Review Article

Role of growth factors in wound healing

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ABSTRACT

The wound healing process involves a series of cellular and biochemical events that ultimately lead to tissue repair and regeneration. These events include hemostasis, inflammation, proliferation, epithelialization, maturation, and remodeling of the scar tissue. Wound healing is an evolutionarily conserved, complex, multicellular process that, in skin, aims at barrier restoration. This process involves the coordinated efforts of several cell types including keratinocytes, fibroblasts, myofibroblasts, smooth muscle cells, endothelial cells, immune cells, macrophages, and platelets. The migration, infiltration, proliferation, and differentiation of these cells will culminate in an inflammatory response, the formation of new tissue and ultimately wound closure. This complex process is executed and regulated by an equally complex signaling network involving numerous growth factors, cytokines, and chemokines. These include epidermal growth factor, fibroblast growth factor (FGF), insulin-like growth factor, keratinocyte growth factor, platelet-derived growth factor, transforming growth factor (TGF), and vascular endothelial growth factor. TGF-β, IL-1, and TGF-α. This review will focus on the specific roles of these growth factors and cytokines during the wound healing process.

KEY WORDS: Growth factors, Types, Phases, Pharmaceutical consideration, Wound healing

INTRODUCTION

Injury to the skin initiates a cascade of events including inflammation, new tissue formation, and tissue remodeling, which finally lead to at least partial reconstruction of the wounded area.[1,2] The repair process is initiated immediately after injury by the release of various growth factors, cytokines, and low-molecular-weight compounds from the serum of injured blood vessels and degranulating platelets. In addition to the importance of cell-cell and cell-matrix interactions, all stages of the repair process are controlled by a wide variety of different growth factors and cytokines. Multiple studies have demonstrated a beneficial effect of many of these growth factors, for example, platelet-derived growth factors (PDGFs), fibroblast growth factors (FGFs), and granulocyte-macrophage colony-stimulating factor (GM-CSF) on the healing process, both in animal models and also in patients suffering from different types of wound healing disorders.[3-7]

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Types of Wound Healing

The three categories of wound closure are primary, secondary, and tertiary

- Primary healing - describes a wound closed by approximation of wound margins. It involves the closure of a wound within hours of its creation.
- Secondary healing - describes a wound left open and allowed to close by epithelialization and contraction. It involves no formal wound closure; the wound closes spontaneously by contraction and re-epithelialization.
- Tertiary wound closure - also known as delayed primary closure, involves initial debridement of the wound for an extended period and then formal closure with suturing or by another mechanism.
- Partial thickness wound - the wound is superficial, not penetrating the entire dermis.

Phases of Wound Healing

Three phases of wound healing are:

- Inflammatory phase.
- Proliferation phase.
- Maturation phase.

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Inflammatory phase

The initial first step starts with the formation of clot. When tissues are injured, circulating blood comes into contact with collagen and other tissue factors that initiate the hemostatic response. Initially, there is vasoconstriction to control hemorrhage within the wound space. Platelets are exposed to collagen within the blood vessel wall beneath the endothelium. Thromboxane and serotonin are released to promote vasoconstriction, keeping locally acting factors within the wound.

As activated platelets aggregate, they initiate the clotting cascade, including both the intrinsic and extrinsic systems. The intrinsic system is activated by factor XII, when it comes into contact with collagen. In the presence of high-molecular-weight kininogen, a precursor of bradykinin and prekallikrein, factor XII activates factor XI, followed by factor IX and factor VIII. The extrinsic system of the coagulation cascade is initiated by thromboplastin, which is formed from phospholipids and glycoproteins released when circulating blood comes into contact with injured tissues. Factor VII is activated in the presence of calcium. The intrinsic and extrinsic systems come to a final common pathway leading to the formation of fibrin and fibrin polymerization.[8,9]

The inflammatory phase begins at the time of injury and lasts 2–4 days. The phase begins with hemostasis and formation of the platelet plug. Platelets release PDGF and transforming growth factor-beta (TGF-β) from their alpha granules to attract neutrophils and macrophages. Neutrophils scavenge for bacteria and foreign debris. Macrophages are the most important mediators of wound healing. Macrophages continue to emit growth factors to attract fibroblasts and usher in the next phase of wound healing.

Proliferative phase

The proliferative phase begins on approximately day 3; the process of fibroplasia begins as the number of macrophages and fibroblasts increases in the wound, while the number of neutrophils decreases. This results in the process of matrix formation, including collagen synthesis. At the same time, the process of angiogenesis begins. At this point, the inflammatory response ends, as inflammatory mediators are no longer produced and mediators already present are inactivated. Inflammatory mediators are also removed from the wound by diffusion or by wound macrophages. Fibroplasia begins about 5 days after injury and may continue for as long as 2 weeks. Fibroblasts migrate into the wound and replicate in response to mediators released during inflammation. These mediators include C5a, fibronectin, PDGF, FGF, and TGF.

