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CONTEMPORARY SURGERY

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Angiogenesis in Wound Healing

by William W. Li, MD, and Vincent W. Li, MD

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Educational objectives: Upon completion of this educational activity, participants will be able to:

- Summarize the "angiogenesis model of wound healing," including the regulation of angiogenesis by endogenous stimulators and inhibitors, the cascade of molecular and cellular events in the wound bed, and the stages of wound healing.
- Describe the role of growth factors, specifically platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF), in wound neovascularization.
- Explain how defects in the angiogenesis process are present in diabetic foot ulcers, venous insufficiency ulcers, and arterial ulcers.
- Select interventions that may promote improved wound granulation to speed healing of chronic wounds.
- Discuss the critical role of sharp debridement for the successful of growth therapy.

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This monograph includes discussion of off-label uses of becaplermin.

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Introduction

By William W. Li, MD

Angiogenesis, the growth of new blood vessels, is an important natural process required for healing wounds and for restoring blood flow to tissues after injury or insult. Angiogenesis therapies—designed to "turn on" new capillary growth—are revolutionizing medicine by providing a unified approach for treating crippling and life-threatening conditions. Currently, more than 200 biotechnology, genomics, and medical device companies and every major pharmaceutical company are racing to develop new angiogenesis-based medicines.

This supplement was written to provide clinicians with an understanding of the mechanisms underlying angiogenic growth-factor therapy. Surgeons and wound-care specialists can use their knowledge of angiogenesis to identify defects and select interventions that may promote improved wound granulation to speed healing.

The first article describes the "angiogenesis model of wound healing," including the regulation of angiogenesis by endogenous stimulators and inhibitors, the cascade of molecular and cellular events in the wound bed, and the stages of wound healing. Defects in the angiogenesis process are present in diabetic foot ulcers, venous insufficiency ulcers, and arterial ulcers.

The second article discusses the role of growth factors, specifically platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF), in wound neovascularization. Both PDGF and VEGF independently initiate angiogenesis and mediate blood vessel growth and behavior. When administered together, they collaborate to form superior blood vessel networks compared with those generated by either growth factor alone.

Impaired circulation is an underlying pathological feature in peripheral arterial disease (PAD), ischemic heart disease, and chronic wounds. Growth factor therapy enhances tissue vascularization, improves local circulation, and promotes healing and regeneration. The third article explains how growth factors activate angiogenesis and the strategies that are being developed for their therapeutic delivery.

The fourth article outlines the clinical experience with recombinant human PDGF-BB (becaplermin, Regranex Gel 0.01%, Johnson & Johnson Wound Management, Ethicon, Inc) the first angiogenic growth factor to receive Food and Drug Administration approval for treating chronic wounds. The critical role of sharp debridement for the successful use of growth factor therapy is specifically discussed.

Finally, a series of case studies demonstrates how becaplermin therapy was used to treat a variety of chronic wounds.

SUPPLEMENT
CONTEMPORARY SURGERY

Angiogenesis: A Control Point for Normal and Delayed Wound Healing

WILLIAM W. LI, MD; DIMITRIS TSAKAYANNIS, MD; VINCENT W. LI, MD

ABSTRACT Angiogenesis is a physiological process required for wound healing. Immediately following injury, angiogenesis is initiated by multiple molecular signals, including hemostatic factors, inflammation, cytokine growth factors, and cell-matrix interactions. New capillaries proliferate via a cascade of biological events to form granulation tissue in the wound bed. This process is sustained until the terminal stages of healing, when angiogenesis is halted by diminished levels of growth factors, resolution of inflammation, stabilized tissue matrix, and endogenous inhibitors of angiogenesis. Defects in the angiogenesis pathway impair granulation and delay healing, and these are evident in chronic wounds. ■

Successful wound healing depends upon angiogenesis, the growth of new capillary blood vessels. Clinically, new capillaries first become visible in the wound bed 3–5 days after injury, and their appearance is synonymous with granulation, the creation of a provisional matrix comprised of proliferating blood vessels, migrating fibroblasts and new collagen.[1] Impaired granulation is a hallmark of the chronic wounds encountered with diabetes, and venous or arterial insufficiency. Angiogenesis has therefore become a major focus of study for wound biologists and surgeons alike.

The field of angiogenesis research began in the 1960s as an inquiry into how new blood vessels support solid tumor growth.[2] Physiologists have long recognized, however, that neovascularization occurs in normal regenerative processes.[3] Proliferating capillaries bring oxygen and micronutrients to growing tissues and remove catabolic waste products. The endothelium comprising these vessels secrete paracrine factors that promote survival of adjacent cells by impeding apoptosis, or programmed cell death.[4]

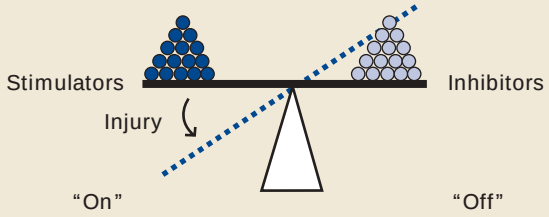
Because angiogenesis is required for wound healing, its induction is beneficial in many clinical situations for achieving wound closure.

PHYSIOLOGICAL CONTROL OF ANGIOGENESIS

The entire skin surface overlies a vast network of capillary blood vessels. Beneath the epidermis, each cell exists no greater than 200 µm from the nearest capillary, the diffusion distance of oxygen.[3] Most blood vessels are formed during fetal development, but adult tissues can induce angiogenesis in response to injury. This capability is governed by pro- and antiangiogenic factors present throughout the body (*Table 1*).

Pro-angiogenic factors consist of a diverse group of molecules including thrombin, fibrinogen fragments, thymosin beta 4, and growth factors. Angiogenic growth factors are proteins that circulate in the bloodstream, are stored in platelets and inflammatory cells, and are sequestered within the extracellular matrix. The production of many of these factors is reg-

FIGURE 1 The Angiogenic Control Switch



Physiological regulation of angiogenesis represents a balance between stimulators (growth factors) and inhibitors.

TABLE 1 Molecular Regulators of Angiogenesis

Endogenous Stimulators of Angiogenesis

- Adrenomedullin
- Angiogenin
- Angiopoietin-1
- Cyr-16
- Del-1
- Fibroblast growth factors: acidic (aFGF) basic (bFGF)
- Follistatin
- Granulocyte colony-stimulating factor (G-CSF)
- Interleukin-3 (IL-3)
- Interleukin-8 (IL-8)
- Leptin
- Midkine
- Placental growth factor (PIGF)
- Platelet-derived endothelial cell growth factor (PD-ECGF)
- Platelet-derived growth factor-BB (PDGF-BB)
- Pleiotrophin (PTN)
- Progranulin
- Proliferin
- Thrombin
- Thymosin beta-4
- Transforming growth factor-alpha (TGF-α)
- Transforming growth factor-beta (TGF-β)
- Tumor necrosis factor-alpha (TNF-α)
- Vascular endothelial growth factor (VEGF)/vascular permeability factor (VPF)

Endogenous Inhibitors of Angiogenesis

- Angioarrestin
- Angiostatic steroids
- Angiostatin
- Antiangiogenic antithrombin III
- Canstatin
- Cartilage-derived inhibitor
- CD59 complement fragment
- Endostatin
- Fibronectin fragment
- Gro-beta
- Heparinases
- Heparin hexasaccharide fragment
- Human chorionic gonadotropin
- Interferon α/β/γ
- Interferon inducible protein
- Interleukin-12 (IL-12)
- Kringle 5
- Metalloproteinase inhibitors
- 2-Methoxyestradiol
- Pigment epithelial-derived factor (PEDF)
- Placental ribonuclease inhibitor
- Plasminogen activator inhibitor
- Platelet factor-4
- Prolactin 16-kd fragment
- Proliferin-related protein
- Retinoids
- Thrombospondin-1v
- Transforming growth factor-beta(TGF-β)
- Tumstatin
- Vasculostatin
- Vasostatin

Source: The Angiogenesis Foundation

ulated by genes expressed in response to hypoxia and inflammation, such as hypoxia-inducible factors (HIF) and cyclooxygenase-2 (COX-2).[5-7]

Angiogenesis inhibitory factors suppress blood vessel growth.[8,9] Some inhibitors circulate in the bloodstream at low physiological levels, while others are stored in the extracellular matrix surrounding blood vessels. A precise physiological balance exists between angiogenesis stimulators and endogenous inhibitors, such that vascular growth is normally suppressed.[9] Immediately following injury, however, angiogenic stimuli are released into the wound bed, and a shift occurs in the balance of regulators favoring vascular growth (*Figure 1*).

THE ANGIOGENESIS CASCADE

Angiogenesis occurs as an orderly cascade of molecular and cellular events in the wound bed (*Figure 2*):

1. Angiogenic growth factors bind to their receptors on the surface of endothelial cells in pre-existing venules (parent vessels).
2. Growth factor-receptor binding activates signaling pathways within endothelial cells.
3. Activated endothelial cells release proteolytic enzymes that dissolve the basement membrane surrounding parent vessels.
4. Endothelial cells proliferate and sprout outward through the basement membrane.
5. Endothelial cells migrate into the wound bed using cell surface adhesion molecules known as integrins ($\alpha_V\beta_3$, $\alpha_V\beta_5$, and $\alpha_5\beta_1$).
6. At the advancing front of sprouting vessels, enzymes known as matrix metalloproteinases (MMPs) dissolve the surrounding tissue matrix.
7. Vascular sprouts form tubular channels which connect to form vascular loops.
8. Vascular loops differentiate into afferent (arterial) and efferent (venous) limbs.
9. New blood vessels mature by recruiting mural cells (smooth muscle cells and pericytes) to stabilize the vascular architecture.
10. Blood flow begins in the mature stable vessel.

These complex growth factor-receptor, cell-cell, and cell-matrix interactions characterize the angiogenesis process, regardless of the inciting stimuli or its location in the body.

BONE MARROW-DERIVED STEM CELLS CONTRIBUTE TO ANGIOGENESIS

Stem cells harbored within adult bone marrow contribute to wound angiogenesis. These cells, known as endothelial progenitor cells (EPCs), can also be isolated in small numbers from the peripheral circulation of normal healthy adults.[10,11] Following injury, EPCs are mobilized into the circulation and they home to sites of

neovascularization where they differentiate into adult endothelial cells. Placental growth factor (PlGF), a member of the vascular endothelial growth factor (VEGF) family, and its receptor flt-1 (VEGF-R1), have been identified as regulators for EPC recruitment in angiogenesis.[12]

THE ANGIOGENESIS MODEL OF WOUND HEALING

Wound healing occurs in 3 major overlapping stages: 1) a hemostatic and inflammatory stage; 2) a proliferative stage; and 3) a remodeling stage. Although granulation is classically assigned to the proliferative stage, angiogenesis is initiated immediately upon wounding and is mediated throughout the entire wound-healing process. We have proposed an "angiogenesis model of wound healing" to more fully describe wound neovascularization.

STEP 1: Angiogenesis Initiation

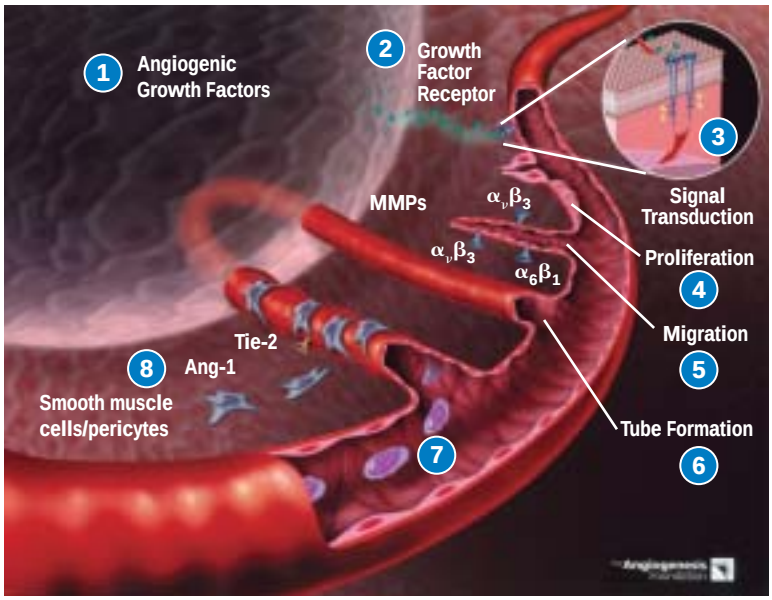
Tissue damage leads to the release of basic fibroblast growth factor (bFGF) normally sequestered within intact cells and the extracellular matrix.[13] Bleeding and hemostasis in a wound also initiates angiogenesis. Thrombin, the first clot element present in a wound, upregulates cellular receptors for VEGF and potentiates this growth factor's effects.[14] Endothelial cells exposed to thrombin also release gelatinase A, which promotes the local dissolution of basement membrane, an essential early step of angiogenesis.[15]

One of the first cells in an acute wound is the platelet. Platelets contain and release multiple growth factors, including platelet-derived growth factor (PDGF), VEGF, transforming growth factor (TGF-α, TGF-β), bFGF, platelet-derived endothelial cell growth factor (PD-ECGF), and angiopoietin-1 (Ang-1). These factors stimulate endothelial proliferation, migration, and tube formation.[16-19]

STEP 2: Angiogenesis Amplification

Wound angiogenesis is amplified by inflammation. Macrophages and monocytes release myriad angio-

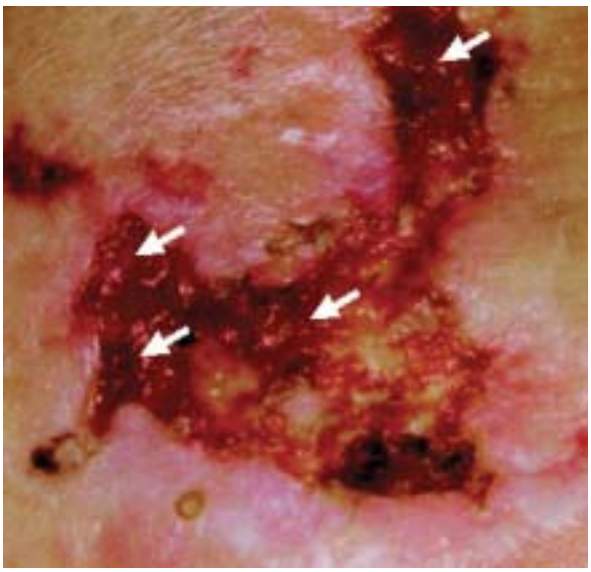
FIGURE 2 The Angiogenesis Cascade of Events



(1) Diseased or injured tissue produce and release growth factors that (2) bind to their receptors on endothelial cells, (3) activating signal transduction pathways and (4) stimulating endothelial proliferation, (5) migration, and (6) vascular tube formation. (7) Bone-marrow derived endothelial stem cells are mobilized and become incorporated into new blood vessels. (8) Stabilization of the vasculature occurs through the recruitment of smooth muscle cells and pericytes.

