3 Basic Principles and Current Approach for Soft Tissue Regeneration

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3.1 Introduction

Due to the ongoing shift in the distribution of the world's population towards old age, we recently experience a dramatic increase in comorbidities like diabetes or venous and arterial insufficiency. This results in a raising number of chronic wounds which have become not only an individual medical but also a significant economic burden, consuming 2–4% of health care budgets worldwide [1].

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Wound healing is a complex system depending on the timed coordination of several cell types, intra- and extracellular mechanisms, proteins, and pathways, but also on several external factors like infections or mechanical irritation (Fig. 3.1). Defect or dominance of one factor can cause to a sudden breakdown of localized healing capacity, leading to formation of chronic wounds. A famous example for the fragility of the cellular mechanism for tissue homeostasis and repair is the connection between vitamin C deficiency and scurvy resulting in nonhealing wounds and spontaneous bleeding known since the sixteenth century [2, 3]. Mentioned first in journey books of Christopher Columbus as a result of monotone diet, the pathomechanism remained unclear until the twentieth century. Then it could be demonstrated that vitamin C represents a main cofactor for collagen cross-linking and an important factor to reduce oxidative stress [4]. This example shows how impactful minimal alterations in our metabolism can be for tissue regeneration. Therefore, a complete understanding of all molecular and cellular players involved in wound healing is pivotal for developing treatment strategies and effective drugs.

In this chapter, we summarize the most promising recent advances in wound healing therapeutics with the corresponding challenges and shed light on possible solutions for effective application.

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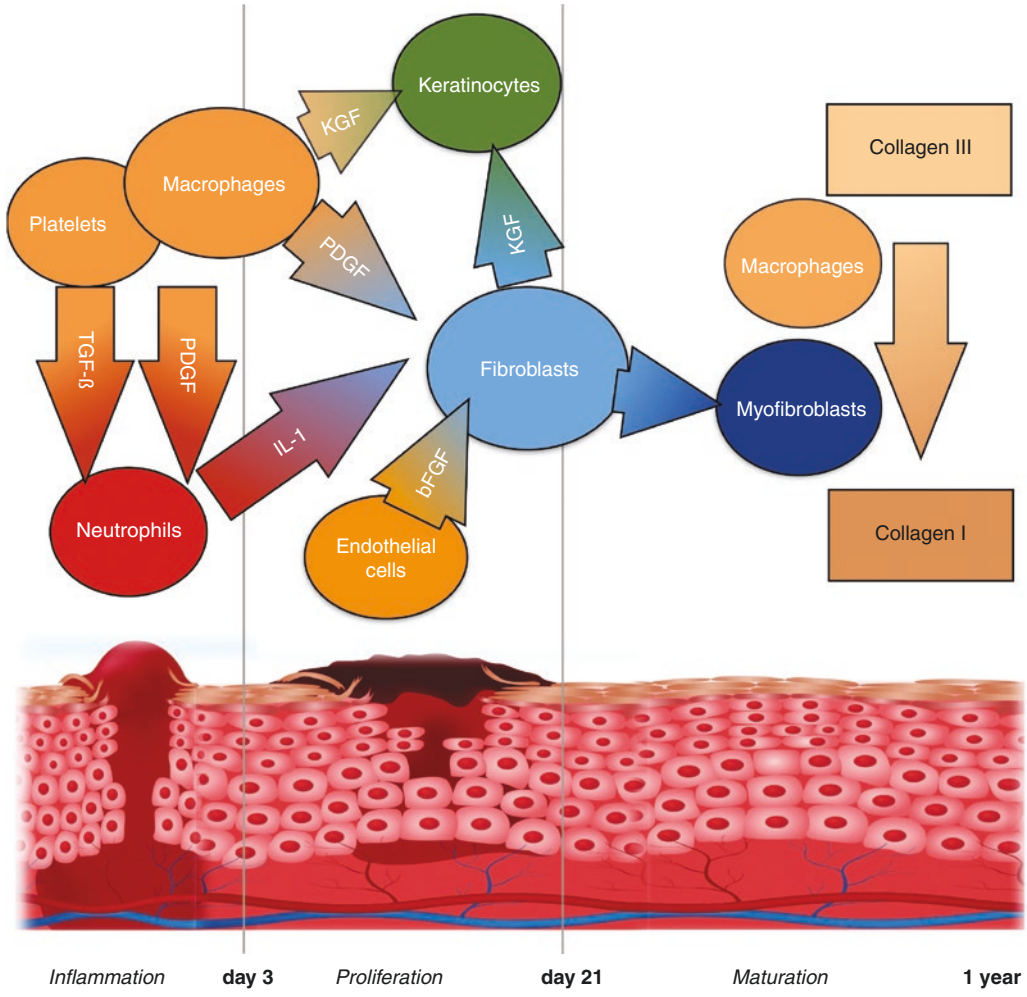

