 (2002) 127–133.
[230] H.J. Kim, S.Y. Jang, J.I. Park, J. Byun, D.I. Kim, Y.S. Do, J.M. Kim, S. Kim, B.M. Kim, W.B. Kim, D.K. Kim, Vascular endothelial growth factor-induced angiogenic gene therapy in patients with peripheral artery disease, Exp. Mol. Med. 36 (2004) 336–344.
[231] K.G. Shyu, H. Chang, B.W. Wang, P. Kuan, Intramuscular vascular endothelial growth factor gene therapy in patients with chronic critical leg ischemia, Am. J. Med. 114 (2003) 85–92.
[232] Y.H. Kusumanto, V. van Weel, N.H. Mulder, A.J. Smit, J.J. van den Dungen, J.M. Hooymans, W.J. Sluiter, R.A. Tio, P.H. Quax, R.O. Gans, R.P. Dullaart, G.A. Hospers, Treatment with intramuscular vascular endothelial growth factor gene compared with placebo for patients with diabetes mellitus and critical limb ischemia: a double-blind randomized trial, Hum. Gene Ther. 17 (2006) 683–691.
[233] D.J. Margolis, L.M. Morris, M. Papadopoulos, L. Weinberg, J.C. Filip, S.A. Lang, S.S. Vaikunth, T.M. Crombleholme, Phase I study of H5.020CMV.PDGF-beta to treat venous leg ulcer disease, Mol. Ther. 17 (2009) 1822–1829.
[234] G. Mulder, A.J. Tallis, V.T. Marshall, D. Mozingo, L. Phillips, G.F. Pierce, L.A. Chandler, B.K. Sosnowski, Treatment of nonhealing diabetic foot ulcers with a platelet-derived growth factor gene-activated matrix (GAM501): results of a phase 1/2 trial, Wound Repair Regen. 17 (2009) 772–779.
[235] R.J. Powell, P. Goodney, F.O. Mendelsohn, E.K. Moen, B.H. Annex, H.G.F.T. Investigators, Safety and efficacy of patient specific intramuscular injection of HGF plasmid gene therapy on limb perfusion and wound healing in patients with ischemic lower extremity ulceration: results of the HGF-0205 trial, J. Vasc. Surg. 52 (2010) 1525–1530.
[236] R. Morishita, H. Makino, M. Aoki, N. Hashiya, K. Yamasaki, J. Azuma, Y. Taniyama, Y. Sawa, Y. Kaneda, T. Ogihara, Phase I/IIa clinical trial of therapeutic angiogenesis using hepatocyte growth factor gene transfer to treat critical limb ischemia, Arterioscler. Thromb. Vasc. Biol. 31 (2011) 713–720.
[237] S. Cui, L. Guo, X. Li, Y. Gu, J. Fu, L. Dong, H. Song, X. Chen, Y. Lu, C. Hu, F. Xiao, D. Zhu, Z. Wu, Q. Zhang, Clinical safety and preliminary efficacy of plasmid pUDK-HGF expressing human hepatocyte growth factor (HGF) in patients with critical limb ischemia, Eur. J. Vasc. Endovasc. Surg. 50 (2015) 494–501.

[238] M.R. Kibbe, A.T. Hirsch, F.O. Mendelsohn, M.G. Davies, H. Pham, J. Saucedo, W. Marston, W.B. Pyun, S.K. Min, B.G. Peterson, A. Comerota, D. Choi, J. Ballard, R.A. Bartow, D.W. Losordo, W. Sherman, V. Driver, E.C. Perin, Safety and efficacy of plasmid DNA expressing two isoforms of hepatocyte growth factor in patients with critical limb ischemia, Gene Ther. 23 (2016) 306–312.

[239] N. Naderi, D. Karponis, A. Mosahebi, A.M. Seifalian, Nanoparticles in wound healing; from hope to promise, from promise to routine, Front. Biosci. (Landmark Ed) 23 (2018) 1038–1059.

[240] L. Sun, L. Xu, H. Chang, F.A. Henry, R.M. Miller, J.M. Harmon, T.B. Nielsen, Transfection with aFGF cDNA improves wound healing, J. Invest. Dermatol. 108 (1997) 313–318.