

Growth Factors in Wound Healing – A Review

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The review is an overview of the features of growth factors involved in cellular signaling mechanisms regulating the wound healing process. Understanding the insights of this mechanism is significant for opening therapeutic and research avenues in wound healing. The review highlights the synergistic functioning of most of the growth factors which would enhance the possibility of these factors being the targets for wound care therapy. The significance of the onset and resolution of inflammation in the healing process is better understood clinically and a range of recombinant growth factors to combat this condition have been identified and used to accelerate healing process. The chemotactic and growth regulating factors act as triggers that take the cellular and biochemical components through the inflammation, proliferation, epithelialization, angiogenesis and tissue remodeling phases. Clinical conditions that create alteration in expression of these factors lead to slow and incomplete healing. The review emphasizes on the clinical use of synthetic and recombinant growth factors whose synergistic effects are remarkable. The review covers the specific signaling mechanisms involved in the regulation of these growth factor expressions, specifically the PI3K/AKT, RAS/MAP and JAK pathways; these could be potential targets for future research expansions in this field.

Keywords: Angiogenesis; Cytokines; Epithelialization; Inflammation;
Growth factors; Wound healing.

This review discusses the basics of wound healing mechanisms and the significance of the growth factors in promoting regeneration of the wounded tissue. The synergistic effects of the growth factors in accelerating the healing process is explored. The growth factors take their role right from the inflammatory phase of the mechanism where their presence is both chemotactic for the infiltrating neutrophils and notable in resolution of inflammation, promoting the onset of macrophage increase at the wound site which is thought to be responsible to kick-start inflammation resolution.

Hallmarks on the existence of growth factors in epithelialization and angiogenesis are also discussed. The review's scope extends to the advancements in clinical usage of the external growth factors. The role of the growth factors in cellular signaling mechanisms like the PI3K/AKT pathway, the RAS/MAPK pathway and the JAK/STAT pathways as studies by pre-clinical and clinical findings is explored in the review. The use of synthetic recombinant growth factors to achieve accelerated post-surgical healing and impaired clinical conditions like diabetic foot ulcers, obesity,

malnutrition, medication-induced impairment, oncological treatment-induced impairments, surgical amputations warrants advancements. The content of this review has updations in technical development for basic science researchers and aids clinicians to take-up synthetic growth factor-based therapeutic interventions to fight healing impairment that otherwise affects the patients life quality during and after the hospital stay. The list of triggers include chemotactic factors, inflammatory cytokines and growth promoting factors. These triggers take the cellular and biochemical components through the inflammation, proliferation, epithelialization, angiogenesis and tissue remodeling phases. Among the various triggers guiding the healing process, a number of growth factors function wholly or in synergy to induce the cellular signaling cascade, including Fibroblast Growth Factor, Epidermal Growth Factor, Platelet-derived Growth Factor, Vascular-endothelial Growth Factor, Transforming Growth Factor, Connective Tissue Growth Factor to name a few. These factors are said to make the structural healing more functional. Impaired healing results due to clinical conditions that influence the expression of any or most of these triggers; finally the impaired condition itself becoming a pathologically significant condition. Many synthetically derived and/or recombinant growth factors have experimentally proved effective and have been in clinical practice over years owing to

their potency to overcome impairment and help accelerate healing as the case may need.

Role of growth factors in wound healing

Wound healing is a complex yet organized process of restoration of the functional structures lost during wounding, due to trauma, post-surgical complications, burns or otherwise. It is a natural repertoire of ordered overlapping series of cellular and vascular events, inflammation, proliferation, epithelialization, granulation tissue formation, maturation, matrix and tissue remodeling, which are triggered by a wide range of mediators from histamine to growth factors like TGF- β and vascular factors like VEGF⁴ from the wound microenvironment. On occurrence of a wound, a complex interplay of cells and proteins take place, the blood platelets aggregate and bind with collagen to prevent blood loss by forming a clot⁵. The incidence of the wound creates a hypoxic environment that promotes inflammation by releasing proinflammatory mediators, the molecular signals that manifold the inflammatory activities, example, by recruiting leukocytes and monocytes which differentiate into tissue macrophages⁶. Neutrophils are the predominant and prime inflammatory cells. Once activated they release enzymes like elastase, protease and collagenase which degrade and remove damaged tissues at the wound site.¹⁰ Platelets also initiate inflammation by producing EGF, PDGF, TGF β 1 and IL-1. These attract neutrophils to the wound