Source: The Angiogenesis Foundation. Copyright © 2003. All rights reserved.

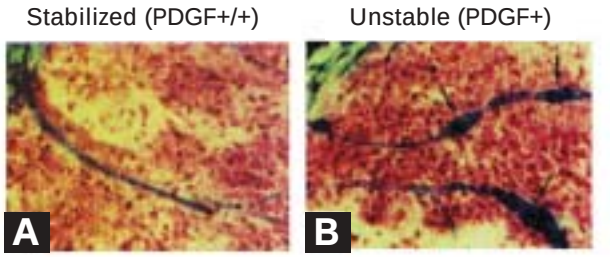
FIGURE 3 Wound Granulation



Granulation tissue in wounds represents intense angiogenesis (arrows).

Source: The Angiogenesis Clinic

FIGURE 4 PDGF Mediates Vascular Stabilization



PDGF mediates vascular stabilization in granulating tissue, creating structurally uniform blood vessels (A, wild-type mice). Deficiency of PDGF leads to abnormal, aneurysmal vasculature (B, PDGF knock-out mice).

Source: Lindahl P, Johannson BR, Leveen P, Beetscholtz D. Pericyte loss and microaneurysm formation in PDGF-B-deficient mice. *Science*. 1997;277:242-245.

TABLE 2 Angiogenesis Defects in Diabetic Wounds

- Decreased PDGF and IGF-1 in early phase of healing
- Decreased macrophage secretion of angiogenic growth factors
- Lower expression of Hox D3
- Overexpression of Ang-2
- Impaired vasa nervorum

genic factors as they marginate into the wound bed, including PDGF, VEGF, Ang-1, TGF-α, bFGF, interleukin-8 (IL-8), and tumor necrosis factor alpha (TNF-α).[20,21] Several growth factors (PDGF, VEGF, and bFGF) synergize in their ability to vascularize tissues.[22]

Proteases that break down damaged tissues further release matrix-bound angiogenic stimulators. Enzymatic cleavage of fibrin yields fibrin fragment E (FnE). This fragment stimulates angiogenesis directly, and also enhances the effects of VEGF and bFGF.[23] Expression of the inducible COX-2 enzyme during the inflammatory stage of healing also leads to VEGF production and other promoters of angiogenesis.[24]

STEP 3: Vascular Proliferation

Wound granulation becomes clinically evident as angiogenesis is sustained (*Figure 3*). Hypoxia is an important driving force for wound angiogenesis. The hypoxic gradient that exists between injured and healthy tissue leads to gene expression of HIF-1α that triggers VEGF production.[21,25] VEGF is present in both wound tissue and wound fluid.[25,26] One property of VEGF is its ability to induce edema through hyperpermeability, hence its alternate name, vascular permeability factor (VPF).[27] Hypoxia also leads to endothelial cell production of nitric oxide (NO). NO promotes vasodilation and angiogenesis to improve local blood flow.[28]

STEP 4: Vascular Stabilization

Newly forming blood vessels must be stabilized or matured. Vascular stabilization is governed by Ang-1, its receptor Tie2, and smooth muscle cells and pericytes. Binding of Ang-1 to Tie2 on activated endothelial cells leads to the production of PDGF and the recruitment of smooth muscle cells and pericytes to the newly forming vasculature.[29-31] A PDGF deficiency leads to abnormal, poorly-formed immature blood vessels (*Figure 4*).[32]

STEP 5: Angiogenesis Suppression

At the terminal stages of healing, angiogenesis is suppressed.[33] Growth factor levels decline as tissue normoxia is restored and inflammation subsides. Endogenous angiogenesis inhibitors become dominant forces. Pericytes that stabilize endothelial cells secrete an inhibitory form of activated TGF-β that impedes vascular proliferation.[34] Epidermal production of interferon-β also inhibits angiogenesis.[35] Endostatin, a cleavage product of collagen XVIII, is present surrounding the vascular basement membrane and inhibits wound vascularity, as does another molecule called vasostatin.[36,37]

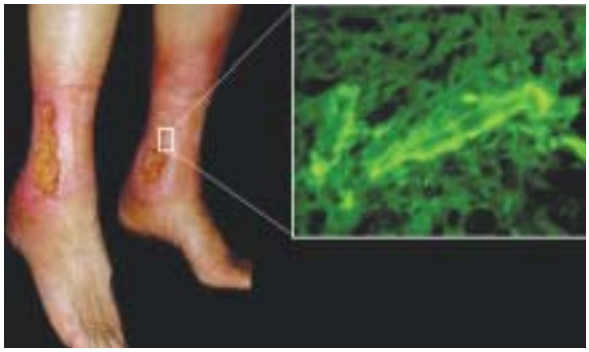
IMPAIRED ANGIOGENESIS IN CHRONIC WOUNDS

Defects in angiogenesis are present in virtually all chronic wounds. When granulation is compromised, further tissue damage results from chronic hypoxia and impaired micronutrient delivery. Specific defects have been identified in diabetic foot ulcers, venous insufficiency ulcers, and ischemic ulcers.

Diabetic Foot Ulcers

While a sensory neuropathy underlies undetected foot injury in diabetic patients, impairments to healing in the angiogenesis pathway have been identified (*Table 2*). Diabetes is associated with a reduction in the expression of growth factors and their receptors.[38-42]

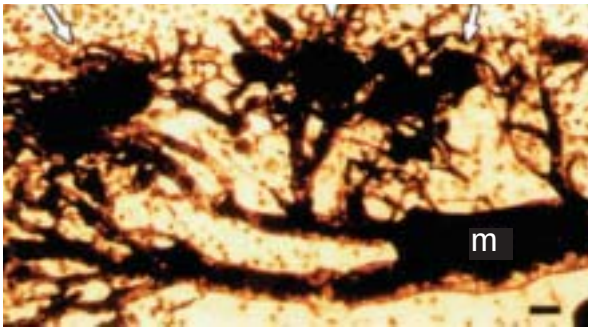
FIGURE 5 Abnormal Vessels in Venous Insufficiency Ulcers



Microvessels in the granulation bed of venous insufficiency ulcers are abnormal and contain fibrin cuffs (bright green) that compromise gas exchange and bind growth factors, sequestering them from the wound bed.

Source: Ouahes N, Phillips TJ. Leg Ulcers. *Curr Probl Dermatol*. 1995;7:109-142; and The Angiogenesis Clinic.

FIGURE 6 Glomeruloid Blood Vessels



Abnormal, non-perfusing glomeruloid blood vessels induced by VEGF (arrows) are similar to these observed in venous insufficiency ulcers (Scale bar = 50 µm).

Source: Sundberg D, Nagy JA, Brown LF, et al. Glomeruloid microvascular proliferation follows adenoviral vascular permeability factor/vascular endothelial growth factor-164 gene delivery. *Am J Pathol*. 2001;158:1145-1160.

Compared with nondiabetic subjects, levels of PDGF in diabetic wounds are decreased in the early phases of healing.[39] The release of growth factors by macrophages in diabetes is also diminished. Diabetic subjects have lower production of insulin-like growth factor-1 (IGF-1),[41] impaired expression of the angiogenic gene Hox D3,[43] and overexpression of angiopoietin-2 (Ang-2) leading to decreased vascular density.[44]

Angiogenic stimulators can be therapeutically administered to diabetic wounds to accelerate neovascularization and promote healing.[45-50] In 1997, the FDA approved the first recombinant human angiogenic growth factor, rhPDGF-BB (becaplermin, Regranex 0.01% gel, Johnson & Johnson Wound Management, Somerville, NJ), for use in promoting healing in full-thickness diabetic foot ulcers.[50] This drug speeds wound closure when used in combination with sharp debridement and good wound care practices.[51]

Diabetic neuropathy is itself associated with an impaired blood supply to the nerves, a microcirculatory network called the *vasa nervorum*.[52] Nerve angiogenesis can be stimulated using gene therapy for VEGF, and this restores blood flow to peripheral nerves, improving nerve conduction in laboratory animals.[52] Clinical studies of angiogenic gene therapy are now underway to reverse neuropathy in diabetic patients.

Venous Insufficiency Ulcers

Venous insufficiency ulcers, or venous stasis ulcers, result from incompetent valves in lower extremity veins, leading to venous stasis and hypertension, and the propensity for skin ulceration. Pathological findings associated with venous ulcers include a microangiopathy, fibrin "cuffing," and the trapping of leukocytes within the microvasculature.[53,54]

Patients with chronic venous ulcers have elevated circulating levels of VEGF.[55] This may explain the vascular permeability and increased transudation of wound fluid associated with their wounds. Biopsies of venous ulcers reveal microvessels that are surrounded by fibrin cuffs thought to compromise gas exchange (*Figure 5*).[56] These cuffs are composed of fibrin and plasma proteins, such as α-macroglobulin, extravasated from leaky capillaries.[57,58] Clinical studies have shown that $TcPO_2$ may be as much as 85% lower in venous ulcers compared with normal skin regions.[57] Hypoxia up-regulates VEGF expression, which further exacerbates vascular permeability, fibrin cuff formation, and compromised gas exchange. Growth factors are trapped within these fibrin cuffs, reducing their availability in the wound.[59,60]

Venous insufficiency ulcers do not form normal capillaries. Instead, the granulation tissue is composed of tortuous, aberrant glomeruloid-like vascular structures.[61] VEGF promotes the formation of such structures (*Figure 6*).[62] Laboratory animals treated with VEGF form glomeruloid vascular structures within 3 days and these are characterized by poor perfusion.[62] Over weeks, these reorganize into normal microvessels

capable of perfusion. In venous ulcers, the persistence of glomeruloid vessels may interfere with oxygen delivery and delay healing.

High levels of proteases are present in wound fluid and tissue from chronic venous ulcers.[63,64] These include neutrophil elastase, matrix metalloproteinases, and urokinase-type plasminogen activator. Concomitantly there are decreased levels of protease inhibitors, such as plasminogen activator inhibitor-2. Excessive protease activity may degrade growth factors and destroy granulation tissue.

Ischemic Ulcers

Arterial ulcers are caused by poor distal perfusion to the limb leading to progressive tissue hypoxia, ischemia, necrosis, and skin breakdown. Unresolved, critical ischemia may result from severe peripheral arterial disease (PAD).[65] In theory, tissue hypoxia should cause compensatory angiogenesis via a physiologic feedback loop by inducing hypoxia-induced factor alpha (HIF-1α) and angiogenic growth factors. In patients with PAD, serum levels of hepatocyte growth factor are elevated 2 times above that found in normal subjects.[66] The tissue compromise caused by severe macrovascular disease, however, may override the angiogenic response. Interindividual differences in the ability to mount angiogenesis under hypoxic conditions also exist among patients with atherosclerosis. Such variations may explain why some patients with PAD are unable to generate adequate collateral circulation, and why others are unable to heal arterial ulcers despite surgical bypass. Patients with a defective angiogenic capacity might benefit from therapeutic growth factors or other clinical methods designed to stimulate angiogenesis. In clinical trials, angiogenic healing of arterial ulcers has been achieved in patients following VEGF gene transfer[67] or autologous transplantation of bone marrow-derived endothelial progenitor stem cells.[68]

SUMMARY

Angiogenesis is a physiological process that is vital for normal wound healing. A number of factors regulate wound angiogenesis, including hypoxia, inflammation, and growth factors. The molecular and cellular events in angiogenesis have been elucidated, and defects in this process are present in chronic wounds. Based on this knowledge, new wound healing strategies are emerging to deliver growth factors to the wound bed. Surgeons and other wound-care specialists can use their knowledge of angiogenesis to identify defects and select interventions that may promote improved wound granulation and healing.

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SUPPLEMENT
CONTEMPORARY SURGERY

The Biology of PDGF and Other Growth Factors in Wound Neovascularization

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ABSTRACT Neovascularization plays a central role in wound granulation and is required for normal healing. This process, known as angiogenesis, occurs through an orderly series of molecular and cellular steps that involve the temporal actions of multiple growth factors in the wound bed. Both platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) independently initiate angiogenesis and mediate blood vessel growth and behavior in unique ways. VEGF also increases vascular permeability, while PDGF also facilitates vascular maturation. PDGF and VEGF have been shown to interact when administered together and form superior blood vessel networks compared to those generated by either factor alone. ■

Neovascularization, or angiogenesis, in the wound is central to healing and involves the growth of new capillary blood vessels. This process is clinically manifest as granulation tissue.[1-4] Wound fluid stimulates vascular endothelial cells to migrate and proliferate in vitro and induces angiogenesis in vivo.[5,6] The angiogenic process involves growth factor activation of endothelial cells, leading to proliferation, migration, tubular morphogenesis, vascular loop formation, and stabilization of vessels to form a mature vascular network.[7,8] Recent advances in wound biology have led to important insights on how growth factors mediate these processes.

The term "growth factor" refers to a broad family of proteins that promote cell proliferation and migration. At least 20 growth factors that stimulate angiogenesis have been identified, sequenced, and had their genes cloned. Among these are platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), fibroblast growth factors (FGFs), and the transforming growth factors (TGFs) (*Table 1*). The mechanisms underlying growth factor gene regulation, signal transduction, and cellular functions are have been elucidated.

GROWTH FACTORS ARE EXPRESSED IN A TEMPORAL FASHION IN WOUNDS

Angiogenesis is regulated by a physiological balance between stimulators and inhibitors of blood vessel growth present in tissues and circulating in the bloodstream. The onset of wound neovascularization reflects a shift in this regulatory balance, temporarily favoring angiogenesis stimulation over inhibition. Early wound healing responses bring a host of growth factors into the wound bed.

