Growth factors and cytokines in wound healing

Stephan Barrientos1,2; Olivera Stojadinovic, MD2; Michael S. Golinko, MD3; Harold Brem, MD3; Marjana Tomic-Canic, PhD2,4

1. University of Rochester School of Medicine and Dentistry, Rochester, New York,
2. Tissue Engineering, Repair and Regeneration Program, Hospital for Special Surgery at Weill Medical College of Cornell University, New York, New York.
3. Wound Healing Laboratory, Columbia University College of Physicians and Surgeons, New York, and
4. Department of Dermatology, Weill Medical College of Cornell University, New York

ABSTRACT

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, endothelial 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. Of particular importance is the epidermal growth factor (EGF) family, transforming growth factor beta (TGF-β) family, fibroblast growth factor (FGF) family, vascular endothelial growth factor (VEGF), granulocyte macrophage colony stimulating factor (GM-CSF), platelet-derived growth factor (PDGF), connective tissue growth factor (CTGF), interleukin (IL) family, and tumor necrosis factor-α family. Currently, patients are treated by three growth factors: PDGF-BB, bFGF, and GM-CSF. Only PDGF-BB has successfully completed randomized clinical trials in the Unites States. With gene therapy now in clinical trial and the discovery of biodegradable polymers, fibrin mesh, and human collagen serving as potential delivery systems other growth factors may soon be available to patients. This review will focus on the specific roles of these growth factors and cytokines during the wound healing process.
complex integration of signals that coordinate cellular processes. These agents are biologically active polypeptides that act to alter the growth, differentiation and metabolism of a target cell. They can act by paracrine, autocrine, juxtacrine, or endocrine mechanisms, and effect cell behavior as a consequence of their binding to specific cell surface receptors or ECM proteins. Binding to these receptors triggers a cascade of molecular events. The endpoint of this signaling is the binding of transcription factors to gene promoters that regulate the transcription of proteins controlling the cell cycle, motility, or differentiation patterns. This review will summarize the major growth factors and cytokines involved in wound healing with particular focus on the EGF family, TGF-β family, FGF family, VEGF, granulocyte macrophage colony stimulating factor (GM-CSF), PDGF-BB, CTGF, IL family, and tumor necrosis factor (TNF)-α family (Table 1).

**EPIDERMAL GROWTH FACTOR (EGF) FAMILY**

Perhaps the best-characterized growth factors in wound healing are those from the EGF family. The ligands

---

**Table 1.** Major growth factors and cytokines that participate in wound healing with cell types and their respective roles in both acute and chronic wounds are listed

<table>
<thead>
<tr>
<th>Growth Factors</th>
<th>Cells</th>
<th>Acute Wound</th>
<th>Function</th>
<th>Chronic Wound</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGF</td>
<td>Platelets</td>
<td>Increased levels</td>
<td>Reepithelialization</td>
<td>Decreased levels</td>
</tr>
<tr>
<td></td>
<td>Macrophages</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fibroblasts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FGF-2</td>
<td>Keratinocytes</td>
<td>Increased levels</td>
<td>Granulation tissue formation</td>
<td>Decreased levels</td>
</tr>
<tr>
<td></td>
<td>Mast Cells</td>
<td></td>
<td>Reepithelialization</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fibroblasts</td>
<td></td>
<td>Matrix formation and remodeling</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Endothelial cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Smooth muscle cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chondrocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGF-β</td>
<td>Platelets</td>
<td>Increased levels</td>
<td>Inflammation</td>
<td>Decreased levels</td>
</tr>
<tr>
<td></td>
<td>Keratinocytes</td>
<td></td>
<td>Granulation tissue formation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Macrophages</td>
<td></td>
<td>Reepithelialization</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lymphocytes</td>
<td></td>
<td>Matrix formation and remodeling</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fibroblasts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDGF</td>
<td>Platelets</td>
<td>Increased levels</td>
<td>Inflammation</td>
<td>Decreased levels</td>
</tr>
<tr>
<td></td>
<td>Keratinocytes</td>
<td></td>
<td>Granulation tissue formation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Macrophages</td>
<td></td>
<td>Reepithelialization</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Endothelial cells</td>
<td></td>
<td>Matrix formation and remodeling</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fibroblasts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEGF</td>
<td>Platelets</td>
<td>Increased levels</td>
<td>Granulation tissue formation</td>
<td>Decreased levels</td>
</tr>
<tr>
<td></td>
<td>Neutrophils</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Macrophages</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Endothelial cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Smooth muscle cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fibroblasts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1</td>
<td>Neutrophils</td>
<td>Increased levels</td>
<td>Inflammation</td>
<td>Increased levels</td>
</tr>
<tr>
<td></td>
<td>Monocytes</td>
<td></td>
<td>Reepithelialization</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Macrophages</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Keratinocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>Neutrophils</td>
<td>Increased levels</td>
<td>Inflammation</td>
<td>Increased levels</td>
</tr>
<tr>
<td></td>
<td>Macrophages</td>
<td></td>
<td>Reepithelialization</td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>Neutrophils</td>
<td>Increased levels</td>
<td>Inflammation</td>
<td>Increased levels</td>
</tr>
<tr>
<td></td>
<td>Macrophages</td>
<td></td>
<td>Reepithelialization</td>
<td></td>
</tr>
</tbody>
</table>
include: EGF, heparin binding EGF (HB-EGF), transforming growth factor-alpha (TGF-α), epiregulin, amphi- 
regulin, beta-catenin, epigen, neuregulin-1 (NRG-1), 
main members involved in wound healing include: EGF, 
TGF-α, and EGF-HB. These ligands bind to the EGF re-
ceptor (EGFR), a tyrosine kinase transmembrane protein, 
resulting in dimerization of the receptor, autophosphoryla-
tion, and tyrosine phosphorylation of downstream pro-
teins.27 In healthy human epidermis, EGFR can be 
localized throughout the entire epidermis, although its membranous 
presence is most prominent in the basal layer.28,29 There are 
also ligands for other receptors, such as β-AR agonists 
catecholamines), angiotensin II, and antimicrobial lisc 
hibin-A1, which can transactivate EGFR.30–32 Ultimately this 
signaling pathway leads to the activation of a number of 
converging pathways promoting cell migration and pro-
liferation.