Fig. 3.1 Phases of adult wound healing with main affecting cells and signals. (Left) Coagulation and inflammation—day 0–3. Platelets: formation of platelet plug and secretion of platelet-derived growth factor and transforming growth factor for chemotaxis of neutrophils. Neutrophils: secrete interleukin 1 for the beginning of chemokine-cascade for chemotaxis of inflammatory cells; macrophages: phagocyte bacteria and secrete paracrine factors for keratinocyte-based epithelization and fibroblast activation. (Middle) Proliferation—day 4–21. Beginning of angiogenesis, dependent on endothelial cells: activated by vascular endothelial growth factor. Formation of the extracellular matrix based on fibroblast activation: activated by basic fibroblast growth factor, interleukin 1, and platelet-derived growth factor. Epithelialization: keratinocyte based as a response to keratinocyte growth factor. (Right) Maturation phase—day 21–1 year. Wound contraction: transformation of fibroblasts to myofibroblast. Collagen remodeling: effected by myofibroblasts and macrophages

3.2 Recent Advances in Wound Therapeutics

3.2.1 Growth Factor Therapy

The wound healing promoting effect of growth factors is broadly known. Although they have shown to act beneficial in preclinical studies, large clinical studies supporting this are still missing. A meta-analysis based on the Cochrane database showed a general benefit of growth factor-induced wound healing without any significant general adverse effects [5]. The platelet-derived growth factors (PDGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), fibroblast growth factor

(FGF), and the transforming growth factor-beta (TGF-β) are in current focus of research.

PDGF-BB, administered in hydrogels and available as "Regranex" (Ortho-McNeil, Raritan, NJ), is the only growth factor therapy that is currently approved for treatment of non-healing wounds [6]. Although there has been shown advantage in hypertensive leg ulcers, the application of this gel should be considered only as ultima-ratio treatment due to a higher rate of malignancies in patients treated with PDGF-BB [7].

As another growth factor VEGF was successfully used for accelerated wound closure in diabetic mice. Preclinical study showed less than half of resurfacing time in VEGF-treated group than in a non-treated group [8]. Also non-treated wounds on the contralateral site of animals with VEGF therapy showed an accelerated wound closure time. This leads to the suggestion of an additional systemic effect of local VEGF treatment with the possibility of interacting with tumor growth and of promoting malignant tendencies. There has been only one clinical trial comparing VEGF treatment and placebo showing no significant benefit for VEGF [9].

Several study groups investigated the effect of intralesional EGF injections on wound closure [10–12]. Although first clinical trials have been performed in Cuba in 2006 and have shown accelerated wound healing of high-grade diabetic foot healing and complete wound closure in up to 85% of cases, it is still no treatment option in western countries. A meta-analysis performed by Yang et al. [13] confirmed these first results strengthening the hopes for a new clinical treatment option for diabetic and avascular wounds.

B-FGF is one of the first growth factors that has been investigated. A clinical study by Richard et al. in 1995 involved 17 patients and could find no promoting effect of b-FGF [14]. Up to now the efficacy of b-FGF in wound healing remains unclear. While there exist clinical studies showing a promotion of diabetic wound healing effected by injectable b-FGF, others deny a significant effect of b-FGF releasing sponges while preclinical trials have shown a promising effect [15–17]. Furthermore, not only b-FGF but also acid-FGF has been tested for wound healing and similarly showed inconclusive results [18].