Studies of tissue biopsies have shown that various growth factors and their receptors appear in the wound bed with distinct temporal patterns. One day after wounding, PDGF expression is detected on the vascular endothelium of injured skin, whereas its presence is minimal in normal intact skin.[9] Between 3 to 7 days after wounding, the expression of VEGF peaks, coinciding with the clinical appearance of granulation tissue.[10] At day 5, basic FGF is expressed at its peak levels, which, by day 7 returns to baseline levels.[11]

Similar patterns in growth factor expression are seen in wound fluid. Studies of skin graft donor sites in patients undergoing reconstructive surgery showed that wound fluid contained high initial peaks of PDGF and basic fibroblast growth factor (bFGF) that decreased over 7 days (*Figure 1*).[12] By contrast, a low concentration of transforming growth factor-beta (TGF-β) was initially detected in wound fluid, but these levels gradually increased over a 1-week period. Differential growth factor expression was also observed in wound fluid from drainage tubes following mastectomy and radical neck dissection surg-

eries.[13] Basic FGF wound fluid levels were highest immediately after wounding and then declined by postoperative day 2. VEGF and TGF-β levels exhibited the inverse pattern, progressively increasing through postoperative day 6.

The nature of the orchestration and the inter-relationships between growth factors in wounds is complex and not precisely understood. How these expression patterns are altered in specific types of chronic wounds is also not known, although deficiencies in certain growth factors are observed in disease states.

ACTIVATION OF GROWTH FACTOR RECEPTORS

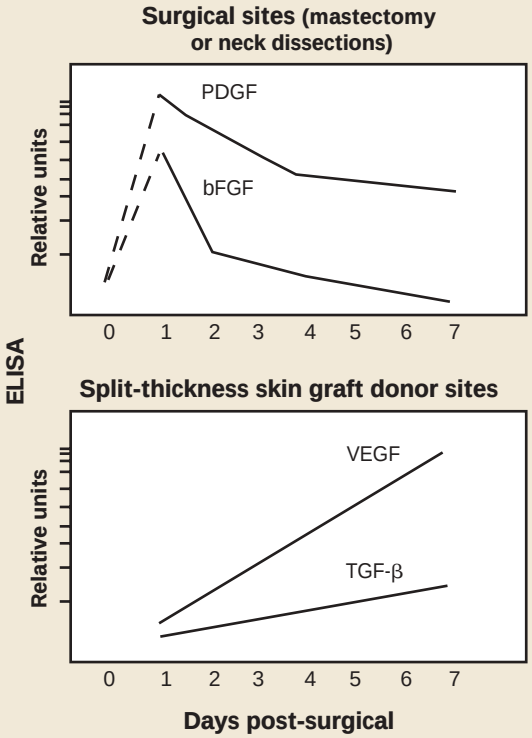
Growth factor receptors are transmembrane structures that facilitate communication from outside of the cell to its cytoplasm and nucleus. Although there are many different growth factors, some common actions are involved with growth factor signaling: 1) binding of the growth factor peptide to its receptor; 2) receptor activation by phosphorylation of the intracellular portion of the receptor; and 3) signal transduction through molecular pathways in the cell cytoplasm to the nucleus. Specific interactions between PDGF and VEGF and their receptors merit further discussion.

The PDGFs represent a family of growth factors consisting of 2 polypeptide chains (A and B) which form dimers, or protein pairs: PDGF-AA, -AB, and -BB. All 3 PDGF isoforms are present in human platelets. The PDGF receptor has a transmembrane structure with extracellular ligand-binding domains and intracellular tyrosine kinase domains. Two PDGF receptors exist, R-α and R-β, with each possessing different specificities for their ligands. The B subunit of PDGF can affiliate with either the PDGFR-α or PDGFR-β subunit, while the A subunit of PDGF can interact only with the PDGFR-α.[14] PDGF-BB can activate any PDGF receptor homodimer or heterodimer. Therefore, therapeutic application of PDGF-BB (becaplermin) can activate both configurations of its receptor.

VEGF also has multiple family members (VEGF-A, VEGF-B, VEGF-C, VEGF-D). VEGFs interact with high-affinity tyrosine kinase receptors, of which the best known are VEGF-R1 (or fms-like tyrosine kinase, Flt-1) and VEGF-R2 (or human kinase insert domain-containing receptor, KDR, and its mouse homologue, Flk-1). These receptors are selectively expressed on angiogenic endothelial cells.[15] The VEGF-R2 (KDR/Flk-1) is thought to be primarily responsible for transducing the signal for endothelial chemotaxis during VEGF-driven angiogenesis. VEGF binding to KDR/Flk-1 is mediated by a co-receptor called neuropilin-1 (Nrp-1), a non–tyrosine kinase that potentiates VEGF-KDR binding. Antagonism of Nrp-1 inhibits VEGF-driven endothelial cell migration. Nrp-1 is abundantly expressed in the wound neovasculature. Treatment of experimental wounds with antibodies against Nrp-1 led to a 67% decrease in vascular density (P = 0.0132).[16] These findings show that Nrp-1 and VEGF play an important role in regulating wound angiogenesis.

Studies of growth factor receptors in normal wound tissue show a temporal appearance during healing.[16] The expression of mRNA levels for VEGF-R1

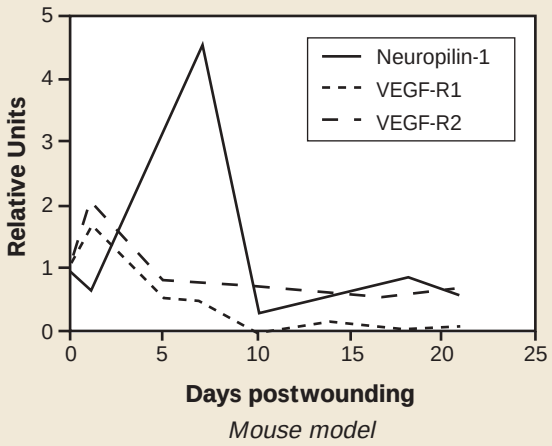
FIGURE 1 Angiogenic Factors in Human Wound Fluid



Multiple growth factors are expressed temporally in human wound fluid.

Source: Vogt PM, Lehnhardt M, Wagner D, et al. Determination of endogenous growth factors in human wound fluid: temporal presence and profiles of secretion. *Plast Reconstr Surg*. 1998; 102:117-123. Nissen NN, Polverini PJ, Koch AE, et al. Vascular endothelial growth factor mediates angiogenic activity during the proliferative phase of wound healing. *Am J Pathol*. 1998;152:1445-1452.

FIGURE 2 Temporal Expression of Growth Factor Receptors During Healing (Northern Blot [mRNA] Analysis)



Cellular receptors for growth factors are temporally expressed in experimental wounds.

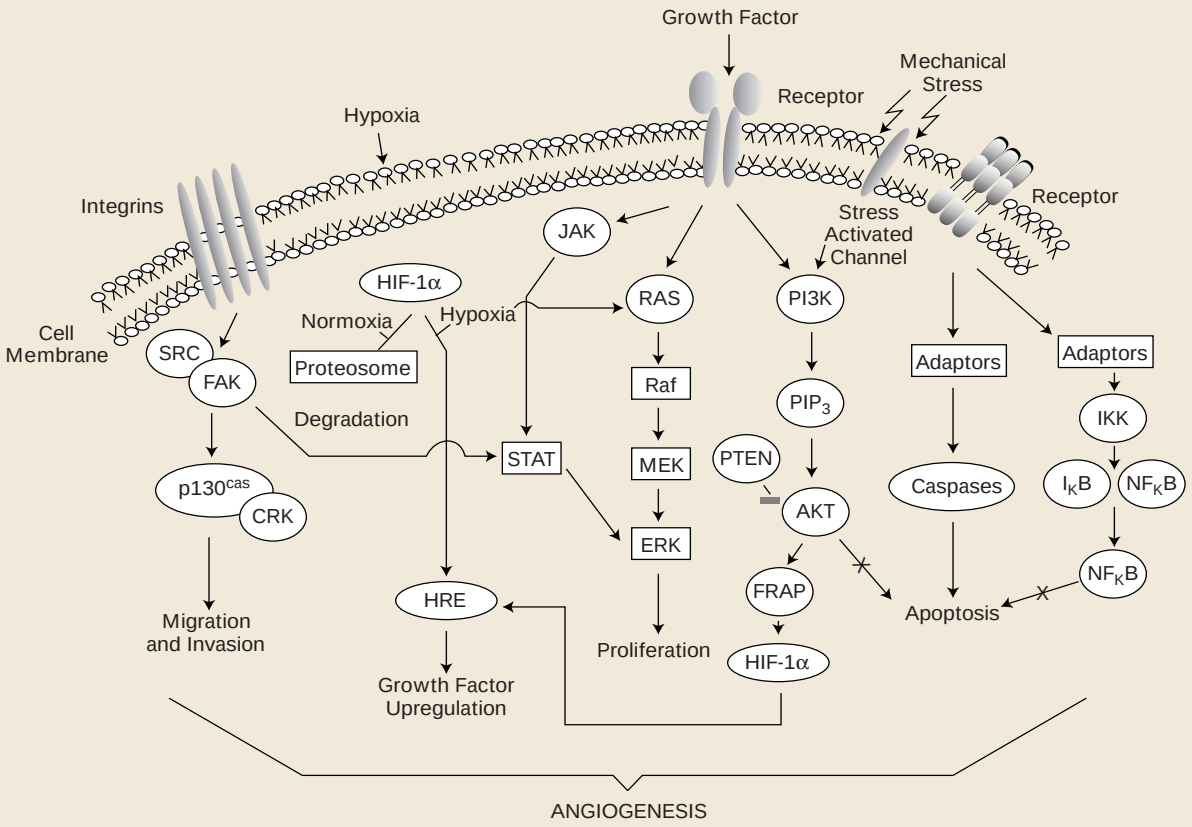
Source: Matthies AM, Low QE, Lingen MW, DiPietro LA. Neuropilin-1 participates in wound angiogenesis. *Am J Pathol*. 2002;160:289-296.

and VEGF-R2 rises modestly during the first day postwounding, then gradually declines from day 2 to 10. By contrast, Nrp-1 mRNA levels rise abruptly after injury and peak at day 6 (*Figure 2*). By day 10, mRNA levels for all 3 receptors decline to or are present below baseline levels. These data illustrate the complexity of both ligand and receptor expression in normal wound healing. Defects in either feature may impair wound angiogenesis.

GROWTH FACTORS TRANSMIT INTRACELLULAR SIGNALS TO THE ENDOTHELIAL CELL NUCLEUS

Once a growth factor binds to its cell surface receptor, a series of signals are transferred from the cell membrane to the nucleus.[17] PDGF activates its receptors by forming dimers. These dimers bind to PDGF receptors, which themselves must dimerize to activate signal transduction by phosphorylation of tyrosine kinase. The intracellular phosphorylation of the receptor modifies protein signals within the cell and instructs the nucleus to begin DNA

FIGURE 3 Growth-Factor Mediated Signal Transduction Pathways



Growth factors bind to their cell-surface receptors and activate a complex series of signal transduction pathways.

Source: The Angiogenesis Foundation

transcription. Docking or adaptor proteins can also bind to the phosphorylated receptor site to transmit signals to the nucleus via alternate pathways. Unbound PDGF receptors remain unpaired and inactive.

Multiple signal transduction pathways in vascular endothelial cells control wound nevascularization (*Figure 3*). In the case of either VEGF or PDGF, receptor tyrosine kinases are responsible for signal transduction.[18] Other pathways are activated by wound hypoxia. Wounds are hypoxic during the early phases of repair.[19] Hypoxia stimulates VEGF production and angiogenesis through induction of the gene for hypoxia-inducible factor-1 alpha (HIF-1α) as well as through other distinct pathways involving Ras/Raf/ERK molecules and the gene for early growth response-1 (EGR-1), a transcription factor produced at the sites of tissue injury.[20-22] These pathways all promote angiogenesis.

Vascular endothelial cells can also be stimulated to proliferate through the MAPK/ERK (mitogen-activated protein kinase/extracellular signal-regulated kinase) and the PI3-K/Akt (phosphatidylinositol 3-kinase/Akt) pathways.[23] Cross-talk can also take place between different signal transduction pathways used by growth factors.[24] For example, the "downstream portion" of the MAPK/ERK pathway acts as the convergence point of both VEGF-initiated and TNF-α-initiated signaling pathways, whereas the "upstream signals" for both growth factors remain distinct.

Cell surface adhesion molecules called integrins facilitate endothelial cell migration. These integrins also influence growth factor-mediated signal transduction.[25,26] Studies of endothelial cells growing in culture have demonstrated that VEGF induces phosphorylation of focal adhesion kinase (FAK) which induces the coupling of FAK to the angiogenic integrin $\alpha_V\beta_5$. This FAK/$\alpha_V\beta_5$ complex mediates endothelial cell migration.[27]

GROWTH FACTORS IMPEDE APOPTOSIS

Apoptosis, the process of programmed cell death, is mediated in normal endothelial cells by caspase-9 via the death receptor and the inflammatory IκB/NFκB cascade (*Figure 3*).[28] Stimulation of angiogenesis inhibits apoptosis through the expression of the integrin $\alpha_V\beta_3$ on endothelial cells. This integrin binds to vitronectin in the extracellular matrix and induces endothelial cells to express the Bcl-2 gene.[29] Bcl-2 is a survival factor that prevents apoptosis.