In vitro studies, show that activation of the EGFR plays 
an important role in reepithelialization by increasing kerati-
nocyte proliferation and cell migration in acute wounds.33,36 The ligands that bind to EGFR are synthe-
sized as membrane-anchored forms, which are proteolyti-
cally processed to bioactive soluble forms. However, EGFR 
ligand shedding is essential for keratinocyte migration and 
it has been established that EGF accelerate keratinocyte 
migration thus promoting reepithelialization.37,38 It is a 
potent mitogen for keratinocytes39,40 and the transmem-
brane forms are able to stimulate growth of keratinocytes 
in a juxtacrine manner, suggesting their participation in re-
epithelialization.41

EGF was originally reported by Dr. Stanley Cohen 42,43 
EGF is secreted by platelets, macrophages, and fibroblasts 
and acts in a paracrine fashion on keratinocytes 44,45 In vi-
tro studies have shown that EGF is up-regulated after 
acute injury significantly accelerating reepithelialization46 
and increasing tensile strength in wounds.47 One mecha-
nism through which EGF functions is by increasing the 
expression of keratins K6 and K16, involved in the prolif-
erative signaling pathway.48,49 One in vitro study demonstrated 
that in the epidermis of nonhealing edges of 
chronic wounds EGFR was found in the cytoplasm of ker-
atinocytes instead of the membrane.50 This suggests that 
the receptor’s down-regulation and mis-localization may 
participate in inhibition of epithelialization in patients 
with chronic wounds. Other in vitro studies demonstrate 
substantial degradation of exogenous EGF and the EGFR 
reversible with the addition of metalloproteinase (MMP) 
inhibitors in chronic ulcers.51,52 This suggests that EGF is 
susceptible to the proteolytic environment found in these 
wounds. Clinical trials for chronic wound therapeutics 
show that the addition of topical EGF increased 
epithelialization and shortened healing time in skin graft 
donor-healing sites, venous ulcers (VU), and diabetic foot 
ulcers (DFU).53–55 Therefore, EGF may still be useful to 
people with chronic wounds if delivered by a system, such 
as gene therapy, polymers, or electrospray nanofibers.56,57 
Such techniques maintain a continuous growth factor con-
centration, sustaining its presence in the wound and pre-
vailing its rapid degradation.