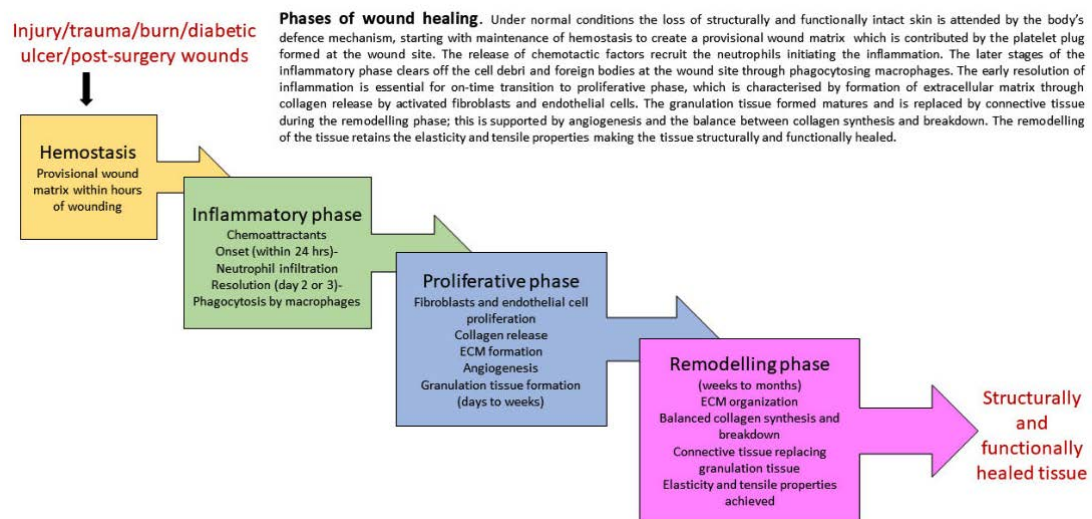


Fig. 1. Phases of wound healing

site; TGFβ induces selective differentiation of monocytes to macrophages which also contribute to the inflammation. Also the monocyte chemotactic protein, TGF-β1 recruits mast cells to the wound site which releases histamines, proteoglycans, proteases and platelet activating factor. Unresolved or prolonged inflammation leads to chronic non healing wounds. Once active the inflammatory cells synthesize TGFβ1 & IL-4 which in turn suppress them leading to reversal of inflammation (Fig. 1).

TNF-α and IL-1 produced by macrophages initiate and regulate proliferation of fibroblasts and endothelial cells. Upon activation the fibroblasts release collagen and other glycosaminoglycans which deposit with fibronectin and forms the ECM, the major component of the granulation tissue. The further growth of the granulation tissue requires angiogenesis, which is initiated by the bFGF and VEGF produced by the endothelial cell, keratinocytes and macrophages. PDGF released by the degranulating platelets holds the important role of increasing the structural integrity of blood vessels during angiogenesis. The balance between the collagen synthesis and breakdown forms an essential part of the remodeling of the collagen fibres. During remodeling the granulation tissue is slowly replaced by the connective tissue which contributes to the elasticity and tensile properties

of the otherwise intact skin, the major contribution being from the connective tissue growth factors (Fig. 2).

Epidermal growth factor (EGF)

Epidermal Growth Factor (EGF) is a growth factor that stimulates cell growth, proliferation and differentiation by binding to the Epidermal Growth Factor Receptor (EGFR). The protein was initially found among nerve growth factors extracted from mouse submandibular gland²⁶. The family consists of other members including Transforming growth factor-α, heparin binding EGF (HB-EGF), amphiregulin, epiregulin, neuregulin and beta-cellulin⁷. EGF is a key regulator of epithelial cell motility thereby influencing the rate of re-epithelialization. It aids wound contraction by stimulating fibroblasts proliferation and migration and induces dermal maturation by binding with the EGFR in the cells at the wound site¹⁴. Topical application of EGF is considered to be a useful therapy for cutaneous wounds. Analysis of the wound fluid gave the first ever evidence of EGF at the wound site. It was studied that EGF exhibited a synergistic role with Insulin-like growth factor-1 (IGF-1) in stimulating in-vitro proliferation of keratinocytes⁷ (Fig. 3).