VEGF also induces Bcl-2 in endothelial cells as well as survivin, another inhibitor of apoptosis.[30,31] Laboratory studies have shown that VEGF protects and may even rescue endothelial cells from senescence.[32] Another growth factor, angiopoietin-1 (Ang-1), inhibits endothelial cell apoptosis by up-regulating survivin, activating the PI-3 kinase/AKT pathway, and inhibiting Smac release from the mitochondria.[33]

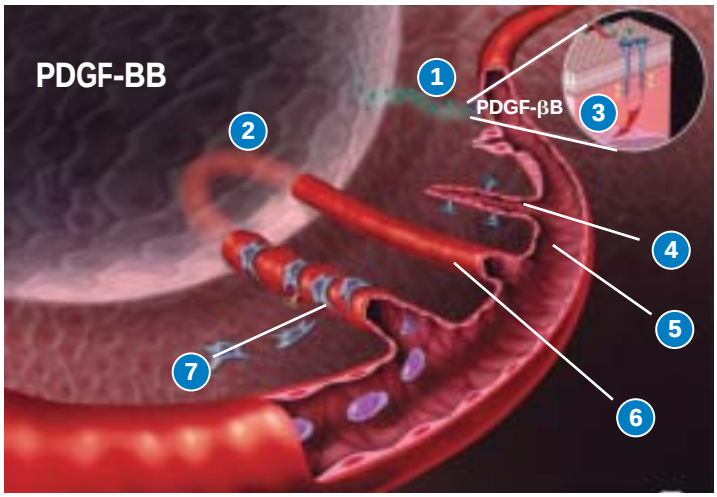
MECHANISMS FOR GROWTH FACTOR-STIMULATED ANGIOGENESIS

PDGF

All isoforms of PDGF stimulate angiogenesis, but PDGF-BB is more highly angiogenic than PDGF-AA. The mechanisms of PDGF-driven angiogenesis are multifold (*Figure 4*).[34]

PDGF-BB binds to the PDGF-β receptor on endothelial cells, but only cells from microvessels and not larger vessels undergo increased DNA synthesis.[35,36] PDGF-BB also induces endothelial migration,[34,37]

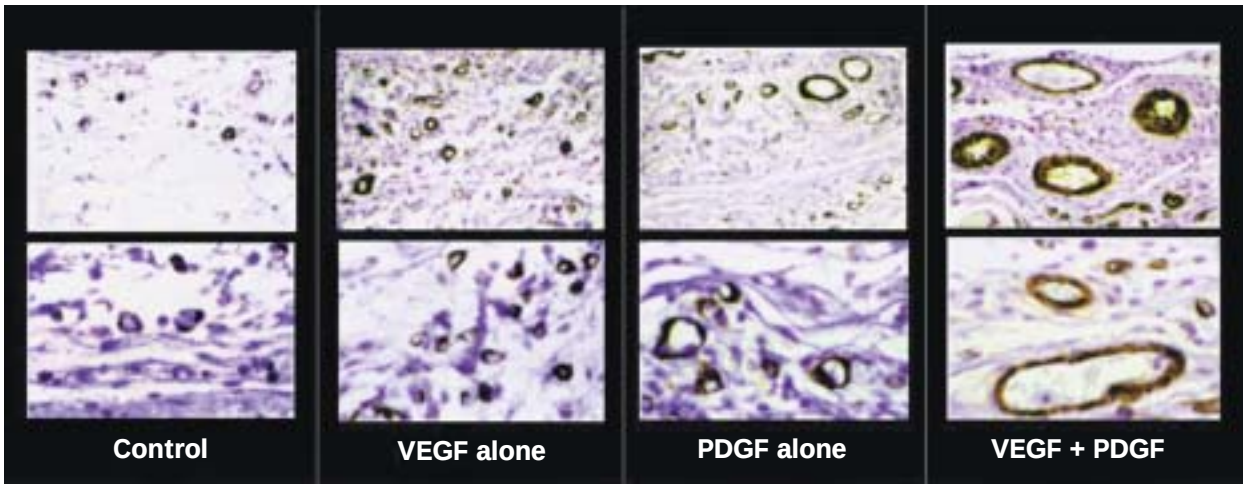
FIGURE 4 PDGF-BB Mediates Angiogenesis



PDGF-BB mediates angiogenesis at multiple points in the angiogenesis cascade by: (1) binding to its receptor PDGF-βR on vascular endothelial cells; (2) inducing production of other growth factors, VEGF and bFGF; (3) activating intracellular signal transduction pathways; 4) stimulating endothelial proliferation; 5) promoting endothelial migration; 6) facilitating vascular tube formation; 7) and recruiting smooth muscle cells and pericytes to stabilize the newly formed vasculature.

Source: The Angiogenesis Foundation. Copyright © 2003. All rights reserved.

FIGURE 5 Growth Factor Collaborations

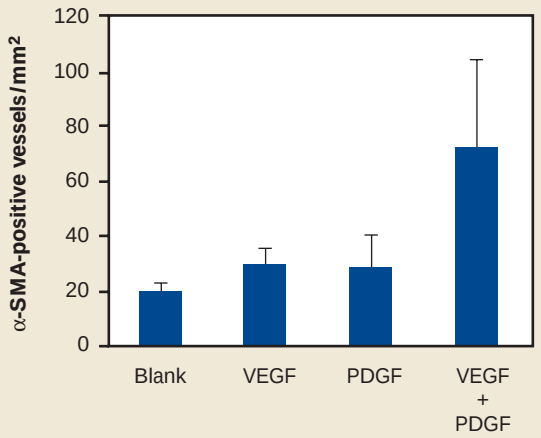


The combination of two growth factors, VEGF and PDGF, induces formation of larger and more mature vessels. Cross-sectional areas of blood vessels at 2 and 4 weeks were measured from hematoxylin and eosin-stained tissue sections (Magnification = 1,000 X).

Source: Richardson TP, Peters MC, Ennett AB, Mooney DJ. Polymeric system for dual growth factor delivery. *Nat Biotechnol*. 2001;19:1029-1034. Reprinted with permission of the Nature Publishing Group.

an activity not seen with PDGF-AA.[37-39] Experiments using time-lapse video microscopy have shown that PDGF-BB elicits not only the migration of single endothelial cells, but also the movement of entire vascular cord-like structures.[37] This vascular migration reflects the repair response when confluent endothelial cell monolayers are wounded in tissue culture. Vascular migration is also facilitated by matrix metalloproteinases (MMPs)[40] and angiogenic integrins ($\alpha_V\beta_3$, $\alpha_V\beta_5$, $\alpha_5\beta_1$).[41] The integrin $\alpha_V\beta_3$ is detected in proliferating microvessels on day 3 after injury but disappears after granulation tissue is matured.[25] PDGF-BB induces integrin expression.[41]

FIGURE 6 Growth Factor Synthesis



Synergistic effects of PDGF and VEGF following dual release from a polymer scaffold in experimental granulation tissue α-smac stains pericytes and smooth muscle cells.

Source: Richardson TP, Peters MC, Ennett AB, Mooney DJ. Polymeric system for dual growth factor delivery. *Nat Biotechnol*. 2001;19:1029-1034.

Vascular tube formation is promoted by PDGF-BB via blockade of the cell cycle from G0 to G1 which enables a 3-dimensional conformational change to occur.[42] Finally, PDGF-BB mediates vascular maturation by recruiting mural cells (pericytes and smooth muscle cells).[7,43-45] The PDGF-β receptor is expressed on pericytes, which require the growth factor ligand for normal development.[43] In PDGF-B knock-out mice, angiogenic capillaries are devoid of pericytes and retain an immature phenotype. By contrast, in wild-type mice, PDGF-Rβ-expressing pericytes proliferate and migrate along PDGF-B-expressing endothelial sprouts. PDGF-BB is therefore a pluripotent angiogenic growth factor.

VEGF

VEGF stimulates angiogenesis by inducing cell proliferation and vascular hyperpermeability, and by promoting vascular survival.[46] Immediately after

wounding, exposure of the endothelium to thrombin causes up-regulation of the receptors VEGF-R1 and VEGF-R2.[47,48] VEGF causes perivascular mural cells (pericytes and smooth muscle cells) to detach from the endothelium of parent vessels. This vascular destabilization requires the coordinated actions of another growth factor, angiopoietin-2 (Ang-2).[45] Numerous new "daughter" vessels then form from a single "parent" blood vessel.[46] Vascular sprouting and endothelial migration are facilitated by integrins and the secretion of MMPs.[40,41,49] VEGF also renders microvascular endothelial cells hyperpermeable to plasma proteins and circulating macromolecules.[13,49] Wound edema and excessive exudation may be caused by these VEGF-mediated effects.[50]

GROWTH FACTOR COLLABORATIONS

Multiple growth factors are expressed during wound healing, and they collaborate and synergize with one another during angiogenesis. The interaction between PDGF and VEGF has been studied in wounds using polymers designed for the controlled delivery of multiple growth factors in subcutaneous tissue.[8] The sustained release of VEGF protein increased vascular density, but the microvessels were of small caliber and immature in phenotype. Due to the properties of VEGF, the tissue surrounding the polymer became edematous. When PDGF was co-released with VEGF, striking changes became evident. Microvascular density increased with striking enhancement of vessel maturity, as reflected by increased cross-sectional area of blood vessels and histological evidence for more smooth muscle cells and pericytes surrounding the neovasculature (*Figures 5 and 6*).[8]

Similar results were observed using non-obese diabetic mice subjected to ischemic limb injury following ligation of the femoral vein and artery.[8] Empty polymer scaffolds (containing no growth factors) were implanted at the site of ligation and only sparse, mostly immature new blood vessels were observed. By contrast, the dual delivery of both PDGF and VEGF proteins in the same location resulted in a statistically significant increase in the density of mature blood vessels compared with either growth factor delivered alone (P <0.05).[8] These data suggest that PDGF and VEGF are 2 important growth factors involved in wound granulation. The delivery of exogenous therapeutic growth factors may be a rational approach to accelerating wound angiogenesis and healing.

CONCLUSION

Growth factors are critical mediators of wound neovascularization expressed during healing in a temporal, orchestrated fashion. By initiating complex signaling pathways in vascular endothelial cells, single and multiple growth factors induce DNA transcription and activate cells to undergo proliferation, migration, tube formation, and vascular maturation. PDGF-BB and VEGF are among the most critical of more than 20 known angiogenic growth factors, because they induce blood vessel growth and guide vascular maturation. Both PDGF and VEGF also promote vascular survival. Exogenous growth factors, delivered as single agents or in combination, may accelerate wound neovascularization and promote more effective healing.

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SUPPLEMENT
CONTEMPORARY SURGERY

Therapeutic Angiogenesis: Using Growth Factors to Restore Circulation in Damaged Tissues

WILLIAM W. LI, MD; VINCENT W. LI, MD

ABSTRACT Impaired circulation is an underlying pathological feature in peripheral arterial disease (PAD), ischemic heart disease, and chronic wounds. Growth factor therapy is an emerging treatment modality that enhances tissue vascularization (granulation), improves local circulation (collateralization), and promotes healing and regeneration. Both recombinant human growth factor proteins and their gene therapy are in human clinical trials to treat limb and myocardial ischemia, and to heal chronic wounds. Topical recombinant human platelet-derived growth factor-BB is now part of the standard of care for treating diabetic foot ulcers. ■

Circulatory impairment is a major cause of morbidity and mortality in the United States, afflicting more than 24 million patients with peripheral arterial disease (PAD), coronary artery disease (CAD), or chronic wounds. While the management of macrovascular disease in these conditions remains the mainstay of therapy, the microvasculature is now a new focus of treatment. "Therapeutic angiogenesis" is defined as the use of biologic agents, bioactive devices, or environmental conditions to stimulate the formation of new blood vessels in vulnerable tissues and organs.[1] The goal of this modality is to restore perfusion, reverse ischemia, and to accelerate repair.

New blood vessels can be induced to form through 4 known mechanisms: 1) by sprouting from pre-existing venules (classical angiogenesis); 2) by dividing single capillaries into multiple branches (intussusceptive microvascular growth); 3) by recruiting vascular stem cells from bone marrow (adult vasculogenesis); or 4) through the remodeling of preexisting arterioles into larger functional arteries (arteriogenesis).[2-5] These related processes are mediated by proteins known as growth factors, and their cellular receptors present within damaged tissues.

GROWTH FACTORS ACTIVATE ANGIOGENESIS

The body's physiological response to injury relies upon hemostasis, inflammation, and the release of growth factors by damaged tissues. Growth factors are also elaborated by platelets, monocytes, macrophages, vascular endothelial cells, and disrupted extracellular matrix. At least 20 growth factors are known to stimulate angiogenesis, and 7 of them—platelet-derived growth factor (PDGF-BB), vascular endothelial growth factor (VEGF), fibroblast growth factors 1, 2, and 4 (FGF1, FGF2, FGF4), keratinocyte growth factor-2 (KGF-2), and transforming growth factor-beta (TGF-β)—have been studied in clinical trials (*Table 1*).

THERAPEUTIC DELIVERY OF GROWTH FACTORS

Two main strategies are being developed for therapeutic angiogenesis using growth factors.[6]

Recombinant Growth Factor Proteins

With recombinant techniques, human growth factor genes can be inserted into yeast cells that then secrete growth factor proteins into industrial fermentation vats. The growth factor proteins are extracted, purified, and formulated into pharmaceutical grade products. The high purity, yield, and the reliability of production are major advantages of recombinant growth factors. Their limitations include a short half-life, temperature sensitivity, and the need for refrigerated storage.

Gene Therapy

Human genes encoding growth factors can be produced as plasmids to create so-called "naked DNA"

for gene transfer to tissues. Alternatively, the genes can be incorporated into non-pathogenic viral vectors. Gene-based therapies offer the theoretical advantage of sustained local expression of growth factors to target tissues.[7]

CLINICAL TRIALS OF GROWTH FACTOR THERAPY

Clinical trials of growth factor therapy are underway for PAD, ischemic heart disease, and chronic wounds. The following is an overview of key clinical data from select trials.

PAD

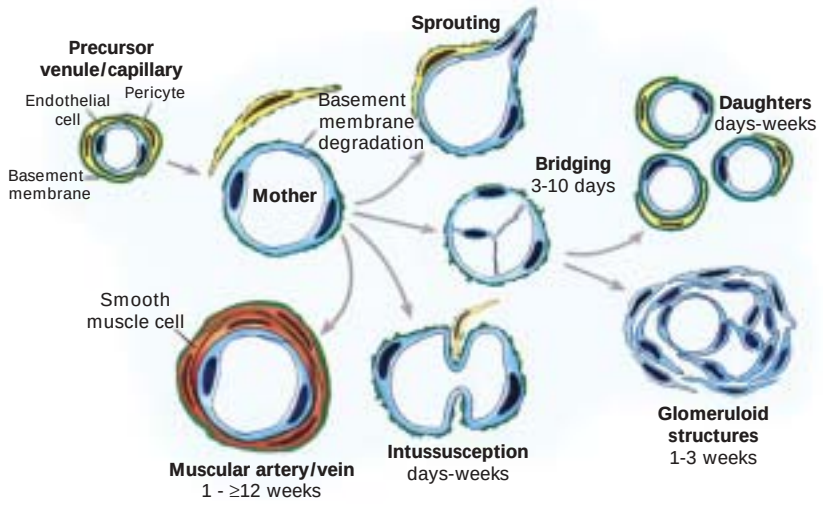
At least 10 million patients in the United States suffer from PAD, a condition characterized by atherosclerotic narrowing or occlusion of the distal arteries. Five million of these patients suffer from exercise-induced leg pain, or intermittent claudication, caused by insufficient blood flow to the lower extremities. While surgical revascularization and angioplasty are effective interventions for many patients, up to 35,000 limb amputations are still performed each year due to life-threatening critical limb ischemia, arterial ulceration,

TABLE 1 Growth Factors or Their Genes in Clinical Development for Therapeutic Angiogenesis

Growth Factor	Protein	Gene Therapy	Developer	Indication
FGF1	Yes		Fulda Medical Center	CAD
		Yes	GenCell/Aventis	PAD
FGF2	Yes		Chiron Corporation	PAD
FGF4		Yes	Schering AG	CAD, PAD
KGF-2	Yes		Human Genome Sciences	Wounds
TGF-β	Yes		Genzyme Surgical	Wounds
VEGF	Yes		Genentech	PAD
		Yes	Vascular Genetics Inc	CAD, PAD
		Yes	GenVec/Pfizer	CAD
PDGF-BB†	Yes		Chiron/Ortho-McNeil	Wounds

† FDA-approved in 1997

FIGURE 1 Angiogenic Pathways



Angiogenic blood vessels activated by growth factors undergo multiple morphological pathways to generate new capillary blood vessels and arterioles.

