Growth Factors and Plastic Surgery

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INTRODUCTION

Plastic surgeons are often asked to manage difficult, complex, or chronic wounds. Additionally, since much of plastic surgery involves moving or changing the shape of tissue, the specialty is particularly dependent on appropriate wound healing. As such, wound healing research has often been within the realm of plastic surgical research. Advances in the understanding of tissue repair have provided improved ability to care for a variety of disease processes, including chronic wounds. The elucidation of growth factors as mediators of tissue repair represents such an advancement that is beginning to translate into new treatment regimens included in the category of "biologic therapy." Such therapy involves the use of a biologically active substance that will stimulate a desired response within tissue. Biologic therapy applicable to plastic surgery includes the use of topical growth factor, gene therapy, and artificial skin or skin substitutes, all of which have seen greatest potential in the treatment of chronic wounds. This article will concentrate on the use of growth factors.

GROWTH FACTORS AND WOUND HEALING

The repair of injured tissue proceeds through a series of events in which cellular and extracellular matrix components act in concert to restore tissue integrity. The orderly interaction of these components is largely due to the modulatory effect of growth factors. For descriptive purposes, the processes of wound healing can be divided into four overlapping phases: hemostasis, inflammation, proliferation and remodeling (Fig. 1) (1). During hemostasis, fibrin is laid down within the wound and serves as a temporary matrix for the influx of subsequent cells. Also, platelets degranulate, releasing the first supply of growth factors that will help recruit other wound healing cells into the site of injury. Inflammation begins with neutrophils, acting as host defense against contaminating bacteria.

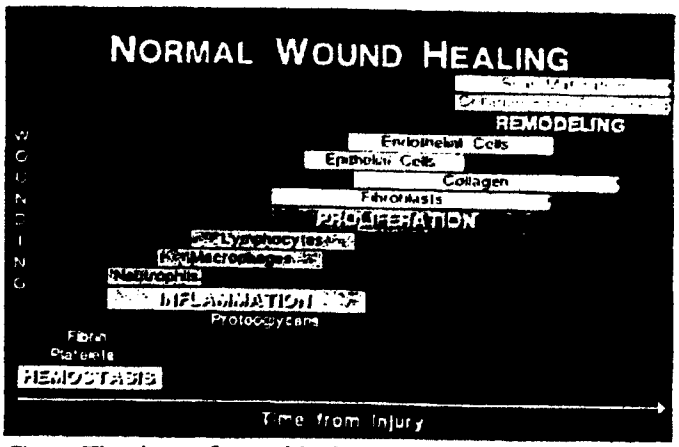

Fig. 1. The phases of wound healing and their constitutive cellular and extracellular matrix components.

Macrophages are present within the wound for up to five days, during which time, they serve as the primary supply of a variety of growth factors that control healing. During proliferation, fibroblasts increase in number and then produce collagen, the main structural protein of skin; epithelial cells migrate and multiply; and endothelial cells partake in angiogenesis. Each of these cells also produces growth factors that control their activity, as well as surrounding cellular activity. Lastly, the remodeling phase lasts longest, during which the time collagen is organized, and degraded if too abundant, cellular density decreases, neovascularization recedes, and the scar fully matures.

Regulation of tissue repair is largely due to an array of soluble mediators that are produced and/or secreted at the wound site (1). These cytokines and polypeptide growth factors act by attaching to specific receptors on the surfaces of target cells. Receptor binding leads to chemical reactions within the cytoplasm. These "second signals" subsequently alter nuclear function, genome transcription and ultimately, cellular behavior. Cytokines and growth factors are chemotactic for some cells, drawing them into the wound site through induced migration; they stimulate cells to pro-

liferate; and/or synthesise particular proteins and enzymes.

Cytokine is a term that refers to a substance that imparts some type of action to a cell. In skin healing, this term is best reserved for proinflammatory cytokines. Tumor necrosis factor-alpha (TNF-) and the interleukins are the most important. These cytokines act to regulate the inflammatory response to tissue trauma. Growth factors are the cytokines that are the principal regulators of healing. There is a plethora of growth factors with tremendous redundancy in cellular sources and function.

TABLE 1 GROWTH FACTORS IN WOUND HEALING

PDGF	Platelet derived growth factor
TGF-beta	Transforming growth factor-beta
TGF-alpha	Transforming growth factor-alpha
FGF	Fibroblast growth factor (acidic, basic)
KGFs	Keratinocyte growth factors
EGF	Epidermal growth factor
IGF-1	Insulin-like growth factor-1
VEGF	Vascular endothelial growth factor

The growth factors that are most important for soft tissue wound healing are listed in Table 1. In terms of tissue repair, the end-point of growth factor action can be considered to be stimulation of connective tissue deposition, promotion of epithelialization or stimulation of angiogenesis. Many growth factors have been shown to stimulate such action in various cell culture and animal models. Only a few growth factors have been shown to be effective in promoting healing in people. Epidermal growth factor (EGF), vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF) hold the greatest potential as therapeutic agents.