Reports on therapeutic usage of the protein highlights the drugs containing the

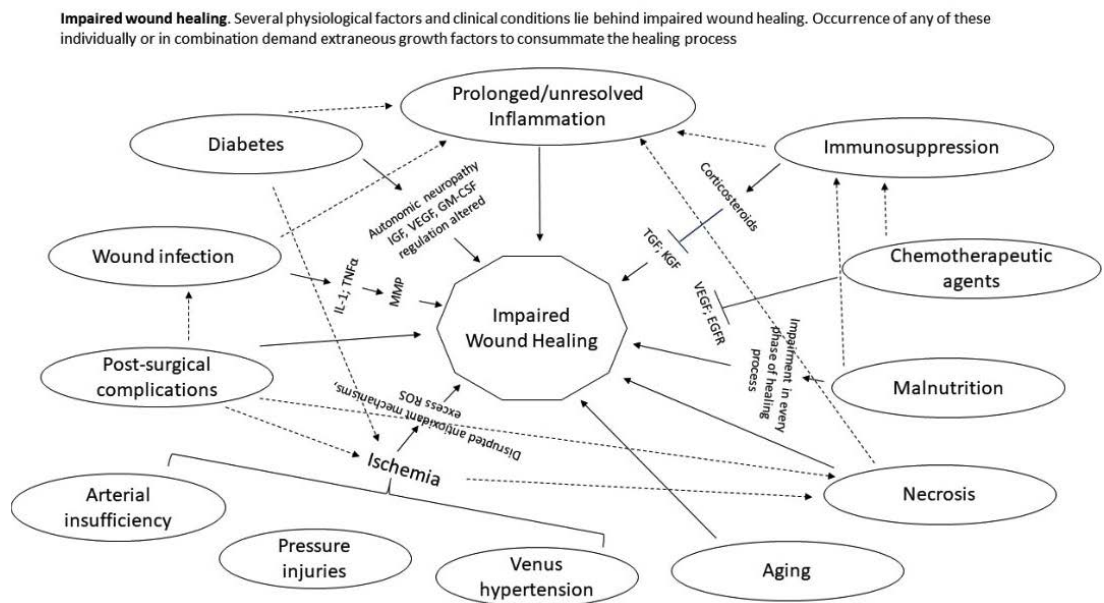


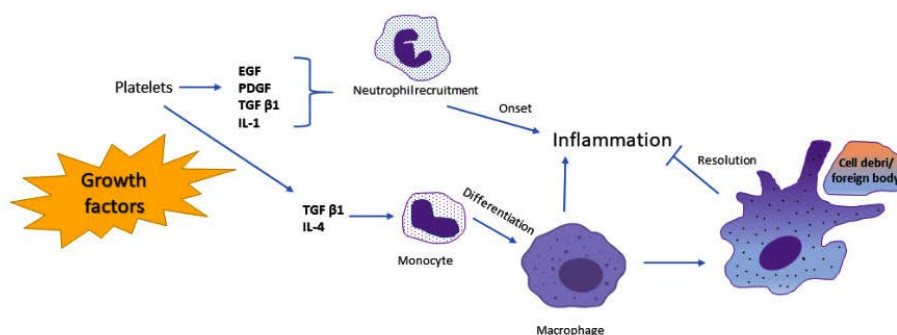
Fig. 2. Impaired wound healing

protein being used for treating diabetic and corneal ulcers in regions of South Korea and Belgium respectively. In early 1990s Okumura et al. demonstrated the use of EGF in combination with nafamostat, a protease inhibitor to treat open wounds in rat models. The treated models showed significant increase in the wound granulation tissue dry weight, hydroxyproline and uronic acid contents, suggesting the need for a protease inhibitor in place to stabilize the presence of EGF thereby establish its healing role¹⁵. A topical treatment of recombinant human epidermal growth factor (rhEGF) ointment was shown to be effective in promoting wound healing by increasing the rate of epidermal proliferation and wound contraction related to myofibroblast proliferation and collagen synthesis¹⁶. To face the challenge during transdermal delivery of EGF Kim *et al.* (2016) succeeded in using Hyaluronate (HA)-EGF conjugate in a patch type HA film. The conjugate allowed an extended residence time of EGF at the wound site and enhanced the regeneration of skin tissues¹⁷. Recombinant human EGF available commercially under the brand name Heberprot-P is used to treat diabetic foot ulcers (DFU)¹³. Recombinant EGFR also rescues the keratinocyte migration suppressed naturally by the shedding of the EGFR in the keratinocyte membranes on wounding³⁵. Recombinant probiotic *Escherichia coli* Nissle 1917 expressing human EGF enhanced human enterocyte migration upon wounding in a murine monolayer model. The EGF secretion by