Although the TGF-β family, including TGF-β-1, 2, and 3, has shown to be involved in both promotion of wound healing and scarring, it has not become a possible treatment option in clinical routine yet [19]. The wound healing ability of TGF-β-1 and TGF-β-2 in murine models are well described. Although first clinical phase I and II trials have shown efficacy and safety of a TGF-β-releasing scaffold for treatment of venous ulcers, there exist no supporting phase III study [20].

Despite the fact that growth factor therapy has shown to be effective in preclinical and some clinical trials, only few of these substances hold promise to enter the clinical routine. A therapy containing only one growth factor is most likely not sufficient to efficiently promote wound healing, especially compared to NPWT, which has shown to significantly enhance a plethora of autologous growth factor levels.

3.2.2 Negative-Pressure Wound Therapy

The use of negative-pressure wound therapies (NPWT = vacuum-assisted closure = VAC) provides an effective and elegant way to close wounds, prevent infections, and simultaneously increase local growth factor levels. NPWT has shown benefit on bacterial contamination rate. It also temporarily creates relative hypoxia in the wound region, resulting in significant higher levels of the main growth factors (VEGF, TGF β, and basic FGF), angiopoietin 1 (essential for neoangiogenesis), and bone morphogenetic protein 2 (BMP 2—involved in cartilage and bone metabolism) [21–25]. Several studies suggest that microdeformation of the wound surface leads to accelerated cell migration and matrix production (Fig. 3.2) [23–25]. Interestingly, the temporary hypoxia induces the osteogenetic differentiation of MSCs. Therefore NPWT seems to be the perfect option for treatment of soft tissue defects involving bone defects and infected or potentially infected wounds. Further research has to be carried out to confirm these hypotheses in a clinical setting.

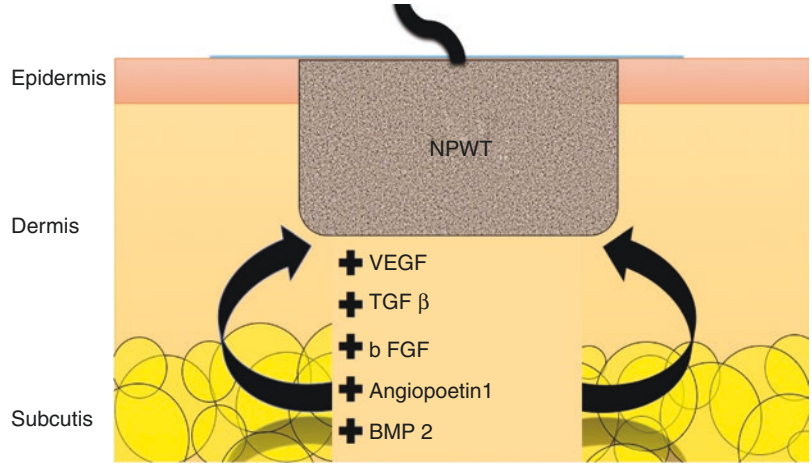

Fig. 3.2 Molecular mechanism of negative-pressure wound therapy

Technical development has led to change in handling of NPWT systems. Dressing changes only three times a week instead of twice daily as recommended in the first trails using NPWT has led to the possibility of a long-term use. By using silver-coated foams, that additionally hinder bacterial growth, NPWT became even more successful [26]. In aggregate, NPWT provides an effective and elegant way to treat difficult wounds by enhancing local growth factor levels and decreasing bacterial contamination of wounds.

3.2.3 Antioxidants and Wnt Modulation

The human skin is constantly exposed to environmental factors such as UV light, radiation, ozone (O_3), or air pollution inducing reactive oxygen species (= ROS). Additionally, cellular metabolism leads to ROS as side products. The causality between free radicals and aging has been described by different groups within the last century [27–31]. By causing accumulation of oxidative toxic products in long-living molecules such as collagen, it leads to peroxidation and dysfunction of these molecules. ROS-induced damaging of the DNA is directly followed by base loss/modification or breakage. ROS can further lead to glycation of proteins followed by degradation. According to this, ROS can significantly inhibit endogenous ability for

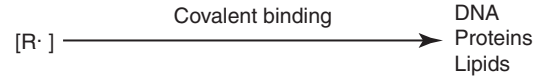

Fig. 3.3 Mechanism of reactive oxygen species (ROS) damage

wound healing by destruction of cells, key proteins, or parts of the ECM (Fig. 3.3).