Another member of this family, TGF-α, is secreted by 
platelets, keratinocytes, macrophages, fibroblasts, and 
lymphocytes and works in an autocrine fashion on kerati-
nocyes.58–61 In vitro studies demonstrate that TGF-
α has the ability to increase keratinocyte migration62 and 
proliferation63–65 and induce the expression of K6 and 
K16.66 In vivo studies suggest a role in early stimulation 
and maintenance of wound epithelialization in partial 
thickness wounds.67 Despite its seemingly important role 
in reepithelialization, absence of this growth factor does 
not hinder wound healing. This can be contributed to a 
certain degree of compensation by the other growth fac-
tors in the EGF-family.68

HB-EGF is also up-regulated in the acute wound.69,70 It 
is secreted by keratinocytes and works in an autocrine 
fashion71 by binding to the EGFR subtypes HER1 and 
HER421 promoting reepithelialization.21 HB-EGF has 
been implicated in vivo as having a role in wound healing 
as a major growth factor found in wound fluid72 and plays 
a role in promotion of keratinocyte migration suggesting 
its important role in early stages of reepithelialization.73 In 
addition, recent in vitro studies demonstrate a possible 
role in angiogenesis.74

**FIBROBLAST GROWTH FACTOR (FGF) FAMILY**

The FGF family is composed of 23 members. Of these, the 
three most important members involved in cutaneous 
chronic 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.58,75–78 The high-affinity FGF receptor (FGFR) fam-
ily, which mediates cellular responses to FGF, comprises 
four members FGFR1–4. These receptors are tyrosine kin-
ase transmembrane proteins, which work much like 
EGFR.79 Essential for activation of the receptor, FGF 
must bind proteoglycans, such as heparin, that incorpo-
rates several ligands together in a web.80

FGF-2, or basic FGF, is increased in the acute wound and 
plays a role in granulation tissue formation, re-
epithelialization, and tissue remodeling.79,81 In vitro stud-
ies have demonstrated that FGF-2 regulates the synthesis 
and deposition of various ECM components, increases 
keraatinocyte motility during reepithelialization,82–84 and 
promotes the migration of fibroblasts and stimulates them 
to produce collagenase.16

Levels of FGF-2 are decreased in chronic wounds.52 
Clinical trials utilizing FGF-2 in the treatment of DFUs 
have failed.85 This is primarily due to FGF-2’s inability to 
maintain its efficacy in these patients. Promising data has 
been obtained from FGF-2-treated pressure ulcer (PU) 
patients showing a trend toward faster wound closure.86

Other important members of this family include FGF-7, 
or keratinocyte growth factor-1 (KGF-1), and its homo-
logue FGF-10, or KGF-2, both of which are expressed in 
acute wounds.87,88 Both FGF-7 and FGF-10 act in a para-
crine fashion through the FGFR2IIIb receptor found only 
on keratinocytes.89 FGF-10 is also able to bind to 
FGFR1IIIb and has been shown to have a mitogenic effect 
on cells containing this receptor.89,90 In vitro studies have 
shown that FGF-7 and FGF-10 stimulate proliferation and 
migration of keratinocytes playing an important role in re-
epithelialization. In addition, FGF-7 and FGF-10 increase 
transcription of factors involved in the detoxification of re-
active oxygen species (ROS). This helps to reduce ROS-
induced apoptosis of keratinocytes in the wound bed preserving these cells for reepithelialization (reviewed in Raja et al.\(^3\)). In vitro studies have also shown FGF-7 to be important during the later stages of neovascularization when luminal spaces and basement membranes are being developed. It is a potent mitogen for vascular endothelial cells and helps in the up-regulation of VEGF. It also stimulates endothelial cells to produce a urokinase type plasminogen activator, a protease required for neovascularization.\(^9\) Because of its potential benefit in reepithelialization, studies have been conducted to evaluate KGF’s effect on chronic wounds. One clinical trial using topical application of Repifermin (rh-KGF-2) resulted in accelerated wound healing in VU patients.\(^9\)

**TRANSFORMING GROWTH FACTOR-\(\beta\) (TGF-\(\beta\)) FAMILY**

The TGF-\(\beta\) family includes the following members: TGF-\(\beta\)-1, -3, bone morphogenic proteins (BMP), and activins. TGF-\(\beta\)-1, TGF-\(\beta\)-2, and TGF-\(\beta\)-3 are the main forms found in mammals, but TGF-\(\beta\)-1 predominates in cutaneous wound healing. They are produced by macrophages, fibroblasts, keratinocytes, and platelets\(^9\) and work by binding to the Smad family of transcription factors.\(^9\) In wound healing, TGF-\(\beta\)-1 is important in inflammation, angiogenesis, reepithelialization, and connective tissue regeneration. It is shown to have increased expression with the onset of injury.\(^9,9\) TGF-\(\beta\)-1 facilitates the recruitment of additional inflammatory cells and augments macrophage mediated tissue debridement (reviewed in Clark\(^8\)). It is also interesting to note that once the wound field is sterilized, TGF-\(\beta\)-1 may be able to deactivate superoxide production from macrophages in vitro.\(^10\) This helps to protect the surrounding healthy tissue and prepares the wound for granulation tissue formation.\(^10\) In vitro studies show that TGF-\(\beta\)-1 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.\(^49,102-106\) It is also involved in up-regulating the angiogenic growth factor VEGF.\(^10\) In addition, in vitro studies show TGF-\(\beta\)-1 playing a role in wound contraction by facilitating fibroblast contraction of the collagen matrix.\(^10\)