the probiotic strain was ABC transporter mediated and the EGF secretion was studied to potentially activate, by phosphorylation, the ERK1/2 and AKT signals finally resulting in the enhancement of the healing process by multifaceted repertoire of cellular events like the proliferation, migration and reepithelialization³⁶. High blood glucose upsets the EGFR/PI3K/AKT signaling pathway in a ROS-sensitive manner and interrupts epithelial wound healing in the cultured porcine cornea. The study by Xu et al. (2009) conclude that the therapeutic combination of EGFR ligands and antioxidants would be promising for diabetic keratopathy³⁷.

Fibroblast growth factor (FGF)

An earlier study demonstrated growth stimulatory activity of a substance in fibroblasts which was later named as “fibroblast growth factor”²⁶. Basic fibroblast growth factor (bFGF) is reported to significantly upregulate the expression of epidermal stem cell markers and Notch1/Jagged1 signaling and down regulate the expression of myofibroblasts markers¹⁹. Delay in re-epithelialization of the full-thickness excision wounds in the absence of the factor breaks open the significance of the cellular mediator. The knockout of the FGF2 gene Sakamoto et al. (2017) have shown that the human cultured epidermis in combination with meshed skin grafts significantly reduced wound area by promoting granulation tissue formation and angiogenesis in a rat model by producing various growth factors like bFGF, IL-1 α , PDGF, TGF and VEGF²⁰. Among the



Role of growth factors in wound inflammation Immediately after wounding platelets aggregate at the wound site, initially to create a fibrin clot (hemostasis) to stop the blood flow from the damaged tissue. The platelets apart from forming the blood clot, also secrete the chemotactic growth factors like epidermal growth factor (EGF), platelet-derived growth factor (PDGF), transforming growth factor β 1 (TGF β 1), Interleukin 1 (IL-1) which induce neutrophil recruitment to the wound microenvironment, resulting in onset of inflammation which prolongs for 1-2 days. The neutrophils function by clearing the wound infection; the chemotactic factors like TGF β 1 and IL-4 mediate differentiation of monocytes to macrophages which contribute to inflammation during the early stages and on later stages, phagocytose cell debris and other foreign bodies thus bringing about resolution of inflammation. The on-time start and early resolution of inflammation is essential for sequential phases of healing to take over.

Fig. 3. Role of growth factors in wound inflammation

various signaling pathways, viz., RAS/MAPK pathway, PI3 kinase/AKT pathway and PLC α pathway, RAS/MAPK signaling pathway is found to predominate²². Farahpour et al. (2017) reported that the accelerative impact of wound healing agents were also due to modulating expression of fibroblast growth factor in BALB/c mice excision wound model²³. The mitogenic and angiogenesis properties of the bFGF are said to induce tissue remodeling, wound healing and neovascularization as tested in animal models²⁴. The rate of wound contraction in diabetic mice wound model was significantly reduced in the absence of FGF-7. The study suggested that the epithelial-mesenchymal interaction which governs the rate of wound closure is directly dependent on the expression levels of fibroblast growth factor²⁵. Ortega et al. studied the ability of bFGF knockout mice to heal full-thickness excision skin wounds. The knockout mice delayed wound contraction and showed reduction in collagen deposition. bFGF promoted fibroblast migration in a dose-dependent manner. Therapeutically, the bFGF drugs had a contraindication in malignant tumor conditions owing to its cell proliferation potency²⁶. mRNA expression studies in mice reveal the relationship between the decline in the FGF with age and impairment in cellular proliferation including keratinocytes, fibroblasts, preadipocytes etc. This decline is understood to be gradual starting from middle age proceeding at higher rates in the

older mice²⁸. Inhibition of the basic FGF receptor tyrosine kinase in rats resulted in dose-dependent delay in healing of experimentally induced tympanic membrane perforation³⁴.