Antioxidant treatment has been used since several years as a product improving the quality of skin. The most popular and most commonly used antioxidant is a polyphenol, also called aloe vera. As the main component of ointments or gels its therapeutic effect is related to the stimulation of collagen syntheses on the one hand and to anti-oxidative effects on the other hand [32]. But the use of anti-oxidants is not only limited on aesthetic treatment options. Some iron chelators like deferoxamine (= Desferal or more potent, desferriexochelin-772SM (D-Exo)) [33], deferiprone, and deferasirox are already in use in different medical fields [34]. Next to being well known as treatment option for beta-thalassemia [35, 36], anti-oxidant drugs have shown benefits mainly due to their anti-inflammatory potential to increase the retention rate of fat grafts, the survival rate of free flaps, and the healing process of diabetic wounds [37, 38]. This can be explained by the ability of free iron to induce the prolyl-hydroxylation of the hypoxia-inducible factor 1α (HIF-1α), a process leading to inactivation and degradation of HIF-1α [39]. Less free iron results in a higher expression of HIF-1α and thereby

leads to benefits in local neovascularization and tissue regeneration. Harnessing these effects, a transdermal delivery system releasing DFO showed accelerated wound healing in diabetic ulcers. Prophylactic use of this system has a preventive effect on ulcer formation [40].

But not only antioxidative agents, also wnt pathway manipulation is a promising new alley of wound healing research. The wnt pathway represents a sequence of factors that can easily be targeted by nanoparticles. The wnt pathway was found to play an important role in embryonic vertebrae development, in the development of different malignancies, and additionally in tissue repair and scarring [41–44]. Pyrvinium, an antihelminthic drug, inhibits the wnt pathway by promoting the effectivity of the casein kinase 1α (CK1α), leading to accelerated degradation of casein, a factor of wnt pathway [45, 46]. Studies using pyrvinium show a 1.4-fold increase of MSC proliferation by simultaneously inhibiting the osteogenic and chondrogenic differentiation. These changes were initiated by a pyrvinium-releasing sponge. Pyrvinium only affected the proliferation rate of MSCs. Other cell lines like HUVECs have shown no significant changes compared to non-treated cells [47].

Additionally, the wnt pathway has also shown to play a significant role in scar formation. By stimulation of the wnt pathway and the FGF pathway, the regeneration of hair follicles could be induced. Hair follicle secretes bone morphogenetic protein (BMP), which again stimulates myofibroblasts to differentiate into adipocytes. This pathway leads to inhibition of scar formation. Targeting and mimicking these three pathways to either prevent scarring or treat hypertrophic scars or keloids is a future goal of drug development [48–50].

3.2.4 RNA Interference-Based Therapy

Gene expression initially starts in the nucleus with transcription—the production of mRNA (messenger-RNA) followed by an export to the cytoplasm. Translation of mRNA leads to the production of proteins. After this process the mRNA is degraded.

The basic principle of RNA interference (RNAi-based therapy) is based on body's own mechanism for mRNA degradation: By binding to mRNAs, endogenous miRNAs (micro-RNAs) or synthetic siRNAs (small interfering RNAs) lead to mRNA degradation or to formation of double-stranded RNA and as a result to suppression of their translation [51, 52]. Synthetically produced siRNA can be used for knocking down factors which inhibit neo-angiogenesis and inhibit keratinocyte migration followed by re-epithelialization [53–55].

For example, endogenous miRNA-21 is one of the best studied miRNAs. It has been shown to regulate re-epithelialization, cell proliferation, wound contraction, and formation of granulation tissue [54, 56, 57]. RNAi is not limited to wound healing applications, but also offers new possibilities in cancer therapy or treatment of genetic diseases like amyotrophic lateral sclerosis (by targeting and destroying wild-type mRNAs) [58, 59].