Source: Pettersson A, Nagy JA, Brown LF, et al. Heterogeneity of the angiogenic response induced in different normal adult tissues by vascular permeability factor/vascular endothelial growth factor. *Lab Invest*. 2000;80:99-115.

and gangrene. Therapeutic angiogenesis is being developed to achieve limb salvage for these situations.

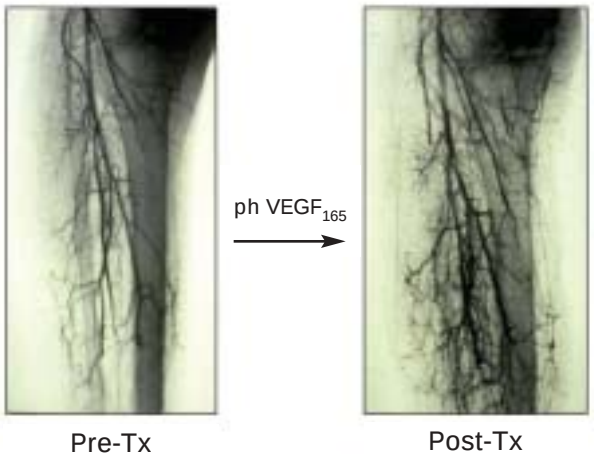
phVEGF$_{165}$

phVEGF$_{165}$ is a non-viral gene therapy using a plasmid encoding the gene for human VEGF$_{165}$. This "naked DNA" approach was first used by Isner and colleagues in Boston to demonstrate proof-of-concept of therapeutic angiogenesis in a patient with critical limb ischemia (*Figure 2*).[8] A follow-up study delivered phVEGF$_{165}$ to 10 limbs in 9 patients with severe PAD, ischemic ulcers and/or rest pain.[9] All patients had baseline ankle-brachial index (ABI) measurements <0.6 and/or toe-brachial index (TBI) <0.3. The phVEGF$_{165}$ (2,000 µg) was injected into the calf or distal thigh at entry into the study, followed by a second identical injection 4 weeks later. VEGF gene expression was observed by transient rises in serum VEGF levels, and this correlated with the development of new collateral vessels (200 to >800 µm diameter) in the treated limb, as demonstrated by digital subtraction angiography. Serial magnetic resonance angiography showed improved distal blood flow in 8 of 10 limbs. The ABI improved significantly from 0.33 to 0.48 (P = 0.02). Exercise tolerance increased in 5 of 5 patients who were able to perform a graded treadmill test, and pain-free walking time at 13 weeks was improved by 152% from baseline following gene therapy (P = 0.43). Ischemic ulcers completely healed or were clinically improved in 4 of 7 limbs, leading to limb salvage in 3 patients who had previously been recommended for below the knee amputation (*Figure 3*). Overall, phVEGF$_{165}$ was well-tolerated, with side effects limited to mild lower extremity edema, consistent with the permeability increasing activity of VEGF. Further studies of phVEGF$_{165}$ are planned.

rhFGF2

A recombinant form of FGF-2 (basic FGF) has been studied in patients with moderate-to-severe intermittent claudication. The multicenter, randomized, double-blind, placebo-controlled study called TRAFFIC (Therapeutic Angiogenesis with FGF-2 for Intermittent Claudication) delivered an intra-arterial infusion of rhFGF-2 (30 µg/kg) to both legs of 190 patients.[10] Patients in this 3-arm trial received either 1 dose of rhFGF-2 (day 1), or 2 doses (day 1 and day 30), or a placebo injection. At day 90, patients treated with a single dose of rhFGF-2 showed a statistically signifi-

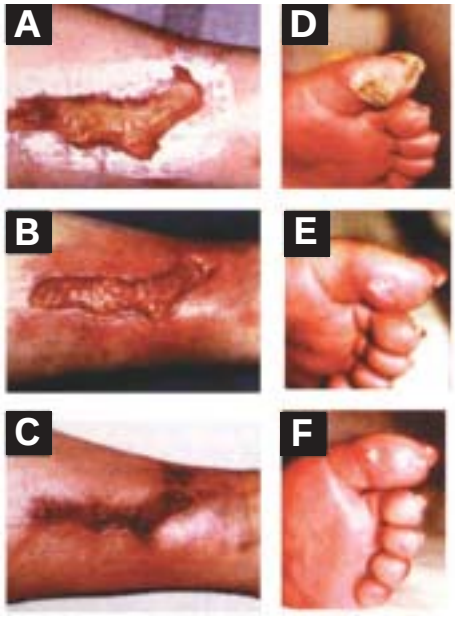
FIGURE 2 phVEGF$_{165}$ Gene Transfer for PAD



New collateral vessels are evident in the limb of a 71-year-old patient with severe PAD 1 month following gene transfer with phVEGF$_{165}$.

Source: Isner JM, Pieczek A, Schainfeld R, et al. Clinical evidence of angiogenesis following arterial gene transfer of phVEGF165. *Lancet*. 1996;348:370-374. Reprinted with permission of Elsevier.

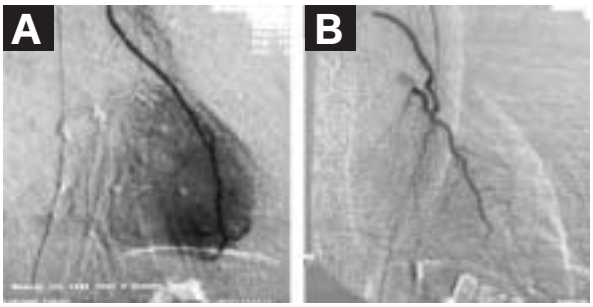
FIGURE 3 Limb Salvage with phVEGF$_{165}$ Gene Transfer



Limb salvage was achieved following phVEGF$_{165}$ gene transfer in a 33-year-old woman with critical limb ischemia, a non-healing calf wound, and ischemic great toe. Healing of the ischemic wound (A through C), and reversal of toe gangrene (D through F) was observed over a 3-month period.

Source: Baumgartner I, Pieczek A, Manor O, et al. Constitutive expression of phVEGF165 after intramuscular gene transfer promotes collateral vessel development in patients with critical limb ischemia. *Circulation*. 1998;97:1114-1123.

FIGURE 4 Coronary Angiogenesis Induction



Coronary angiogenesis is induced following myocardial injections of FGF1 in a patient with 3-vessel coronary artery disease, as evidenced by the dark blush of dye seen on angiography at 12 weeks (A). By comparison, heat-activated FGF-1 injected into another patient did not induce angiogenesis (B).

Source: Schumacher B, Pecher P, von Specht BU, Stegmann TH. Induction of neoangiogenesis in ischemic myocardium by human growth factors. *Circulation*. 1998;97:645-650.

cant increase in peak walking time (PWT) compared with placebo (P = 0.026), but the difference was not sustained at day 180. Compared with baseline values, a positive trend was seen at day 90 toward improvement in PWT following rhFGF-2 treatment. Positive trends were observed for ABI improvement and quality-of-life measures.

NV1FGF

NV1FGF is a non-viral gene therapy in which a plasmid encodes the gene for human FGF-1 (acidic FGF). A multicenter Phase I study enrolled 51 patients with severe, unreconstructible lower-extremity ischemia with rest pain or tissue necrosis.[11] NV1FGF was injected directly into thigh and calf muscle at various single (0.5, 1, 2, 4, 8, and 16 mg) and repeat (2 x 0.5 mg, 2 x 4 mg, 2 x 8 mg) doses. NV1FGF treatment appeared to be well-tolerated with no serious associated adverse events reported. No increase in serum FGF1 was observed, suggesting localization of gene expression. At 12 weeks, arteriography clearly showed visible new collateral blood vessels in the limbs of 33% of patients compared with pre-treatment angiograms. NV1FGF significantly reduced ischemic pain (P <0.001), increased $TcPO_2$ (P <0.01), and improved ABI scores (P <0.01) at 6 months. Healing of ischemic leg ulcers was observed in most patients, and aggregate ulcer size decreased significantly compared with pre-treatment values (P <0.01).[11]

At 6 months, 4 (7.8%) patients had died, 8 (15.7%) were alive with amputation required, and 39 (76.5%) were alive without requiring amputation.[12] Historical comparison of 6-month outcomes in similar PAD patients under standard care showed a death rate of 20%, a rate of patients alive with amputation of 35%, and only 45% of patients alive without amputation.[12] A Phase 2 study of NV1FGF is underway.

Ischemic Heart Disease

Twelve million patients in the United States have coronary artery disease, 7 million of whom suffer from angina pectoris.[13] More than 1 million patients will have a new or recurrent heart attack each year, and 40% will die as a result. While coronary artery bypass grafting and percutaneous coronary angioplasty are effective interventions, not all patients are suitable candidates for these procedures. Angiogenic growth factors and their gene therapies are being developed to treat ischemic heart disease.

rhFGF1

Recombinant human FGF-1 (acidic FGF) was produced from *Escherichia coli* at the Fulda Medical Center, Germany. After its angiogenic activity was confirmed in ischemic rat hearts, the growth factor was studied in 20 patients undergoing elective coronary bypass surgery for 3-vessel CAD.[14] FGF1 (0.01 mg/kg) was directly injected into the myocardium near the anastomosis of the internal mammary artery or left anterior descending artery after surgical vessel attachments were completed. A control group of patients underwent similar surgery, but received injections of heat-denatured, biologically inactive FGF-1. At 12 weeks post-surgery, angiography was performed and digital gray-value analysis was used to assess coronary angiogenesis. In all patients treated with biologically active FGF-1, an accumulation of contrast material was observed around the growth factor-injection site extending 3-4 cm around the anastomoses, indicative of new blood vessel growth (*Figure 4*). Capillary networks were seen sprouting from the coronary artery nearest the injection site into the myocardium. This was not seen in patients treated with inactivated FGF-1. Quantitative analysis showed a two- to three-fold increase in local blood flow in active FGF-1-treated but not control (inactive FGF-1-treated) hearts.

This landmark 1996 study was the first human clinical trial of therapeutic angiogenesis for ischemic myocardium. A 3-year clinical follow-up demonstrated persistence of the angiographic findings in treated but not control patients.[15] Echocardiography showed that the left ventricular ejection fraction was more greatly improved in active FGF-1-treated patients (13.5% increase) versus control patients (7.9% increase). There was also greater improvement in the New York Heart Association classification in treated patients over 3 years.

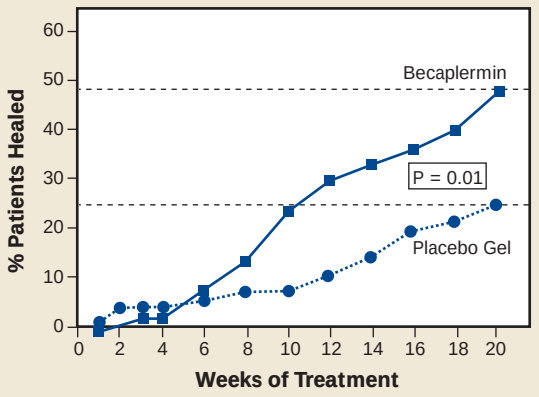
$Ad_{GV}VEGF121.10$

$Ad_{GV}VEGF121.10$ is a form of gene therapy in which a replication-deficient adenovirus encodes $VEGF_{121}$. In a randomized, controlled Phase 2 trial involving 71 patients with severe CAD, 36 patients received an intramyocardial injection of $Ad_{GV}VEGF121.10$ using a mini-thoracotomy, whereas a control group (n = 35) received the best standard of care (no injection).[16] The primary efficacy endpoint was time required to achieve an additional 1-mm ST-segment depression on EKG on an exercise treadmill, reflecting the change in myocardial reserve before ischemia. At 12 weeks, there was a trend toward improvement in the $Ad_{GV}VEGF121.10$-treated group (24 seconds), which increased to statistical significance at 26 weeks (1.1 minutes) compared with control patients (P = 0.024). At 26 weeks, $Ad_{GV}VEGF121.10$-treated patients experienced a 1.5-minute improvement in time-to-angina with exercise compared with baseline measures (P = 0.002), as well as a statistically significant increase in total exercise time compared with control groups (P = 0.014). Significant improvements were also observed in quality-of-life assessments at 6, 12, and 26 weeks using the Seattle Angina Questionnaire (SAQ) and the Canadian Cardiovascular Society (CCS) Angina Class Questionnaire. In 4 patients, serious cardiac events were associated with the mini-thoracotomy/$Ad_{GV}VEGF121.10$ injection. On the basis of positive clinical data and safety considerations, $Ad_{GV}VEGF121.10$ with delivery of the gene by cardiac catheterization is being further studied.

Ad5-FGF4

Ad5-FGF4 is a gene therapy in which the human gene encoding FGF4 is incorporated into an adenovirus. A randomized, double-blind, placebo-controlled Phase 1/2 trial called AGENT (Angiogenic GENe Therapy) was conducted in 79 patients with chronic stable angina (CCS class 2 or 3).[13] Sixty patients received single intracoronary infusions of Ad5-FGF4 at 5 ascending doses (3.3×10^8 to 10^{11} viral particles in half log increments), and 19 patients received a placebo infusion. Approximately 87% of Ad5-FGF4 was extracted by the heart on first-pass in the circulation, and no growth-factor protein was detected in the plasma, urine, or semen of patients. Overall, exercise treadmill testing (ETT) at 4 and 12 weeks showed a greater improvement in Ad5-FGF4-treated patients. A statistically significant improvement was observed between placebo-treated patients and the subgroup of 50 patients with baseline ETT ≤10 minutes (more severe disease) at 4 weeks (0.6 vs 1.6 minutes, P = 0.01) and 12 weeks (1.27 versus 1.86 minutes, P = 0.047), respectively. In this subgroup, the time to angina with exercise was significantly improved at 4 weeks (1.7 minutes) compared with placebo-treatment (0.7 minutes, P = 0.003). Ad5-FGF4 appeared safe and well-tolerated up to 399 days after treatment, with the rare occurrence of transient fever and mild, reversible elevation of liver enyzmes. A Phase 3 trial (AGENT 3) is in progress in the United States and Europe.