Transforming growth factor (TGF)

Human TGF- β superfamily consists of about 33 different proteins that play significant roles in cellular signaling mediated regulation of tissue homeostasis in multicellular organisms²⁹. Platelets, macrophages, neutrophils, fibroblasts and few other cell types produce TGF- β in abundance. This is the key cytokine that initiates intracellular signaling pathways by binding with type II serine threonine kinase receptor influencing transcription of genes whose product affect all the phases of wound healing²¹. TGF- β signals through the transmembrane serine/threonine kinase receptors. Signal to the receptor induces cross phosphorylation among the two types of receptors which facilitates binding of the intracellular receptor-regulated Smad proteins. Smad knockout mice models lacked in TGF- β 1 signaling which in turn resulted in a series of cellular events like increase in inflammation, increase in peripheral lymphocytes and immature neutrophils and insufficient keratinocyte migration and irregular contraction of fibroblasts and hence improper wound closure (Fig. 4).

TGF- β 1 enhances angiogenesis by promoting the endothelial progenitor cells to facilitate blood supply to the wound site³⁰. TGF- β 1

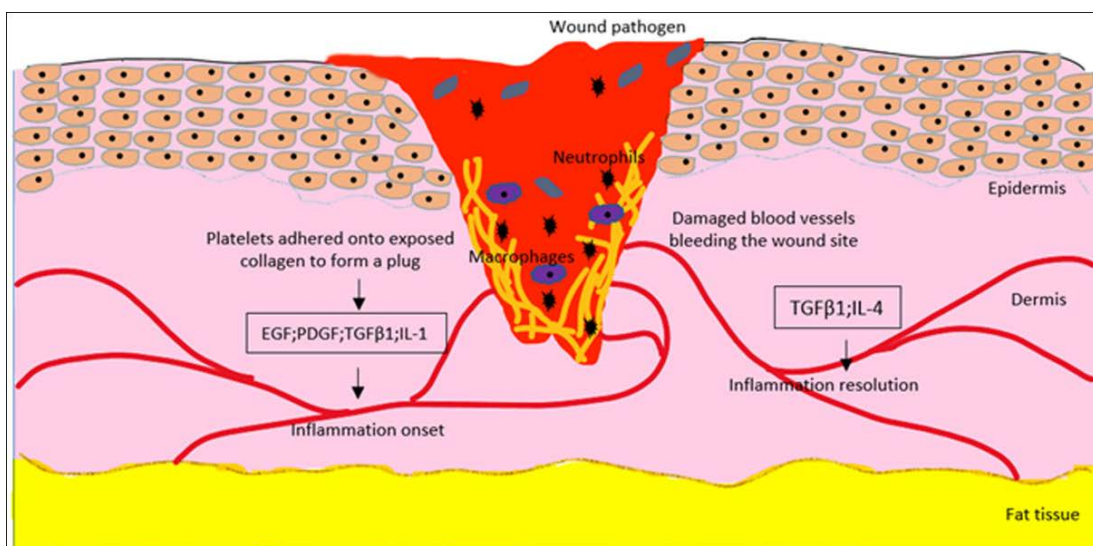


Fig. 4. Hemostasis and Inflammatory Phase

plays a major role in myofibroblast differentiation which is a potent target for treating hypertrophic scars and keloids. Binding of these and other isoforms to TGF- β receptors is found to induce the overproduction of collagen I, collagen III, fibronectin and other cytokines¹⁸. Hayashi et al. (1989) investigated the effect of the peptide in corneal healing of a Vitamin A deficient rat model and observed the infiltrated acute inflammatory cells in the peripheral stroma which gradually spread to the central cornea indicating the role in reepithelialisation, collagen remodeling and neovascularization. The significance of TGF- β expression in cellular growth differentiation and function was understood when Clark and Coker (1998) studied the TGF- β 1 knock out mice. 50% of these mice died in utero and the rest suffer from uncontrolled inflammation after birth²⁸. Exogenous TGF- β 1 implantation in male albino rabbits with a standard surgical incision in the diestema region regulated the oral mucosal wound healing process better than the controls through alteration in the production levels of Nitric Oxide (NO).