Difficulties for the application of this novel therapy include delivery to specific cells and problems with internalization of i-RNA to certain cell types [60, 61]. Additionally, a high degradation rate through RNases leads to a short intracellular half-life. However, further development of this technique may lead to new ways to enhance tissue regeneration and to bring us closer to the holy grail of scarless wound healing [62, 63].

3.2.5 Stem Cell-Based Therapy

Mesenchymal stromal cells (MSCs) can be utilized to treat challenging wounds, such as wounds followed irradiation, ischemic or diabetic wounds. The basic principle is a potential differentiation of stromal cells and a higher local level of growth factors. Although preclinical and clinical studies showed promising results, there still remain several problems. Irregularities are caused by patient's individual factors, such as diabetes or age [64, 65]. These uncertainties still limit the clinical use.

But stem cell-based therapy is not only limited to the regenerative potential of MSCs harvested from bone marrow or adipose tissue. Also peripheral blood cells (PBCs) have shown to secrete a mixture of the pro-angiogenetic factors VEGF and HIF-1, when being temporally conditioned under hypoxic stress [66]. These findings have been used for developing both an implantable and an injectable wound healing system and seem to be a promising approach to accelerate wound healing [67].

3.2.6 Scaffolds and Skin Equivalents

Bioactive dressings are engineered from components that are naturally present in the ECM or composed of polymers to mimic this unique matrix [68]. Biomimetic collagen hydrogels have been shown to accelerate early wound healing by modifying cell recruitment and augmenting granulation tissue formation [69]. Recently biologic matrices have evolved from being simple ECM replacements towards drug and cell delivery vehicles. Novel regenerative matrices are capable of both skin replacement and stimulation of endogenous cells [70]. For example, pullulan (a polysaccharide polymer)-collagen matrices was seeded with mesenchymal stem cells (MSCs) and showed to enhance cell survival [71]. MSCs delivered in such a structured matrix environment have demonstrated enhanced efficacy by increased angiogenic cytokine expression [72]. Therefore, scaffolds can represent an intelligent and efficient drug-delivery vehicle to overcome certain problems of cell-based therapies [73].

In addition to improving wound healing and skin regeneration by increased neovascularization, scaffold-seeded progenitor cells can also enhance tissue repair by inducing a specific immune response. Delivery in the correct niche environment can further enhance the immunemodulatory effects of MSCs and have positive impact on scar formation. ASCs delivered to cutaneous excisional wounds via an ECM patch attenuate wound fibrosis more effectively than ASCs applied without scaffold support [74].

Despite significant scientific advancements and early clinical trials, clinical translation of progenitor cell-seeded biomimetic scaffolds for skin regeneration still remains a challenge. However, innovative therapies based on emerging concepts arising from the intersection of engineering, molecular signaling, and stem cell biology will potentially result in the transformation of fibrotic healing into skin regeneration. Looking ahead, understanding the genetic and epigenetic indicators that might predispose a patient to impaired wound healing or excessive scarring may enhance tissue regenerative approaches further.

3.3 Conclusions

A growing number of patients suffering from chronic wounds have brought soft tissue regeneration into spotlight of current research. The ideal treatment for wound healing is cheap and effective for most wounds. Furthermore, it is essential for upcoming devices to avoid side effect, to provide long-lasting application and should not be impaired by patient-dependent factors such as chronic systemic diseases. Several different approaches have been developed and have shown promising results in preclinical studies. Nevertheless, only NPWT has made the step to clinical routine. Possible reasons for this big gap between basic science and clinical implementation include several uncertain factors such as possible malignancy (growth factor-based therapy), high degradation rate (RNAi, growth factor, and stem cell-based therapy), systemic side effects (pyrvinium), uncertain retention rates, and individual limitations (stem cell-based therapy). Further approached should strive for a holistic attempt to correct the plethora of molecular defects that lead to nonhealing wounds rather than a one-factor replacement therapy.

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