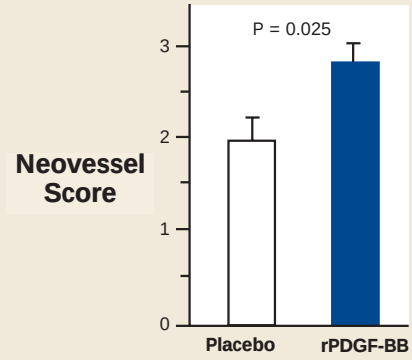
FIGURE 5 Treatment of Diabetic Foot Ulcers: Becaplermin Versus Placebo

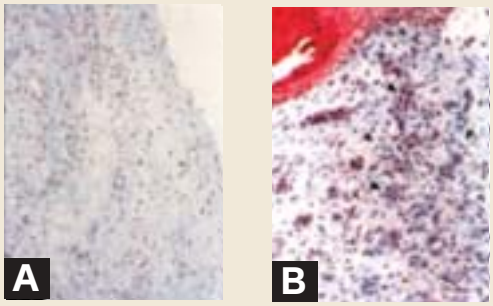


Becaplermin (recombinant human platelet-derived growth factor-BB 0.003%) increases the incidence of complete closure of diabetic foot ulcers compared with placebo-treated subjects.

Source: Steed DL. Clinical evaluation of recombinant human platelet-derived growth factor for the treatment of lower extremity diabetic ulcers. Diabetic Ulcer Study Group. *J Vasc Surg*. 1995;21:71-81.

FIGURE 6 Treatment of Pressure Ulcers Using Becaplermin (rhPDGF-BB)





Pressure ulcers in patients treated with rhPDGF-BB (0.01%) exhibit significantly increased angiogenesis during healing (B, and blue bar), compared with wounds treated with placebo gel (A, and white bar).

Source: Pierce GF, Tarpley JE, Allman RM, et al. Tissue repair processes in healing chronic pressure ulcers treated with recombinant platelet-derived growth factor-BB. *Am J Pathol*. 1994;145:1399-1410.

CHRONIC WOUNDS

Delayed-healing wounds comprise a diverse group of conditions including diabetic foot ulcers, venous insufficiency ulcers, arterial ulcers, and pressure-related ulcers. Chronic wounds may lead to cellulitis, osteomyelitis, gangrene, and septicemia.[17] Limb amputation may be required to prevent or treat these complications. Therapeutic growth factors that stimulate wound angiogenesis (granulation) have been shown to accelerate healing.

KGF-2 (Repifermin)

Repifermin is recombinant human keratinocyte growth factor-2 (also known as FGF-10). Structurally, it is most closely related to FGF-7, with which it shares a 57% homology. KGF stimulates both epithelialization and granulation.[18] Interestingly, KGF stimulates endothelial cells only from small vessels, and it protects endothelial cells against VEGF-induced increases in vascular permeability.[19]

A randomized, double-blind, parallel-group, placebo-controlled, multicenter Phase 2 study of topical repifermin (20 µg/cm^2 and 60 µg/cm^2) was conducted in 94 patients with venous insufficiency ulcers.[20] The growth factor was administered as a twice weekly topical spray for 12 weeks in conjunction with standard compression therapy. A statistical difference was observed in patients who achieved 75% wound closure following repifermin treatment (71% and 63%, respectively, for the low and high dose) versus placebo (45%). The growth factor effect was more pronounced, achieving 90% closure, in patients whose wounds were ≤15 cm^2 in size and whose wound age was ≤18 months old. The drug was well-tolerated by patients and showed minimal immunogenicity. Phase 3 studies of repifermin are underway.

THE FIRST FDA-APPROVED GROWTH FACTOR FOR THERAPEUTIC ANGIOGENESIS

Becaplermin is recombinant human platelet-derived growth factor-BB (rhPDGF-BB). PDGF is a potent stimulator of angiogenesis that also stabilizes newly formed blood vessels.[21,22] Commercially sold under the tradename Regranex Gel 0.01% (Johnson and Johnson Wound Management, Somerville, NJ), becaplermin was the first angiogenesis-stimulating growth factor to become FDA-approved (December 1997) as a topical agent for full-thickness diabetic neuropathic foot ulcers. To achieve its optimal efficacy, becaplermin must be used in conjunction with good standard wound care practices, including sharp debridement, infection control, off-loading, and maintaining a moist wound environment.

In a pivotal clinical trial involving 118 patients with wounds of at least 8 weeks' duration, 61 were randomized to receive becaplermin (0.003%) once daily and 57 received placebo gel. After 20 weeks of treatment, 29 (48%) of becaplermin-treated patients had complete healing of their ulcer, compared with only 14 (25%) of those receiving placebo (P = 0.01) (*Figure 5*).[23] Multicenter, randomized, placebo-controlled efficacy studies verified the incidence of complete healing in becaplermin-treated (0.01%) patients was higher than in placebo groups.[24] Overall rates of healing were also increased with becaplermin treatment. Sharp debridement was shown to improve the efficacy of becaplermin.[25,26] In all studies, the drug was

safe and well-tolerated and there were no adverse systemic effects.[23,26]

Because angiogenesis is requisite for healing for all wounds, the wound care community has used becaplermin in off-label fashion to achieve successful healing in a wide spectrum of wound types. These include venous stasis ulcers, arterial insufficiency ulcers, burns, ischemic ulcers from thrombotic thrombocytopenia and sickle cell anemia, traumatic wounds, dehisced surgical incisions, and pressure ulcers. In a placebo-controlled study of chronic pressure ulcers, becaplermin treatment increased fibroblast content, collagen deposition, and angiogenesis, as observed by light and electron microscopy of wound biopsies (*Figure 6*).[27] More than 500,000 prescriptions of becaplermin have been written since FDA approval, and this drug is now considered an essential part of the modern standard of care for diabetic foot ulcers.

SUMMARY

Growth factor therapy can be used to stimulate angiogenesis in vulnerable tissue and improve perfusion. PAD, ischemic heart disease, and chronic wounds are prime indications for this form of intervention. Growth factors may be delivered as topical or injected proteins, or by gene therapy. Becaplermin is the first FDA-approved angiogenesis-stimulating drug for healing diabetic foot ulcers. Other growth factors are now in clinical development as well. Data from Phase 1 and 2 clinical trials of these agents appear promising, but further well-designed Phase 3 clinical trials are required to confirm their efficacy. As one of the most successful areas of modern biotechnology, growth factor therapy has been successfully translated from a laboratory concept into mainstream clinical practice in modern wound management.

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SUPPLEMENT
CONTEMPORARY SURGERY

Angiogenic Therapy for Chronic Wounds: The Clinical Experience with Becaplermin

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ABSTRACT Growth factors can be clinically applied to promote angiogenesis and heal the chronic wound. This article describes investigational recombinant human growth factors and related gene therapies in clinical trials as well as well as outlines clinical experience with the first FDA-approved growth factor. Randomized, placebo-controlled clinical trials of this drug in patients with full-thickness, chronic, lower extremity diabetic foot ulcers have established that daily application of topical recombinant human PDGF-BB results in a statistically significant increase in the incidence of complete healing and reduced healing time when used with good standard-wound care practices. ■

Chronic wounds affect more than 6.5 million patients each year and cause significant morbidity and impaired quality of life. Diabetes, venous or arterial insufficiency, and chronic pressure are common etiologies of delayed-healing wounds prevalent in the United States. Conventional wound care has relied primarily upon passive interventions, such as dressings, antimicrobial agents, compression, and off-loading devices.

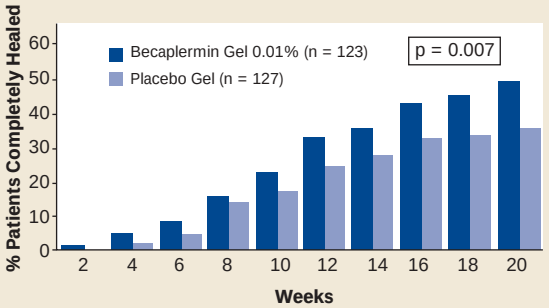
Biologically active wound interventions emerged in the late 1990s as technologies became available for the biotechnology industry to manufacture therapeutic growth factor proteins and tissue-engineered products. In 1997, recombinant human platelet-derived growth factor-BB (rhPDGF-BB, becaplermin, Regranex Gel 0.01%) became the first therapeutic growth factor to receive FDA approval for healing chronic wounds.

PDGF is a pluripotent peptide present in platelets, macrophages, fibroblasts, and endothelial cells, and it plays a critical role in normal wound healing. PDGF stimulates angiogenesis, new blood vessel growth, for wound granulation. Recombinant human PDGF can be manufactured by genetically engineering yeast to express the PDGF gene. The protein is extracted as a pharmaceutical product for topical formulation. This drug can be applied using a cotton applicator onto the wound bed, where it binds to its receptor, stimulates angiogenesis (granulation), and promotes healing. Becaplermin is the first prescription growth factor therapy for angiogenic healing.

CLINICAL TRIALS FOR DIABETIC FOOT ULCERS

The clinical efficacy of becaplermin in diabetic foot ulcers (DFUs) was established through 4 multicenter, randomized, parallel-group clinical trials evaluating the effects of once-daily topical becaplermin gel in 922 patients with nonhealing DFUs of at least 8 weeks' duration.[1] Patients in these studies were ran-

FIGURE 1 Becaplermin Improves Healing



Becaplermin gel increased complete healing of chronic diabetic foot ulcers in 4 clinical trials.

Source: Smiell JM, Wieman TJ, Steed DL, et al. Efficacy and safety of becaplermin (recombinant human platelet-derived growth factor-BB) in patients with nonhealing, lower extremity diabetic ulcers: a combined analysis of four randomized studies. *Wound Repair Regen.* 1999;7:335-346.

domly assigned to a standardized regimen of good wound care alone, or good wound care plus becaplermin gel at either 30 µg/g or 100 µg/g, or a placebo gel (sodium carboxymethylcellulose). Treatment was continued for 20 weeks or until the wound healed with the primary endpoint of complete healing, defined as 100% epithelialization with no drainage. Becaplermin gel (100 µg/g) significantly increased the incidence of complete healing compared with the placebo gel (50% vs 35%, respectively; $P = 0.007$), based on an analysis of patients with a baseline ulcer area (≤ 10 cm^2) common to all trials, representing 95% of all patients (*Figure 1*).[1]

CLINICAL BENEFITS OF BECAPLERMIN

Becaplermin increases the incidence of complete wound healing. Additionally, although 100% wound closure is the only clinical endpoint currently accepted by the FDA for drug approval in wound studies, becaplermin also increases the rate of wound closure. Time-to-healing and ease of closure are important clinical parameters.[2] Reduced healing time has a number of meaningful benefits—including less risk of infectious complications (cellulitis, osteomyelitis); decreased overall costs due to reduced hospitalizations, visiting nurse expenses, and requirement for wound dressing supplies and medications; and an improved quality of life for patients.

In clinical trials, becaplermin gel (100 µg/g) significantly decreased the overall time to complete healing compared with placebo gel ($P = 0.01$), with the 35th percentile of time to complete healing reduced by 6 weeks (from 127 to 86 days). In another subset of patients with smaller ulcers (≤ 5 cm^2 at baseline), becaplermin also significantly increased the incidence of complete healing with a corresponding decrease in the time to healing. Patients who observe accelerated healing of their wounds may also be motivated to increase their compliance with prescribed treatment regimens.

BECAPLERMIN CAN REDUCE OVERALL COST OF WOUND HEALING

A retrospective analysis of cases from a Phase 3 clinical trial of becaplermin indicated that growth factor treatment can result in cost savings of as much as $1127 over good wound care alone.[3] A pharmacoeconomic analysis conducted in our own clinic compared the cost of wound healing based solely on the need to purchase common outpatient wound care supplies and treatments, such as dressings, antibiotics, pain medications, and sterile saline. We compared these costs over a 2-month period in patients treated with and without becaplermin and found significant cost savings by using growth factor therapy compared to conventional wound care due to faster wound closure (*Table 1*).

TABLE 1 Cost Analysis of Wound Care Supplies

Therapy*	Cost per unit	Cost for 2 months
Fixed Costs:		
Saline (1 L)	$14.00	$112.00
Sterile gauze (4 x 4", 10/box)	$5.29	$38.08
Multilayered compression (3 times/week)	$35.07	$841.68
Subtotal		$991.76
Variable Costs:		
Hydrocolloid (DuoDerm, 3 times/week)	$81.59	$391.64
Antimicrobial topicals (bacitracin ointment 30 g)	$3.48	$6.96
Oral antibiotic (levofloxacin 500 mg once daily, 14 days)	$137.99	$137.99
Unna boot (as needed)	$20.65	$20.65
Pain management (tramadol 50 mg 3 times/week)	$60.50	$103.68
Subtotal		$660.92
Total		**$1,652.68**
versus becaplermin (15 g)	$431.99	$863.98

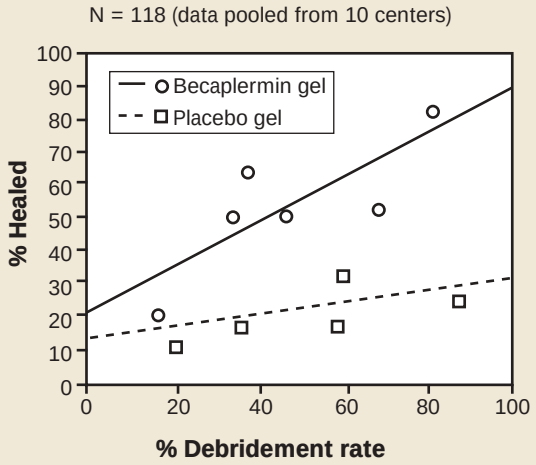
** For venous ulcers*

Source: The Angiogenesis Clinic

CRITICAL ROLE OF SHARP DEBRIDEMENT

Sharp debridement is an absolute requirement for successful use of becaplermin. Clinical studies have shown a direct correlation between sharp debridement and the rate of complete healing of DFUs. A meta-analysis of a randomized, prospective, double-

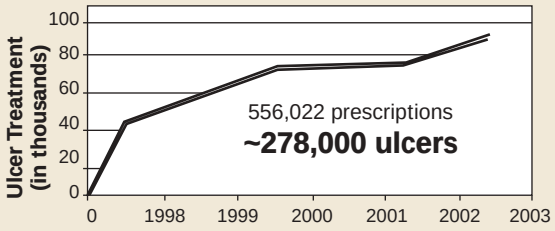
FIGURE 2 Debridement Improves Growth Factor Therapy



Sharp debridement improves healing by growth factor therapy.