During reepithelialization, TGF-\(\beta\)-1 shifts keratinocyte integrin expression toward a more migratory phenotype.\(^62\) There are conflicting data as to the role of TGF-\(\beta\)-1 in keratinocyte proliferation. Several studies both in vitro and in vivo have demonstrated that TGF-\(\beta\)-1 inhibits keratinocyte proliferation.\(^109-111\) Furthermore, animal in vivo studies have shown that Smad3-null (Smad3\(^-\)/-\) mice have accelerated cutaneous wound healing compared with wild-type mice, characterized by an increased rate of reepithelialization and significantly reduced local infiltration of monocytes.\(^112\) However, other studies show that overexpression of TGF-\(\beta\)-1 increases the proliferative phenotype of keratinocytes particularly during the late stages of wound healing.\(^111,113\) This illustrates the complexity of signaling necessary to coordinate cellular processes participating in wound healing, emphasizing the importance of tight spatio-temporal control, in which small changes in levels and timing of any growth factor may have a completely different outcome.

Finally, in the matrix formation and remodeling phase of wound healing, TGF-\(\beta\)-1 is involved in collagen production (particularly type I and III). It is also a potent inhibitor of metalloproteinase MMP-1, MMP-3, and MMP-9 and a promoter of tissue inhibitor of metalloproteinase TIMP-1 synthesis, thus inhibiting collagen breakdown.\(^49,104-106\)

TGF-\(\beta\)-1’s ability to stimulate collagen production is so potent that it can result in pathology. TGF-\(\beta\)-1 plays a major role in the pathogenesis of fibrosis by inducing and sustaining activation of keloid fibroblasts.\(^115\) When overexpressed, TGF-\(\beta\)-1 has been shown to stimulate connective tissue growth factor (CTGF) also shown to play an important role in the development of hypertrophic and keloid scars.\(^116\) It has been shown that localized increase in the release and activation of TGF-\(\beta\)-1 in burn injuries inhibits reepithelialization and enhances fibrosis.\(^117\) Furthermore, in the fetal wound the fetal fibroblast responds to its hypoxic environment by decreasing TGF-\(\beta\)-1 transcription that could explain, in part, the scarless healing seen in the fetus.\(^118-120\)

The second isoform, TGF-\(\beta\)-2, has also been shown to have a role in wound healing. Like TGF-\(\beta\)-1, TGF-\(\beta\)-2 is involved in all stages of wound healing. It is involved in recruiting inflammatory cells and fibroblasts to the wound site. In vivo experiments show that TGF-\(\beta\)-2 stimulates the formation of granulation tissue by inducing angiogenesis.\(^121,122\) It also has been shown to accelerate reepithelialization in vivo.\(^121,123\) During matrix formation and remodeling, TGF-\(\beta\)-2 increases protein, DNA, and collagen production. By stimulating recruitment of fibroblasts to the wound site, the combined result is increased collagen deposition (particularly type I and III) and scar formation in vivo.\(^121,124\)

The third isoform, TGF-\(\beta\)-3, has been shown to play a role in wound healing. In vivo studies have shown that TGF-\(\beta\)-3 promotes wound healing by recruiting inflammatory cells and fibroblasts to the wound site and by facilitating keratinocyte migration. TGF-\(\beta\)-3 has also been shown to be a potent stimulant of neovascularization and vascular rearrangement.\(^125,126\) Furthermore, it has been demonstrated that TGF-\(\beta\)-3 is a potent inhibitor of DNA synthesis in human keratinocytes. These findings along with the observation of constitutive TGF-\(\beta\)-3 expression in the intact epidermis support the hypothesis that activation of TGF-\(\beta\)-3 may be an important stop signal for terminal differentiation in this tissue.\(^125,127,128\) It has also been shown that unlike the other two isoforms which promote scar formation, TGF-\(\beta\)-3 inhibits scarring and promotes better collagen organization in vivo.\(^124\)

In chronic wounds, TGF-\(\beta\)-3s are significantly decreased\(^129\) possibly due to degradation from proteolytic enzymes, particularly neutrophil elastase.\(^29\) It has also been shown that TGF-\(\beta\)-3s can be sequestered by molecules like decorin, fibrinogen, albumin and alpha2-macroglobulin, limiting their bioactivity.\(^130,131\) Early work on clinical trials using exogenous TGF-\(\beta\)-2 on venous stasis ulcers was promising.\(^132\) Nevertheless, TGF-\(\beta\)-3 has failed multiple clinical trials for treatment of chronic wounds.
ACTIVINS