Platelet derived growth factor (PDGF)

Platelet derived growth factor (PDGF) is a helping hand in regulating a lot of cellular activities related to wound healing process including mitogenesis of fibroblasts and many cells, angiogenesis and chemotaxis. The protein is made of A and B polypeptide chains, combining to generate three isoforms, AA, AB and BB. Located

on the chromosomes 7 and 22, the genes for A and B chains express to generate proteins of varying degrees of affinities to cell surface receptors belonging to the tyrosine kinase family³⁸. The expression is stimulated by conditions like low oxygen tension, thrombin levels and presence of other growth factors or cytokines. PDGF binds to the specific receptors and activates them through ligand-induced dimerization of α and β receptors, forming homo- and heterodimers of different signaling strengths. Receptor dimerization induces autophosphorylation of intracellular receptor components, change in conformation in-turn and activation³⁹. Activation of the PDGF receptors tends to induce chemotaxis in certain cell types, however PDGF α receptors are studied to inhibit chemotaxis of a few selected cell types like the fibroblasts and the smooth muscle cells. Both the receptor types induce increase in intracellular Ca^{2+} concentrations. In connection with this the receptors are understood to inhibit communication between cells through gap junctions and present an anti-apoptotic effect. A group of intracellular adaptor molecules involve in interacting with the receptor molecule including PI3-K, Src, Stat5, Grb2, Grb7, Nck, Crk etc. These molecules contain the conserved SH2 domain which binds with a phosphorylated tyrosine, which is achieved in this case by ligand dimerization⁴⁰.

PDGF treated incisional and excisional wounds at 20-200 picomoles concentration by accelerating the infiltration of inflammatory cells

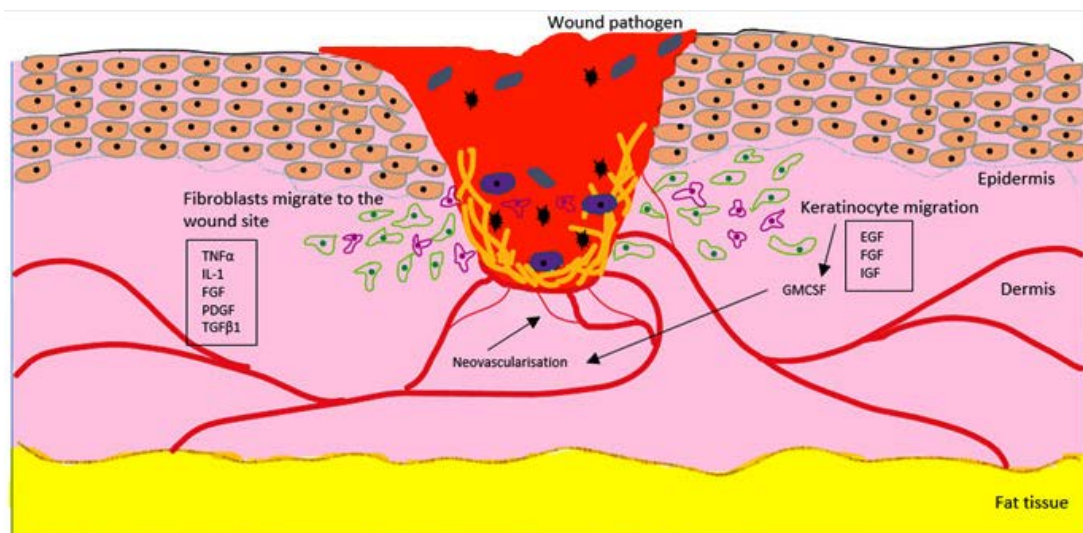


Fig. 5. Proliferative Phase

and fibroblasts, extracellular matrix deposition and collagen formation; the effect was reported to be an exaggerated one resulting in accelerated healing process as compared to the untreated models⁴¹. Earlier Lynch et al. (1987) reported that the platelet-derived growth factor worked better as a partially purified fraction when compared with a pure fraction in terms of inducing fibroblasts migration, collagen and glycosaminoglycan production and DNA synthesis. Purified PDGF in combination with equal volumes of epidermal growth factor (EGF) showed 44% increase in width of the epidermal layer with a thicker keratinocyte layer. The growth factor in combination with insulin-like growth factor (IGF-I) resulted in 2.5 fold wider connective tissue layer. These effects were not seen when either of the synergistic growth factors were added alone to the surgical wound created in Yorkshire pigs⁴². Similar synergistic role of PDGF was described by Giselle Hosgood (1993) when exogenously administered in combination with transforming growth factor (TGF- β). Though both factors work with a different model of action, synergistically they induce chemotaxis of the inflammatory cells involved in the healing process. In 2008, the US Food and Drug Administration approved indications for use of Regranex Gel, a human recombinant platelet-derived growth factor for topical application for treating diabetic foot ulcers; however serious mortality rate was recorded

and warning information were disseminated among the users and medical professionals (Fig. 5, 6).