Source: Steed DL, Donohoe D, Webster MW, Lindsley L. Effect of extensive debridement and treatment on the healing of diabetic foot ulcers. Diabetic Ulcer Study Group. *J Am Coll Surg*. 1996;183:61-64.

FIGURE 3 Impact of Becaplermin Gel Therapy 1998–2002



More than 270,000 ulcers have been treated with rhPDGF-BB (becaplermin gel) since its FDA approval for diabetic ulcers in December 1997.

Source: The Angiogenesis Foundation.

blind, multicenter trial of 118 patients treated with topical becaplermin or placebo showed an improved response rate with more frequent sharp debridement even in the placebo-controlled group (*Figure 2*).[4]

The benefits of sharp debridement can be understood on a cellular level. Debridement eliminates wound eschar and fibrinous slough, and exposes growth factor receptors that are required for cellular activation. Removing fibrinous slough facilitates wound epithelialization, because keratinocytes cannot migrate on fibrin.[5] Debridement eliminates senescent cells at the periphery of chronic wounds. These cells are unresponsive to growth factors, so their removal enables normal responsive cells to be exposed at the wound margin.[6] The surgical manipulation of tissue also liberates growth factors that are normally stored in the extracellular matrix.[7] The mild bleeding caused by debridement brings thrombin and platelets into the wound bed. Thrombin is a potent angiogenic stimulus, while platelets release endogenous PDGF and other growth factors. Sharp debridement upregulates the expression of PDGF and its receptor in fibroblasts and epithelium.[8] Finally, debridement may also mobilize the recruitment of bone marrow-derived endothelial progenitor cells to the wound bed, where these stem cells contribute to angiogenesis in granulation tissue.[9]

DRESSING CHANGES–A PRACTICAL APPROACH

The goal of dressing the wound is to protect the wound bed from external contamination and to maintain a moist environment that facilitates healing. In its package insert, becaplermin is recommended for daily application with twice-daily changes of saline-moistened dressings, based on the regimen used in its clinical trials.[10,11] In clinical practice, however, wound dressings employed with becaplermin have evolved toward a simpler, more practical once-daily regimen. Less frequent dressing changes are desirable because of the reduced need for home nurse visits, better patient compliance, and less traumatic manipulation of the wound site.

An open-label clinical study was conducted in 134 patients with full-thickness, lower extremity diabetic ulcers who received becaplermin and only once-daily dressing change.[12] Entry criteria and efficacy endpoints were similar to prior studies. Becaplermin with once-daily dressing changes resulted in complete healing in 57.5% of patients with a mean time to closure of 63 days. The recurrence rate was 21% at 6 months. A once-daily dressing change therefore results in an efficacy rate similar to twice daily changes. The firsthand experience of the wound care practice community supports this conclusion.

GROWTH FACTOR THERAPY FOR NON-DIABETIC CHRONIC WOUNDS

Since becaplermin's introduction to the marketplace in 1998, more than 275,000 chronic wounds are estimat-

ed to have been treated with this agent, including many non-diabetic wounds (*Figure 3*).[13] Off-label use of becaplermin is both common and effective in many clinical situations. Because angiogenesis is required for healing regardless of wound etiology, we have successfully treated more than 24 different types of delayed-healing wounds, including venous insufficiency, pressure ulcers, and ischemic wounds, in our Angiogenesis Clinic using becaplermin (*Table 2*).

Clinical trials of becaplermin have been conducted in both pressure and venous insufficiency ulcers.[14-16] These trials have proven to be more complex to design and evaluate than DFU studies due to the heterogeneous characteristics of pressure and venous ulcers. An overview of these studies is presented.

Pressure Ulcers

A multicenter, double-blind Phase 2 study was conducted enrolling 124 patients with chronic, full-thickness pressure ulcers (ulcer volume ranged from 10 to 150 mL).[14] Patients were randomized to receive becaplermin 100 µg/g once daily or twice daily, 300 µg/g once daily, or a placebo gel, with all groups receiving a standardized regimen of good wound care. Treatment was continued for 16 weeks or until healing was complete. The results showed that once-daily treatment with becaplermin (100 µg/g) significantly increased the incidence of complete healing (23% versus 0%) and ≥ 90% healing (58% versus 29%) compared with placebo gel (*Figure 4*). Treatment also significantly reduced the median relative ulcer volume at 16 weeks (P <0.025 for all comparisons).[14]

Data from a subset of patients (n = 28) was analyzed to investigate the outcome of salvage surgery after incomplete wound closure in the context of becaplermin or placebo gel.[15] Patients who failed to heal after 16 weeks of treatment underwent surgical repair using myocutaneous flaps, primary closure, or skin grafts. An intent-to-treat analysis found that 92% (11 of 12) of patients treated with becaplermin and salvage surgery ultimately healed within 1 year after the start of the clinical trial, whereas no patients (0 of 3) treated with placebo plus salvage surgery progressed to full healing within the same time period.

A further retrospective study of 83 patients from the Phase 2 pressure ulcer trial found that becaplermin (100 µg/g once daily) facilitated wound closure.[2] Four independent evaluators, blind to the intervention used, were shown clinical photographs of patients before and after becaplermin or placebo treatment and asked to provide an "ease of closure" score (0 = no need to close/healed to 13 = not possible to close), based on their clinical judgment. The study detected a 1.5-times greater improvement in ease of closure score in patients treated with becaplermin compared with placebo gel at 16 weeks. This improvement was independent of initial wound area or volume.

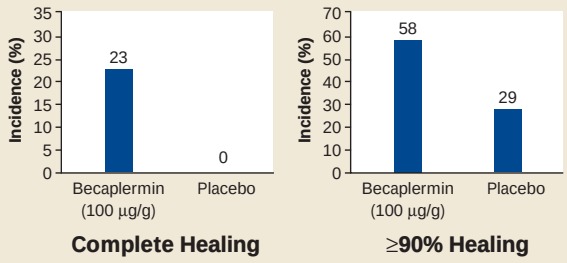
Pressure ulcers encompass a heterogeneous group of lesions, due to anatomic location, ulcer etiology, stage, size, and underlying disease. The clin-

TABLE 2 Ulcers Successfully Treated by Becaplermin

- Diabetes
- Venous stasis
- Arterial insufficiency
- Decubitus (pressure)
- Rheumatoid (vasculitis)
- Sickle cell anemia
- Thrombotic thrombocytopenia
- Protein C deficiency
- Deep venous thrombosis
- Multiple sclerosis
- Chronic radiation scar
- Livedoid vasculopathy
- Scleroderma
- Lymphedema
- Lipodermatosclerosus
- Necrobiosis lipoidica diabeticorum
- Sarcoidosis
- Sjögren's syndrome
- Neurotic excoriation
- Surgical excision
- Trauma
- Animal bites
- Calciphylaxis
- Pyoderma gangrenosum

Source: Angiogenesis Clinic

FIGURE 4 Treatment of Pressure Ulcers: Becaplermin Versus Placebo



Becaplermin (100 µg/g) significantly increased the incidences of complete and >90% healing of pressure ulcers, compared with placebo gel.

Source: Rees RS, Robson MC, Smiell JM, Perry BH. Becaplermin gel in the treatment of pressure ulcers: a phase II randomized, double-blind, placebo-controlled study. *Wound Repair Regen.* 1999;7:141-147.

ical studies suggest that becaplermin may be useful in the treatment of pressure ulcers, especially prior to initiating surgical closure. Becaplermin may prime the local wound milieu by increasing angiogenesis and rendering the tissue more responsive to complete healing with surgical treatment. Although becaplermin has not yet been approved for use in pressure ulcers, the drug is commonly used in the nursing home, chronic care, and neurological unit setting as an adjunct to good wound care, pressure relief, and surgical intervention.

Venous Insufficiency Ulcers

Venous insufficiency ulcers result from incompetent valves in the veins leading to venous hypertension and eventual skin breakdown. The vasculature within venous ulcers is abnormal in structure and is hyperpermeable, leading to the leak of macromolecules and fibrin that form a cuff around microvessels. Endogenous growth factors are thought to be trapped within these cuffs, rendering them unavailable for tissue repair.[16] Lower extremity compression is therefore vital in the standard care of venous ulcers by transferring fluid from tissues back into veins.

Becaplermin has been successfully used through off-label prescription to treat venous insufficiency ulcers. The delivery of this therapeutic growth factor may help to generate more normalized, less leaky capillary blood vessels in venous ulcer granulation tissue.

Two randomized, placebo-controlled studies of becaplermin in patients with venous insufficiency ulcers were performed using daily or twice-weekly regimens of becaplermin gel for 16 weeks.[17] All patients received a standardized regimen of good wound care, including sharp debridement and compression wraps. An intent-to-treat analysis of data from the clinical trials found a higher incidence of complete healing with becaplermin (100 µg/g) compared with placebo for wounds ≥5 cm^2 (46% versus 7% in Study 1; and 38% versus 23% in Study 2). Although the studies were not powered for statistical significance, these findings are consistent with the clinical experience of wound care centers using becaplermin as part of the treatment armamentarium for venous ulcers. As with pressure ulcers, venous ulcers are heterogenous lesions, with a number of underlying etiologies, e.g., incompetent perforators, post-thrombotic syndromes, and saphenous vein insufficiency. Future clinical studies of becaplermin for venous ulcers must be carefully designed to control for these variables and to evaluate a range of clinically useful endpoints.

In our Angiogenesis Clinic, we routinely use becaplermin as part of a successful, comprehensive approach to the treatment of venous insufficiency ulcers. Sharp debridement and compression are absolute requirements. Pulse therapy employing becaplermin once or twice weekly promotes healing in our experience, and vigorous wound angiogenesis generally becomes clinically evident within weeks following the start of treatment. Becaplermin can be applied by a visiting nurse during the change of compression strappings (Unna boot or multi-layered compression wraps). We have found the concomitant use of high-dose pentoxifylline at 800 mg 3 times per day to be beneficial for venous ulcer healing.[18]

COMBINING BECAPLERMIN WITH OTHER MODALITIES

Becaplermin is often used in clinical practice in combination with other wound healing technologies such as vacuum-assisted closure (VAC), hyperbaric oxygen therapy (HBO), electrical stimulation, and tissue engineering products (Apligraf, Organogenesis, Canton, MA; Dermagraft, Smith & Nephew Wound Management, Largo, FL). While clinical trials of such combined regimens have not yet been conducted, each of these modalities also stimulates neovascularization by upregulating the expression of angiogenic genes in local tissue.

VAC applies sustained negative pressure to the wound bed and removes wound exudate. Negative pressure alters the shape of cells within the wound. Such changes in endothelial cell shape can induce angiogenesis by initiating cytoskeleton-mediated signal transduction and changes in the cell cycle.[19] HBO therapy elevates oxygen tension (pO_2) within the wound bed during treatment leading to tissue hyperoxia. While hyperoxia is thought to be the major stimulus for healing, the periods of relative hypoxia between treatment sessions are equally important. Hypoxia is a potent stimulus for blood vessel growth, and switching between hyperoxic and hypoxic conditions induces angiogenesis.[20] HBO-stimulated angiogenesis has been shown to be synergistic with growth factor effects.[21] Electrical stimulation also promotes neovascularization in injured tissues by stimulating growth factor production.[22,23] Finally, tissue engineered

products can stimulate angiogenesis by producing growth factors in the wound bed and by providing a 3-dimensional scaffold for the ingrowth of new capillaries.[24-27] Use of these modalities may be useful for amplifying the angiogenic effects of becaplermin.

OPTIMIZING BECAPLERMIN USE

Becaplermin must be used with good wound management practices, and it is important to understand that the drug is not a stand-alone intervention. Our clinical experience with becaplermin has led to critical insights for optimizing its use. The top 10 success factors are:

- Properly executed sharp debridement.
- Decreasing microbial bioburden.
- Management of excessive protease activity.
- Strict, enforced off-loading (for DFUs).
- Adequate use of compression therapy (for venous leg ulcers).
- Education of patients to refrigerate becaplermin to avoid temperature inactivation.
- Patient compliance with becaplermin use.
- Optimizing nutritional status.
- Proper management of macrovascular disease.
- Minimizing the concomitant use of medications possessing antiangiogenic properties (*Table 3*).

A clinical checklist of these key considerations should be incorporated into wound practices to optimize becaplermin's efficacy.

GROWTH FACTOR THERAPY FOR WOUND CARE: THE FUTURE

A number of angiogenic growth factors are in preclinical and clinical development for wound healing including vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), keratinocyte growth factor (KGF), and transforming growth factor-beta (TGF-β). Although PDGF-BB is the first growth factor to become FDA-approved for wound care, it is likely that multiple growth factors will eventually become available.

Some important questions remain to be addressed for growth factor therapy. What are the precise roles of different growth factors and their temporal sequence of expression in the wound healing cascade? Can multiple growth factors optimize healing, and if so, for what types of wounds?[28] Are there specific molecular markers in wounds that can predict which patients are at greatest risk for delayed healing? Are there markers that can differentiate between responders and nonresponders to growth factor therapy? Can the tools of genomics and proteomics be used to profile individual wounds in order to tailor therapy for patients? Will therapeutic growth factors be optimally used in combination or in sequence? Will gene therapy or cell-based therapy delivering angiogenic growth factors offer advantages over recombinant protein therapy?

Novel growth factor delivery systems are now under investigation, such as angiogenic gene sutures, autologous stem cell transplantation, genetically-modified tissue engineered constructs, and growth factor impregnated dressings or sprays.[29-32] As wound research and technology development continue, these efforts will undoubtedly reveal new opportunities for accelerating wound angiogenesis and healing.