Activins are members of the TGF-β superfamily produced by fibroblasts and keratinocytes. Their biological functions are mediated by serine/threonine kinase receptors. During wound repair there is up-regulation of activin where it plays a role in reepithelialization. In vitro studies suggest that activin effects keratinocyte proliferation in an indirect fashion by inducing the expression of growth factors in dermal fibroblasts. Activin by itself inhibits keratinocyte proliferation and induces terminal differentiation of keratinocytes. Therefore, a theoretical therapeutic approach for healing chronic wounds could be delivering activin to a wound in the presence of dermal fibroblasts.

BONE MORPHOGENIC PROTEINS (BMPs)

BMPs are also members of the TGF-β superfamily. They also work via a heterodimeric serine/threonine kinase receptor. BMP-2, 4, -6, and -7 are all expressed in the wound tissue. 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 epithelium. In vitro studies have shown it to be important in keratinocyte differentiation. Furthermore, overexpression of BMP-6 has been shown to severely delay reepithelialization in vivo. There is evidence showing that BMP-6 levels are elevated in chronic wounds perhaps contributing to the pathology of these ulcers.

PLATELET DERIVED GROWTH FACTOR (PDGF)

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. These ligands bind to two different transmembrane tyrosine kinase receptors (alpha and beta). Ligand binding causes receptor dimerization, leading to autophosphorylation of the receptors. This creates a docking site for SH2 (Src homology 2) domain-containing signaling molecules, whereby several signaling pathways are then activated.

PDGF plays a role in each stage of wound healing. Upon injury PDGF is released from degranulating platelets and is present in wound fluid. This stimulates mitogenicity and chemotaxis of neutrophils, macrophages, fibroblasts, and smooth muscle cells to the wound site. It also stimulates macrophages to produce and secrete growth factors such as TGF-β. Much like TGF-β, PDGF also augments macrophage-mediated tissue debridement and granulation tissue formation. The effects of PDGF on inducing angiogenesis are organ dependent. For example, production of PDGF in cardiac microvascular cells leads to induction of VEGF and VEGF-receptor-2 suggesting an important role in cardiac angiogenesis. With regard to wounding, it has been shown in vitro that PDGF works synergistically with hypoxia to increase the expression of VEGF as seen in ischemic injury. PDGF is particularly important in blood vessel maturation. In vivo experiments demonstrated that PDGF is important in recruiting pericytes to the capillaries and thus increase the structural integrity of these vessels. In addition, in vivo studies show that PDGF in combination with VEGF not only increases pericyte recruitment but also smooth muscle cells further enhancing the integrity of the capillaries. It should be noted however that PDGF’s angiogenic effect is weaker than that of FGF and VEGF and does not appear to be essential for the initial formation of blood vessels. PDGF also plays a role in reepithelialization by up-regulating the production of IGF-1 and thrombospondin-1 in vitro. IGF-1 has been shown to increase keratinocyte motility and thrombospondin-1 delays proteolytic degradation and promotes a proliferative response in the wound in vitro. PDGF has also been shown to enhance the proliferation of fibroblasts and thus the production of ECM. In addition, it stimulates fibroblasts to contract collagen matrices and induces the myofibroblast phenotype in these cells. During tissue remodeling, PDGF helps to break down old collagen by up-regulating matrix metalloproteinases.

Levels of PDGF are decreased in chronic wounds. It has been shown that PDGF is susceptible to the proteolytic environment found in the chronic wound and its degradation can be reversed with the addition of MMP inhibitors. It is the increased MMP activity that degrades the ECM inhibiting cell migration and collagen deposition. MMPs also break down growth factors and their target cell receptors.

Recombinant human variants of PDGF-BB (Beclaporin) have been successfully applied in diabetic and PU’s and it is the only FDA approved drug for chronic wound treatment. Margolis et al. was the first to demonstrate that gene delivery of PDGF can successfully and safely be tested in patients with chronic wounds. Recently, a clinical trial using Adenovirus-PDGF-BB has been initiated for persons with diabetic ulcers. These advances herald in a new era in the treatment of ulcers and growth factor therapy that may enable many of the growth factors that accelerate healing experimentally to be effective in patients, i.e., by safely testing a new delivery system gene therapy.

VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF)

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. It binds to the tyrosine kinase surface receptors Flt-1 (VEGF receptor-1) and KDR (VEGF receptor-2 [VEGFR-2]) localized to the endothelial surface of blood vessels. These receptors have different functions. KDR is an important mediator of chemotaxis and proliferation of endothelial cells in vitro. It is also responsible for inducing endothelial cell differentiation. In comparison, Flt-1 is required for organization of blood vessels.

VEGF-A is important in wound healing because it promotes the early events in angiogenesis, particularly endothelial cell migration and proliferation as seen in ischemic injury. VEGF-A is important in wound healing because it promotes the early events in angiogenesis, particularly endothelial cell migration and proliferation as seen in ischemic injury.
in several in vitro studies, VEGF-A transcription and secretion along with the VEGFR are elevated in the acute wound. In animal studies, macrophages release VEGF-A during wound healing, which induces VEGF-A expression in keratinocytes and fibroblasts. Other cytokines and growth factors that act as paracrine factors enhancing VEGF-A expression include TGF-β, EGF, TGF-α, KGF, bFGF, PDGF-BB, and IL-1β. 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, reestablishes microcirculation, and increases oxygen tension at the wound site. In particular, hypoxia enhances VEGF-A expression in monocytes, fibroblasts, keratinocytes, myocytes, and endothelial cells. It also increases the expression of Flt-1 receptors on endothelial cells. As a result, there is a gradient of VEGF-A expression that parallels the hypoxic gradient. In addition to its angiogenic effects, VEGF-A plays a role in lymphangiogenesis during wound healing. One in vitro study proposed that VEGF-A promotes lymphatic vasculature formation via activation of VEGFR-2.

Chronic wounds such as DFUs, venous stasis ulcers, and PUs have areas of local skin ischemia making VEGF-A a possible therapeutic modality. In animal studies, it has been shown that the administration of VEGF-A restores impaired angiogenesis found in diabetic ischemic limbs. Other in vivo experiments show that VEGF-A improves reepithelialization of diabetic wounds associated with enhanced vessel formation. Despite these improvements, however, exogenous administration of VEGF induces sustained vascular leakage and promotes the formation of disorganized blood vessels as well as malformed and poorly functional lymphatic vessels. In human studies, intramuscular gene transfer of VEGF165 to patients with ischemic ulcers and or rest pain secondary to peripheral arterial disease resulted in limb salvage significantly decreasing rest pain.

VEGF-C is also up-regulated during wound healing. This growth factor is primarily released by macrophages and is important during the inflammatory stage of wound healing. VEGF-C works mostly through the VEGF receptor-3 (VEGFR3), which is expressed in lymphatic endothelium, fenestrated endothelia, and monocytes/macrophages. However, the proteolytically processed mature form of VEGF-C can also activate VEGFR-2 in blood vessel endothelium. In vitro studies show this growth factor playing a role in facilitating hematopoietic and inflammatory cell recruitment to the wound site both directly and indirectly by binding to VEGFR-2 increasing vascular permeability. In vitro studies also show VEGF-C playing a role in lymphangiogenesis by binding to VEGFR-3 and angiogenesis after proteolytic cleavage by binding to VEGFR-2. Because DFUs are a result of insufficient blood perfusion coupled with impaired angiogenesis, treatment with VEGF-C has been proposed. In an in vivo animal model VEGF-C was administered via an adenoviral vector to genetically diabetic mice resulting in accelerated healing rate. These results suggest potential therapeutic function in treatment of diabetic wounds.

Placental growth factor (PLGF) is a proangiogenic molecule that is up-regulated during wound healing. In the skin, this growth factor is expressed by keratinocytes and by endothelial cells. This growth factor acts by binding and activating the VEGFR-1. Like VEGF-C, PLGF plays a role during the inflammatory stage of wound healing. It has been shown, in vitro, to promote monocyte chemotaxis and bone marrow-derived precursor cell mobilization. It is also involved in promoting granulation tissue formation, maturation, and vascularization. It is thought to work synergistically with VEGF by potentiating its proangiogenic function. In addition, PLGF has been shown to directly stimulate cultured fibroblast migration, suggesting a direct role in accelerating granulation tissue maturation. In DFUs, it has been shown that PLGF expression is significantly reduced. The observation that PLGF specifically enhances adult pathophysiological neovascularization does not interfere with lymphatic vessel function, and induces augmented permeability only when administered at high concentration. This makes it an ideal candidate for therapeutic modulation for adult angiogenesis. Animal models using genetically diabetic mice have shown that diabetic wound treatment with an adenovirus vector expressing the PLGF gene significantly accelerated the healing process compared with wounds treated with a control vector.