Angiogenesis begins as endothelial cells migrate and proliferate through a healing wound. Capillaries form through budding from existing capillary networks, stimulated by angiogenesis factors such as FGF. TGF-α also can stimulate endothelial cell growth. The endothelial cells then enable connections between capillaries, forming a capillary network in the healing wound. Angiogenesis is critical in wound healing in that it provides a mechanism for new healing factors to be brought into the wound. The process of angiogenesis stops as the wound receives an adequate blood supply and may be regulated by oxygen tension. It appears that hypoxia stimulates angiogenesis, whereas normal oxygen tensions in the wound may stop it. Granulation tissue, formed in this phase, is particularly important in wounds healing by secondary intention. When collagen synthesis and breakdown become equal, the next phase of wound healing has begun.[8,9]

Remodeling phase

Increased collagen production and breakdown continue for 6 months to 1 year after injury. The initial type III collagen is replaced by type I collagen until a type I: type II ratio of 4:1 is reached, which is equal to normal skin. Furthermore, fibroblasts differentiate into myofibroblasts, causing tissue contraction during this phase of wound healing.

The scar becomes less hyperemic as the vascularity is reduced. There is progressive organization and maturation of the tissues, leading to an increase in wound strength for up to 2 years. Despite the increase in wound strength, the collagen content of the wound does not increase.

Growth factors are synthesized and released by many cell types. Once a growth factor is released, it may:

- Act on the same cell that produced it (autocrine stimulation).
- Act on cells that are adjacent to the producer cell (paracrine stimulation).
- Enter the circulation to be transported to cells that are distant from the producer cell (endocrine stimulation).

GROWTH FACTORS WHICH TAKES PLACE IN DIFFERENT PHASE OF WOUND HEALING ARE [TABLES 1 AND 2]

PDGF family

PDGF is the major human serum polypeptide growth factor and is a potent mitogen for cells of mesenchymal origin, such as fibroblast and arterial-smooth muscle cells. PDGF stimulates many metabolic processes, including general protein and collagen synthesis, collagenase activity, and chemotaxis of fibroblast, and smooth muscle cells.

PDGF, delivered by platelets to the site of injury, may play an important role in the initiation of the

Table 1: Growth factors and its functions in wound healing

Growth factors	Source	Wound healing related functions
PDGF	Platelets, macrophages, endothelial cells, injured cells, smooth muscle cells	Chemotaxis, fibroblast proliferation, collagenase production, endothelial cell proliferation, smooth muscle proliferation
VEGF	Endothelial cells	Endothelial cell proliferation, angiogenesis
TGF-β	Macrophages, platelets, neutrophils, lymphocytes, fibroblasts, epithelial and endothelial cells, injured cells.	Fibroblast proliferation, chemotaxis, collagen metabolism
EGF	Plasma, platelets, macrophages, epithelial cells	Epithelial cell proliferation, granulation tissue formation
TGF-α	Activated macrophages, epithelial cells, injured cells	Epithelial cell proliferation, granulation tissue formation
HGF	Mesenchymal cells	Proliferation of epithelial cells
HB-EGF	Macrophages, mesenchymal cells	keratinocytes replication
KGF	Fibroblast	Keratinocyte migration, differentiation, and proliferation
FGF	Pituitary, macrophages, fibroblast, endothelial cells	Fibroblast proliferation, matrix deposition, wound contraction, angiogenesis

KGF: Keratinocyte growth factor, FGF: Fibroblast growth factor, EGF: Epidermal growth factor, HB-EGF: Heparin-binding Epidermal growth factor, HGF: Hepatocyte growth factor, TGF-alpha: Transforming growth factor-α, TGF-β: Transforming growth factor-β, VEGF: Vascular endothelial growth factor, PDGF: platelet-derived growth factors

Table 2: Growth factors and cytokines affecting various steps in wound healing

Monocyte chemotaxis	Chemokines, TNF, PDGF, FGF, TGF-β
Angiogenesis	VEGF, angiopoietins, FGF
Fibroblast migration/ replication	PDGF, EGF, FGF, TGF-β, TNF, IL-1
Keratinocytes replication	HB-EGF, FGF-7
Collagen synthesis	TGF- β, PDGF

KGF: Keratinocyte growth factor, FGF: Fibroblast growth factor, EGF: Epidermal growth factor, HB-EGF: Heparin-binding epidermal growth factor, HGF: Hepatocyte growth factor, TGF-alpha: Transforming growth factor-α, TGF-β: Transforming growth factor-β, VEGF: Vascular endothelial growth factor, PDGF: Platelet-derived growth factors

wound repair process. PDGF comprises a family of homo or heterodimeric growth factors including PDGF-AA, PDGF-AB, PDGF- BB, PDGF-CC, and PDGF-DD. PDGFs are produced by platelets, macrophages, vascular endothelium, fibroblasts, and keratinocytes.[8-10] They exert their functions by binding to three different transmembrane tyrosine kinase receptors, which are homo- or heterodimers of an α- and a β-chain.[11,12,91,93]