TABLE 3 Common Medications Possessing Antiangiogenic Properties

Bumetanide	Imiquimod
Captopril	Interferon-α,-β,-γ
Celecoxib	Isosorbide dinitrate
Clarithromycin	Lovastatin
Dopamine	Rofexocib
Doxycycline	Simvastatin
Etanercept	Tamoxifen
Furosemide	Tetracycline

Source: The Angiogenesis Foundation

SUMMARY

Topical becaplermin (recombinant human PDGF-BB) stimulates angiogenesis and promotes the healing of chronic wounds. Full-thickness diabetic foot ulcers are the first FDA-approved indication for becaplermin use, although off-label prescription is common and a diverse range of delayed healing wounds have been successfully treated using this drug. Clinical trials of becaplermin in pressure ulcers and venous leg ulcers suggest improved healing occurs with growth factor therapy, although these trials were limited by their endpoint of incidence of complete healing. Increased rate of wound healing, improved quality of life, and

decreased overall cost of wound care following becaplermin use are important beneficial factors to consider. Other modern wound treatment modalities, such as the VAC, HBO, electrical stimulation and tissue engineering, have been successfully used with becaplermin, and these may be rationally employed to synergize with the angiogenic response initiated by growth factor therapy. Specific critical factors have been identified to optimize the results of growth factor therapy using becaplermin. When clinicians understand the mechanisms underlying growth factor therapy, their ability to provide modern wound care becomes state of the art.

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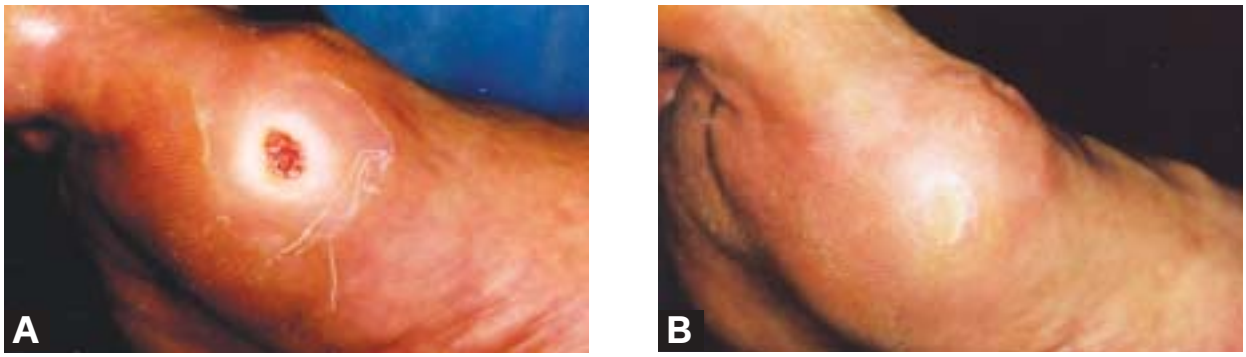
CASE STUDIES

CASE STUDY 1 Nonhealing Diabetic Foot Ulcer

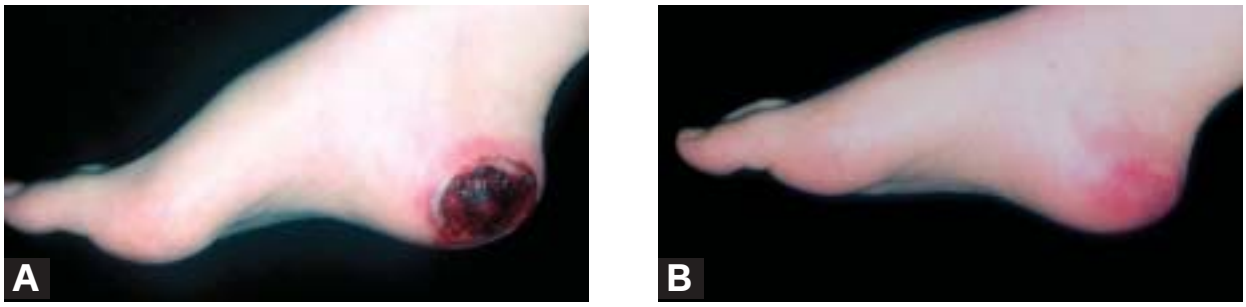
A 74-year-old male with diabetes, sensory neuropathy, and poorly fitting footwear presented with a 15-month nonhealing ulcer of the foot (A). On his initial visit, surgical debridement of the ulcer was performed. The patient was treated with topical becaplermin gel once-daily in conjunction with local wound care involving infection control and enforced off-loading. The chronic ulcer completely healed in 6 weeks (B).



Source: The Angiogenesis Clinic

CASE STUDY 2 Nonhealing Wound Associated with Multiple Sclerosis

A 38-year-old female with multiple sclerosis and absent motor or sensory function from the waist down presented with nonhealing ulcers on both heels of 11-months' duration (A). Treatment consisted of sharp debridement, off-loading, and daily topical application of becaplermin gel followed by Adaptic as a primary dressing. The ulcers healed in 7 weeks with good cosmesis (B).



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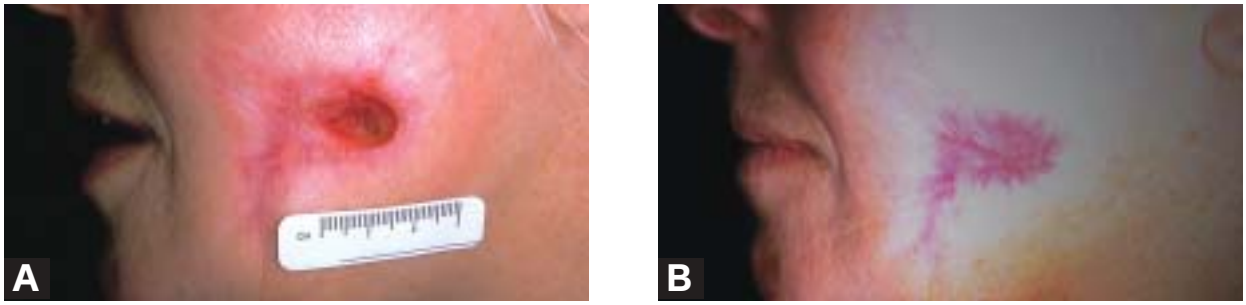
CASE STUDY 3 Self-induced (Neurotic) Facial Ulcer

A 51-year-old female psychiatric patient presented with bilateral 1-cm-deep self-inflicted ulcers of the cheek (A). The ulcers had been present for over a decade. The patient was placed on an anxiolytic (oral buspirone, 10 mg 3 times daily), and received daily treatment with topical becaplermin gel. The ulcer completely healed in 2 weeks in response to treatment (B). The patient was maintained on buspirone to manage the underlying psychiatric disorder.



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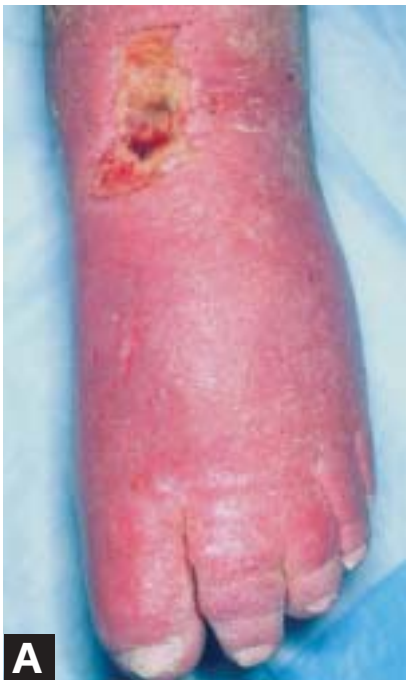
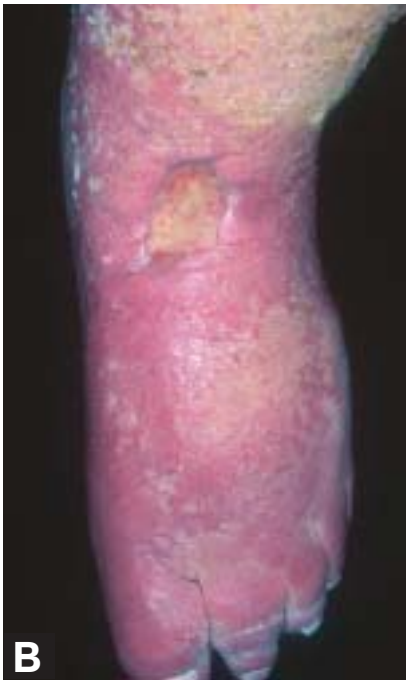
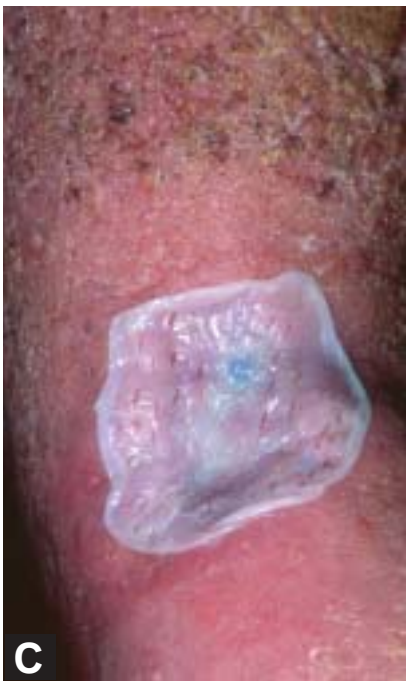
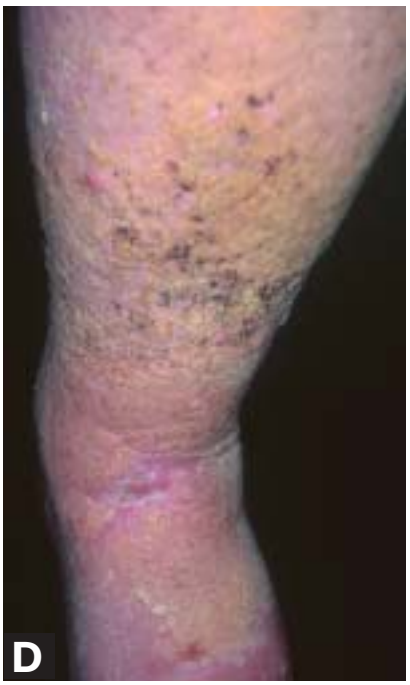
CASE STUDY 4 Venous Stasis and Lymphedema

A 58-year-old male with diabetes, venous stasis, lymphedema, and elephantiasis nostra verrucans presented with characteristic soft tissue hypertrophy and a 7 x 4-cm excavating wound at the dorsal flexion of the foot, unhealed for 4.5 years (A). After 6 weeks of treatment with daily topical becaplermin gel, a good granulating wound bed was achieved, along with a reduction in the ulcer size (B). A tissue-engineered bilayered skin equivalent was implanted at the ulcer site (C). After an additional 7.5 weeks, the ulcer healed completely (D). There was no recurrence up to 4 years of follow-up. This case illustrates that growth-factor therapy can be combined with other modalities such as tissue-engineered skin to achieve complete healing.









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Angiogenesis in Wound Healing
CME QUESTIONS AND POST-TEST ANSWER FORM

To receive CME accreditation, circle the correct response below, complete the program evaluation and registration form on the back page and then submit this form to Medical Education Resources. Certificates will be mailed to the address listed on the back page. Please allow 3 weeks for processing.

1. The field of angiogenesis research began as an inquiry into:
a) Why diabetic foot ulcers fail to heal normally
b) How new blood vessel growth supports tumor growth
c) How to increase local blood flow in the treatment of coronary artery disease
d) How to control endometrial proliferation during the female reproductive cycle

2. Physiological regulation of angiogenesis represents a balance between:
a) Stimulatory and inhibitory factors
b) Fibrinogen fragments and thrombin molecules
c) Hypoxia-inducible factor (HIF) and cyclooxygenase-2 (COX-2)
d) Matrix metalloproteinases and integrins

3. The angiogenesis model of wound healing includes these stages:
a) initiation, amplification, proliferation, stabilization, and suppression
b) inflammation, production, and stabilization
c) inflammation, proliferation, and remodeling
d) none of the above

4. Impaired granulation is a hallmark of the chronic wounds encountered with:
a) diabetes
b) venous insufficiency
c) arterial insufficiency
d) all of the above

5. Proteins that promote cell proliferation and migration in regenerating tissue are called:
a) Growth factors
b) Regenerators
c) Apoptic factors
d) None of the above

6. Studies show that growth factors appear in the wound bed with distinct temporal patterns. The following is correct:
a) VEGF expression peaks one day after wounding, and expression of PDGF peaks 3 to 7 days after wounding.
b) PDGF expression is detected immediately after wounding, while expression of VEGF peaks 3 to 7 days after wounding.
c) Basic fibroblast growth factor is not present.
d) None of the above.

7. When administered together, platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF)
a) Impede vascularization
b) Synergize in their ability to vascularize tissues
c) Compete to regulate EPC recruitment
d) Cancel out each other's effects

8. Growth factor therapy
a) Enhances tissue vascularization
b) Improves local circulation
c) Promotes healing and regeneration
d) All of the above

9. Becaplermin is
a) A non-viral gene therapy
b) Recombinant human platelet-derived growth factor-BB
c) Recombinant human keratinocyte growth factor-2
d) Recombinant fibroblast growth factor-2

10. Which of the following statements is not true:
a) All isoforms of platelet-derived growth factor (PDGF) stimulate angiogenesis.
b) PDGF-AA is more highly angiogenic than PDGF-BB
c) When PDGF-BB binds to the PDGF-beta receptor on endothelial cells, only cells from microvessels undergo increased DNA synthesis
d) PDGF-BB induces endothelial migration, an activity not seen with PDGF-AA

11. When treating chronic wounds with becaplermin, sharp debridement
a) May be helpful
b) Is critical
c) Is not necessary
d) Is contraindicated

12. In clinical practice, becaplermin is often used in combination with:
a) Vacuum-assisted closure
b) Hyperbaric oxygen therapy
c) Tissue engineering products (ie, Apligraf, Dermagraft)
d) All of the above

SUPPLEMENT

CONTEMPORARY SURGERY

Angiogenesis in Wound Healing
PROGRAM EVALUATION

Please check the box that best reflects your opinion on the statements below, using the rating scale defined at right.	Strongly disagree 1	Disagree 2	Agree 3	Strongly agree 4
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2. The program content was useful and relevant	❒	❒	❒	❒
3. The program was educational and not promotional	❒	❒	❒	❒
4. The program was presented in an organized manner	❒	❒	❒	❒
5. The program will change the way I practice	❒	❒	❒	❒
6. The authors demonstrated expertise in the topic	❒	❒	❒	❒
7. The material was presented at an acceptable level of expertise	❒	❒	❒	❒

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