Vascular endothelial growth factor (VEGF)

Angiogenesis, a key event in various natural cellular processes including wound healing is induced by an interesting proangiogenic factor called vascular endothelial growth factor (VEGF). All the seven members of the VEGF family share a homology domain and are thought to transduce signals by binding to specific vascular endothelial growth factor receptors. Activated platelets at the wound site release VEGF-A which through binding with VEGFR-1, on the inflammatory cells, attracts circulating neutrophils and monocytes. The recruited neutrophils further bring into the loop various proinflammatory cytokines like IL-1 α and TNF- α . In addition the VEGF-A is studied to have a hand in regulating the plasminogen activator production during the re-epithelialization phase⁴⁴. VEGF induces local vascular regeneration in radius fracture model of rabbits. It is studied to be a very important factor to promote vascularity and closure in hypoxic chronic wounds. The impaired healing of diabetic wounds is studied to be due to diminished production of VEGF and thus decreased angiogenesis. In addition to increasing and maintaining vasculature in the experimental full-thickness mice wound models, the vascular permeability factor, VEGF, also accelerates wound healing by attracting the inflammatory

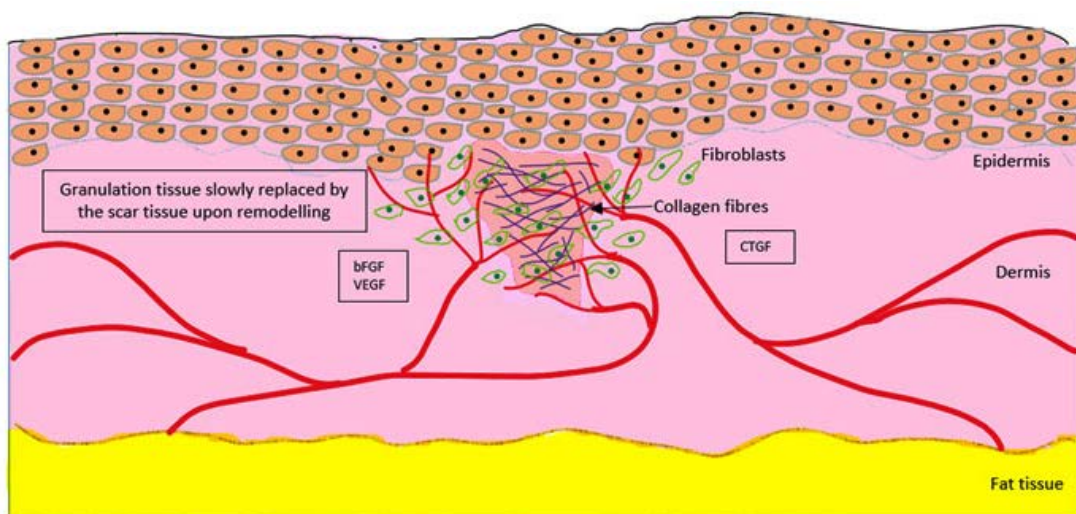


Fig. 6. Remodelling Phase

cells at the site of injury and inducing migration and proliferation of the endothelial cells⁴⁵. The expression of VEGF and their receptors is thought to be regulated by hypoxia, a condition created during traumatic wound or ischemic injury. Developmental studies in mouse embryos have revealed that loss of at least a single allele of VEGF gene results in abnormal blood vessel formation and embryonal death. The absence of VEGF receptors result in failed differentiation of haemangioblast precursor cells to endothelial cells, a crucial cellular developmental process⁴⁶. The importance of VEGF in wound healing was understood from the Cheng et al's. (2016) effort to review the use of anti-VEGF agents to control wound healing process in patients who have undergone glaucoma filtration surgery, done to manage intraocular pressure, as scarring during regular wound healing process in these patients would lead to failure in the surgery⁴⁷.