PDGF was the first growth factor shown to be chemotactic for cells migrating into the healing skin wound, such as neutrophils, monocytes, and fibroblasts. In addition, it enhances proliferation of fibroblasts and production of extracellular matrix (ECM) by these cells. Finally, it stimulates fibroblasts to contract collagen matrices and induces the myofibroblast phenotype in these cells.[12,13] Thus, it has long been suggested to be a major player in wound healing.

Vascular endothelial growth factor (VEGF) family

The members of the VEGF family include: VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placenta growth factor. VEGF-A is produced by endothelial cells, keratinocytes, fibroblast smooth muscle cells, platelets, neutrophils, and macrophages. They exert their biological functions by binding to three different transmembrane tyrosine kinase receptors, designated VEGFR-1, VEGFR-2, and VEGFR-3.[14] VEGF-A is important in wound healing because it promotes the early events in angiogenesis, particularly endothelial cell migration [15-17] and proliferation.[18-22] VEGF-A transcription and secretion along with the VEGFR are elevated in the acute wound.[23-25] On injury activated platelets release VEGF-A.[26,27] In addition, macrophages release VEGF-A during wound healing[24] as well as releasing TNF-α. A major stimulus for the release of VEGF-A in the acute wound setting is hypoxia due to metabolic derangements in the wound environment. The resulting angiogenesis restores tissue perfusion, re-establishes microcirculation, and increases oxygen tension at the wound site. In addition to its angiogenic effects, VEGF-A plays a role in lymphangiogenesis during wound healing.

TGF family

The TGF-β family includes the following members: TGF- β1-3, bone morphogenic proteins (BMP), and activins. TGF- β1,TGF- β2, and TGF- β3 are the main forms found in mammals, but TGF- β1 predominates in cutaneous wound healing. They are produced by macrophages, fibroblasts, keratinocytes, and platelets [28-32] and work by binding a heteromeric receptor complex consisting of one type I and one type II receptor, both of which are serine-threonine kinases. In addition, they bind to a nonsignaling type III receptor, which functions in presenting TGF-β to the type II receptor. In wound healing, TGF- β1 is important in inflammation, angiogenesis, re-epithelialization, and connective tissue regeneration. It is shown to have increased expression with the onset of injury.[33,34] TGF-β1 facilitates the recruitment of additional inflammatory cells and

augments macrophage mediated tissue debridement [reviewed in Clark].[35] It is also interesting to note that once the wound field is sterilized, TGF- β1 may be able to deactivate superoxide production from macrophages *in vitro*.[36,91] This helps to protect the surrounding healthy tissue and prepares the wound for granulation tissue formation.[37] *In vitro* studies show that TGF-β1 helps initiate granulation tissue formation by increasing the expression of genes associated with ECM formation including fibronectin, the fibronectin receptor, and collagen, and protease inhibitors.[38-43,93]

BMP

In addition to TGF-βs and activins, BMPs have also been suggested to play a role in wound repair. BMPs are also members of the TGF-β superfamily. They also work through a heterodimeric serine/threonine kinase receptor. BMP-2, -4, -6, and -7 are all expressed in the wound tissue.[44] In particular, BMP-6 is highly expressed in regenerated keratinocytes as well as in fibroblasts in the acute wound. After wound closure, BMP-6 accumulates throughout the suprabasal layer of the newly formed epidermis.[45] There is evidence showing that BMP-6 levels are elevated in chronic wounds perhaps contributing to the pathology of the ulcers.[46,91]

FGF family

The FGF family is composed of 23 members. Of these, the three most important members involved in cutaneous wound healing are FGF-2, FGF-7, and FGF-10. FGFs are produced by keratinocytes, fibroblasts, endothelial cells, smooth muscle cells, chondrocytes, and mast cells.[47-51] FGF is increased in the acute wound and plays a role in granulation tissue formation, re-epithelialization, and tissue remodeling.[52,35]

Most members of the FGF family have a broad mitogenic spectrum. They stimulate the proliferation of various cells of mesodermal, ectodermal, and also endodermal origin. The only exception is FGF7 keratinocyte growth factor, which seems to be specific for epithelial cells, at least in the adult organism.[53] In addition to their mitogenic effects, FGFs also regulate the migration and differentiation of their target cells. Some FGFs have been detected at the wound site, indicating that the endogenous proteins are also regulators of wound healing.