**CONNECTIVE TISSUE GROWTH FACTOR (CTGF)**

CTGF is an ECM-associated heparin-binding protein that binds directly to integrins. It is synthesized by fibroblasts and stimulates proliferation and chemotaxis of these cells. CTGF expression is increased after injury and is involved in granulation tissue formation, reepithelialization, and matrix formation and remodeling. In vitro experiments have shown that CTGF promotes endothelial proliferation, migration, survival, and adhesions in angiogenesis. It has also been demonstrated that CTGF is required for reepithelialization in wound healing by promoting cell migration. It is thought to be induced by TGF-β through the Ras/MEK/ERK MAPK signalling pathway. In addition, CTGF is a strong inducer of ECM proteins, such as collagen type I and fibronectin and their integrin receptors, and acts as a mediator of TGF-β. Much like TGF-β, CTGF also has increased expression in hypertrophic and keloid scars.

**GRANULOCYTE MACROPHAGE-COLONY STIMULATING FACTOR (GM-CSF)**

GM-CSF has been shown to be increased in the epidermis in wounded skin. It is particularly important during the inflammatory stage of wound healing increasing the number of neutrophils and enhancing their function at the wound site. In vitro studies have shown GM-CSF to increase keratinocyte proliferation and thus enhance reepithelialization. It has been suggested that GM-CSF works directly on the keratinocyte but also indirectly by up-regulating IL-6. In addition, in vitro studies have demonstrated this growth factor to increase migration and proliferation of endothelial cells suggesting a role in

---

**Growth factors and cytokines in wound healing**

Barrientos et al

PROINFLAMMATORY CYTOKINES

Proinflammatory cytokines, particularly IL-1 and interleukin-6, and TNF-α are up-regulated during the inflammatory phase of wound healing. IL-1 is produced by neutrophils, monocytes, macrophages, and keratinocytes. Upon wound healing it is immediately released by keratinocytes. In addition to having a paracrine effect, it also works in an autocrine fashion increasing keratinocyte mitogenesis and proliferation (reviewed in Raja et al.). IL-1 has been shown to induce the expression of K6 and K16 in migrating keratinocytes. In addition, IL-1 activates fibroblasts and increases the secretion of FGF-7.

IL-6 is produced by neutrophils and monocytes and has been shown to be important in initiating the healing response. Its expression is increased after wounding and tends to persist in older wounds. It has a mitogenic and proliferative effect on keratinocytes and is chemotactic to neutrophils.

Much like IL-1, TNF-α can induce the production of FGF-7, suggesting that it can indirectly promote reepithelialization. Alone, TNF-α has been shown to inhibit wound reepithelialization. The effects of exogenous TNF-α are dependent on concentration and duration of exposure emphasizing the importance of balancing the proinflammatory signals controlling wound healing. TNF-α, at low levels, can promote wound healing by indirectly stimulating inflammation and increasing macrophage produced growth factors. However, at higher levels, especially for prolonged periods of time, TNF-α has a detrimental effect on healing. TNF-α suppresses synthesis of ECM proteins and TIMPs while increasing synthesis of MMPs (MMP-1, MMP-2, MMP-3, MMP-9, MMP-13, and MT1-MMP). In addition, elevated levels of IL-1β have a similar response to that of TNF-α. Both TNF-α and IL-1β have been shown to perpetuate each others expression and therefore amplify this signal.

Levels of TNF-α and IL-1β are elevated in chronic wounds. In addition, infection that is common in chronic wounds further contributes to prolonged inflammation. Furthermore, nonhealing wounds also exhibit elevated levels of interstitial collagenases, gelatinases, and stromelysins that have been shown to be induced by TNF-α and IL-1β. It has, therefore, been hypothesized that in chronic wounds, chronic inflammation causes inflammatory cells to secrete TNF-α and IL-1β that synergistically increase production of MMPs while reducing synthesis of TIMPs. It is increased MMP activity that degrades the ECM inhibiting cell migration and collagen deposition. MMPs also break down growth factors and their target receptors.

CHEMOKINES

Chemokines are also active participants in the wound healing process because they stimulate the migration of multiple cell types in the wound site particularly inflammatory cells. In addition, the presence of chemokine receptors on resident cells suggests that they also contribute to the regulation of reepithelialization, tissue remodeling, and angiogenesis (reviewed in Raja et al.). The CXC, CC, and C families of ligands act by binding to G protein-coupled surface receptors, CXCR-receptors and the CC-receptor.