Granulocyte macrophage colony stimulating factor (GM-CSF)

The keratinocytes, upon injury, secrete the granulocyte macrophage colony stimulating factor (GM-CSF) which acts in an autocrine manner to enhance epidermal proliferation. The significance of GM-CSF in normal wound healing was understood when GM-CSF antagonists over-expressing mice were experimentally observed; the mice showed delayed epithelialization and neovascularization⁴⁸. GM-CSF enhances keratinocyte's migratory capabilities thereby supporting the influx of these cells at the wound site, a remarkable event that ends up in epithelialization and granulation tissue formation⁴⁹. GM-CSF deficiency resulted in defective vascular collagenous matrix production suggesting the significance in maintaining vascular integrity. In diabetic mice exogenous GM-CSF enhanced wound healing by increasing production of IL-6 and monocyte chemoattractant protein-1 production. Topical application of GM-CSF was found to treat decubital ulcers in cancer patients at a concentration of 200µg/mL⁵⁰. At a lesser concentration, Gulcelike et al. (2006) proved a local injection of GM-CSF helped enhance healing of an incisional wound in the Adriamycin-treated rats⁵¹. In addition GM-CSF happens to be chemotactic for a wide range of inflammatory cells and mediators, indirectly contributing to the wound inflammation and repair. GM-CSF thus

meeting all the requirements to be an active agent accelerating wound healing mechanisms, achieved interests among scientists to be synthesized in lab by recombinant technology. rHuGM-CSF has been through clinical trial cases, intradermal infiltration of the same resulted in complete healing of the chronic plebostatic ulcerative lesion in about eight weeks of treatment. Experimental studies have also recorded GM-CSF has a stimulatory effect on the phagocytotic and bactericidal properties of macrophages⁵². Local injections of GM-CSF healed sacral pressure ulcer in a patient with bilateral hemiplegia in an observational case study. A firm granulation tissue formed within few days; biopsy of the granulation tissue showed inflammatory cells and fibroblasts, the site of injections showing higher infiltration. Randomised control trials in patients with diabetic foot ulcers evidenced the role of GM-CSF in increasing the release of neutrophil endothelial progenitor cells from bone marrow thus being significant in infiltrating the inflammatory cells. Systemic administration of GM-CSF has proved to be effective even in healing dystrophic epidermolysis bullosa wounds in a pilot trial study. At molecular level, binding of recombinant GM-CSF to its specific receptor creates a signaling complex that activates Janus Kinase (JAK) and signal transducer and activator of transcription (STAT) proteins; through a stream of signaling molecules like MAPK and c-fos and c-jun genes the factor influences regulation of hematopoietic differentiation⁵³⁻⁵⁶.

Connective tissue growth factor (CTGF)

Connective tissue growth factor is a protein regulating a number of biological processes like cell proliferation and differentiation, adhesion and angiogenesis. Exogenous CTGF has been extensively researched in animal and human trials for healing potency. Streptozotocin-induced diabetic Sprague-Dawley rats were used as diabetic wound models with experimentally created full-thickness excision wounds. Topical administration of recombinant CTGF for a period of seven days showed a difference in wound closure in the diabetic group though not statistically significant^{56,57}. Another experimental excision wound models were observed after long-term administration of recombinant CTGF which revealed enhanced fibroblasts proliferation and collagen deposition at the granulation tissue. The vascularity was better

in the treated model compared to the controls. The wound cellular microenvironment was influenced by the CTGF treatment, especially in enhancing the macrophage counts. The macrophage population is inevitable for onset and resolution of wound inflammation. In addition CTGF expression was observed in the early stages of acute burn injury; however experimental evidences for its role need to be deduced. Collagen biosynthesis and deposition determines the extent of healing and thus the quality of healing⁵⁸. CTGF treated diabetic wounds markedly showed an increase in collagen IV, which is significant to take the healing forward to a remodeling phase. Since biosynthesis of collagen involves formation of hydroxyproline, the measure of the later serves as an indication of tissue collagen. Radio-active proline injected into the wound site traces the net rate of collagen synthesis and deposition in experimental rat models. Reepithelialisation though occurred in the CTGF knockout mice was found to be impaired; CTGF inhibition delayed wound closure and stromal scarring. CTGF apart from being chemotactic for fibroblasts, also regulates scarring. CTGF is understood to be a part of the positive feedback loop, inducing thrombospondin to convert the latent TGF- β precursor to active form^{59,60}.

CONCLUSION

Managing impairment in regular healing mechanism and improving life quality, in patients with specific surgical or clinical conditions, is a major challenge for clinicians. The therapeutic use of synthetic extraneous growth factors like EGF, FGF, VEGF, GM-CSF etc have been increasing since the past few years. Recombinant proteins replaced the synthetic preparations to overcome the drawbacks associated. Future research and pre-clinical testing can target molecular interactions between intracellular proteins triggered by these signal; mechanisms like PI3K/AKT pathways and JAK/STAT pathway could be considered to customize these growth factors on a case to case basis.

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Conflict of interest

No competing interests exist.

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