To provide functional evidence for the role of FGF2 in wound repair, Broadley *et al*.[54] used a neutralizing polyclonal antibody that was raised against human FGF2. They incorporated the purified immunoglobulin G (IgG) into pellets, which were placed in the center of a polyvinyl alcohol sponge disk, and the disks were then implanted subcutaneously under ventral panniculus carnosus of rats. The continuous release of the antibody caused a striking reduction in cellularity and vascularization compared with the granulation tissue formed in the control IgG sponges. In addition, DNA, protein, and collagen levels in the anti-FGF2 sponges were reduced by 25–35% relative to control at day 7 after implantation. This study strongly suggested an important role of endogenous FGF2 in wound repair.

EGF family

The best characteristic growth factor in wound healing is those from the EGF family. The ligand includes: EGF, heparin binding EGF (HB-EGF), (TGF-a), epiregulin, amphiregulin, betacellulin, epigen, neuregulin-1 (NRG-1), NRG-2, NRG-3, NRG-4, NRG-5, and NRG-6.[55-67] The main members involved in wound healing include: EGF, TGF-a, and EGF-HB. These ligands bind to the EGF receptor (EGFR), a tyrosine kinase transmembrane protein, resulting in dimerization of the receptor, autophosphorylation, and tyrosine phosphorylation of downstream proteins.[68] In healthy human epidermis, EGFR can be localized throughout the entire epidermis, although its membranous presence is most prominent in the basal layer.[69,70] There are also ligands for other receptors, such as b-AR agonists (catecholamines), angiotensin II, and antimicrobial hCAP- 18, which can transactivate EGFR.[71-73] Ultimately this signaling pathway leads to the activation of a number of converging pathways promoting cell migration and proliferation.

EGF, TGF-α, and HB-EGF exert their function through binding to the EGFR, a transmembrane protein tyrosine kinase that is expressed on many different cell types. Consistent with the expression of the ligands at the wound site, EGFR mRNA, and protein were also detected in healing wounds. With the use of enzyme-linked immunosorbent assay and histological methods, an increase in the number of immunoreactive receptors was found in a tape stripping wound model before an increase in epidermal thickness. This early increase was followed by a decline in EGFR levels, which was followed by a decline in epidermal thickness.[74] This expression pattern suggested a role of the EGFR in re-epithelialization of skin wounds.

EGF is up-regulated after acute injury significantly accelerating re-epithelialization and increasing tensile strength in wounds.[75] One mechanism through which EGF functions is by increasing the expression of keratins K6 and K16, involved in the proliferative signaling pathway.[76,77].One *in vitro* study demonstrated that in the epidermis of nonhealing edges of chronic wounds EGFR was found in the cytoplasm of keratinocytes instead of the membrane.[78] This suggests that the receptor's downregulation and mislocalization may participate in the inhibition of epithelialization in patients with chronic wounds.[93]

Pharmaceutical Consideration

Most products containing growth factors or cytokines either are under investigation or still in pre-clinical stages of development.[79] This is mainly due to formulation difficulties, pre-clinical concerns (such as toxicology and pharmacology) and problems with clinical trial development such as selecting a suitable patient population according to defined criteria and ensuring that response variables are kept to a minimum. The usual concentration of exogenously applied growth factors *in vivo* ranges from 10 to 1000 mg/ml.[80] This is much higher than the concentrations that are needed to stimulate DNA synthesis and the migration of wound cells *in vitro*, possibly due to diffusion from the wound area and degradation by proteinases.[81] A common opinion is that a cocktail of growth factors may be more effective than individual growth factors.[81,82] It may be that certain combinations of growth factors may act synergistically to accelerate wound healing.[82,83]

There is a number of safety issues surrounding the use of exogenously applied growth factors. As they are protein drugs, they are likely to cause immunogenicity, especially with repeated application. It has been hypothesized that neoplastic transformation may occur through the unregulated expression of growth factors,[84] thus raising fears about the carcinogenic potential of exogenously applied agents. The prolonged exposure to growth factors to wound cells could induce excessive tissue proliferation, leading to pathologies such as atherosclerosis or hypertrophic scarring.[85-93]

CONCLUSION

Growth factors, cytokines, and chemokines are crucial for coordinating multiple cell types during the healing process, making cutaneous wound healing possible. Proper wound healing is guided by stringent regulation of these agents as well as a wound environment that favors their activity. The most promising growth factors that require clinical testing are VEGF, bFGF, and GM-CSF. PDGF-BB has already been approved by the FDA and is currently used in the treatment of chronic ulcers. Living cell therapy, which is also FDA approved, may be considered as sustained, simultaneous multiple growth factor therapy. Both healthy keratinocytes and fibroblasts produce at least 17 different growth factors and secrete these factors stimulating patients' cells to participate in the healing process.

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