Macrophage chemo-attractant protein (MCP-1 or CCL2) is a CC family chemokine. MCP-1 is induced in keratinocytes upon wounding. It is a chemotactant for macrophages, T-cells, and mast cells. Sustained expression of this chemokine permits a prolonged presence of neutrophils and macrophages in the chronic wound contributing to a prolonged inflammatory response. However, lack of MCP-1 in vivo significantly delays wound healing particularly with reepithelialization, angiogenesis, and collagen synthesis as seen in mouse models. This suggests that in the mouse MCP-1 may be influencing gene expression/protein synthesis of growth factors in murine macrophages. However, in humans MCP-1 does not seem to regulate growth factor production by these cells. Addition of exogenous MCP-1 to wounds in animals yielded only moderate improvements in wound healing.

Interferon inducible protein 10 (IP-10 or CXCL10) is another cytokine part of the CXC family. In acute wounds and chronic inflammatory states, there is increased expression by keratinocytes. IP-10 has been demonstrated to negatively impact wound healing. Overexpression of IP-10 results in a more intense inflammatory response by recruiting lymphocytes to the wound site. In vitro studies show that IP-10 delays reepithelialization and prolongs the granulation phase. This cytokine inhibits the migration of dermal fibroblasts by blocking their release from the sub-stratum regulated by IP-10 inhibition of EGF and heparin-binding EGF-like growth factor receptor-mediated calpain activity. In addition, it has been shown that IP-10 inhibits angiogenesis (reviewed in Belperio et al.). A suggested mechanism can be seen in the related cytokine, PF4. PF4 inhibits endothelial cell migration, proliferation, and angiogenesis in response to bFGF. PF4 inhibits bFGF binding its receptor by forming heterodimeric complexes via heparin binding. It has been suggested that IP-10 might work in a similar fashion.

Interleukin-8 (IL-8 or CXCL8) is a member of the CXC family. Its expression is increased in acute wounds and it has been shown to play a role in reepithelialization by increasing keratinocyte migration and proliferation. It also induces the expression of MMPs in leukocytes, stimulating tissue remodeling. It is, however, a strong chemotactant for neutrophils, thus participating in the inflammatory response. High levels of this chemokine accumulate in non-healing wounds. Furthermore, addition of IL-8 in high levels decreases keratinocyte proliferation and collagen lattice contraction by fibroblasts. It has been shown that there are relatively low levels of IL-8 in the fetus. This finding may be responsible for the lack of inflammation during the fetal wound healing and contribute to scarless wounds.

The GRO-α (CXCL1) chemokine is also a member of the CXC family. This cytokine is a potent regulator of neu-
Growth factors and cytokines in wound healing

Barrientos et al

SUMMARY

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. In the acute wound, the healing process is controlled by spatio-temporal action of these growth factors, cytokines and chemokines leading through progression of healing and resulting in the reestablishment of the skin’s barrier function. This is contrasted by the chronic wound, which is arrested in a state of chronic inflammation. As a consequence, the generation of a proteolytic environment by inflammatory cells infiltrating the wound site as well as prolonged up-regulation of pro-inflammatory cytokines and chemokines inhibits normal progression of wound healing. This environment subjects various growth factors and cytokines to degradation and sequestration in the wound site inhibiting their activity.

Topical delivery of growth factors to chronic wounds must be resistant to rapid degradation form the wounds proteolytic environment and have sustained release. This is readily being accomplished using gene therapy. Currently, multiple novel delivery systems, including adenovirus and slow-releasing polymers are being investigated as growth factor delivery systems. 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 factors273 and secrete these factors stimulating the expression of growth factor receptors that are not properly expressed at the nonhealing edge of chronic ulcers, making cells unresponsive to exogenous growth factor therapy.56,275

ACKNOWLEDGMENTS

Our research is supported by the National Institutes of Health grants NR08029 (M.T.-C.), AG030673 (M.T.-C.), a pilot award (M.T.-C.) from the UL1RR024996 Center for Translational Science Award of the Weill Cornell Medical College, DK59424 (H.B.), LM008443 (H.B.). We are very grateful to Mr. Esteban J. Barrientos for editing the manuscript.

REFERENCES

Growth factors and cytokines in wound healing

Barrientos et al


Growth factors and cytokines in wound healing


Growth factors and cytokines in wound healing

Barrientos et al


118. Chen SM, Ward SI, Olutoye OO, Diegelmann RF, Kelman I. Ability of chronic wound fluids to degrade peptide growth factors is associated with increased levels of elastase activity and diminished levels of protease inhibitors. Wound Repair Regen 1997; 5: 23–32.


Growth factors and cytokines in wound healing


252. Wallace HJ, Stacey MC. Levels of tumor necrosis factor-alpha (TNF-alpha) and soluble TNF receptors in chronic