EPIDERMAL GROWTH FACTOR

Epidermal growth factor or EGF was the first growth factor to be described in 1962 (2). EGF is a potent keratinocyte mitogen, thus inducing epithelialization. It is elicited in the wound by platelets and macrophages. EGF also stimulates fibroblast proliferation, so that collagen and connective tissue deposition may also be affected. Based on the effect of EGF on keratinocytes, this growth factor was studied to determine if a clinical effect could be garnered on wounds that heal primarily by epithelialization. Accordingly, the donor sites for split thickness skin grafts were treated with EGF or with placebo in two different studies from two different institutions. Brown and colleagues (3) found that EGF caused a more rapid healing of skin graft donor sites as documented by serial photography and biopsy. A similar study by Cohen and co-investigators showed no difference in wounds treated with EGF versus placebo control (4). Venous stasis ulcers also heal largely by epithelialization. A small, randomized study demonstrated that venous stasis ulcers treated with EGF showed no statistical improvement in healing compared to placebo (5). To date, no other studies have been shown to demonstrate any clinical wound healing effect of EGF and there is no current clinical role for its use in cutaneous wound healing. However EGF has been showing promise as a biologic therapy in fields other than plastic surgery. Clinical trials using EGF have been encouraging in the fields of oncology for neoplastic conditions (6); ophthalmology for the repair of corneal ulcerations (7); and otolaryngology for the treatment of chronic perforations of the tympanic membrane (8).

VASCULAR ENDOTHELIAL GROWTH FACTOR

Angiogenesis or new blood vessel formation is an integral part of tissue repair. Several growth factors stimulate angiogenesis during wound healing including: vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), transforming growth factor-ß and fibroblast growth factor. The most important of these factors is likely VEGF due to its ability to be secreted by intact cells, and its mitogenic specificity for endothelium (9). A large body of data generated from animal experiments has shown VEGF to be a potent inducer of neovascularization in ischemic conditions. Indeed, when VEGF has been administered in the form of gene therapy, improved vascularity has been documented in animal studies of myocardial ischemia (10) and limb ischemia (11) (12). Furthermore, topical application of VEGF, as well as gene therapy application has resulted in increased survival of ischemic flaps in animals (13).

Management of chronic wounds of the lower extremity necessitates the ability to recognize ulcerations that result from arterial insufficiency. Such wounds are provided treatment in conjunction with lower extremity revascularization by mean of bypass surgery or angioplasty. However, some patients are not candidates for revascularization due to the extent of their distal arterial insufficiency or co-morbid medical conditions. Many of these patients undergo amputation. Biologic therapy with angiogenic growth factors is being investigated as a means to help such patients. Animal models of limb ischemia have shown VEGF to be quite effective in improving limb vascularity (14) (15).

This led to a clinical trial using gene therapy to supply VEGF to several patients with Buerger's disease (thromboangiitis obliterans) who had end-stage lower extremity ischemia and whose conditions were not amenable to angiographic or surgical revascularization (16). Five of the seven patients had healing of ischemic ulcers or improvement in rest pain. Angiography demonstrated marked improvement of collateral blood supply, presumably due to neovascularization. The marked clinical improvement in these patients is a dramatic example of the potential benefit of growth factor therapy. It is highly probable that biologic therapy with VEGF will find a true clinical application in the near future.

PLATELET DERIVED GROWTH FACTOR

Platelet derived growth factor (PDGF) was named for the cell from which the growth factor was first isolated. However, PDGF is supplied within the wound site by platelets, macrophages, endothelial cells and smooth muscle cells. Broad reaching effects on wound healing result from PDGF, including chemoattraction of neutrophils, macrophages and fibroblasts; stimulation of fibroblast proliferation; induction of connective tissue deposition; and stimulation of angiogenesis. Due to its far-reaching effects, PDGF has been studied as a potential therapeutic agent for chronic wounds.

Earliest studies did not use isolated PDGF, but rather an autologous growth factor solution derived as a platelet releasate that is obtained from the patient's blood using a proprietary method. There is extensive experience using this solution called platelet derived wound healing fluid (PDWHF or Procuren®) which contains multiple molecules (growth factors and cytokines), which are normally released from platelet granules. Several individual studies have been performed resulting in data that variably supports the efficacy of the PDWHF (17). This platelet releasate has not been subjected to the rigors of FDA required research due to it being an autologous bloodderived formula administered to the patient from whom it is derived. The actual efficacy of PDWHF therapy is not well defined due to the absence of large prosective randomized studies.

Human recombinant PDGF has been studied extensively as a therapy for chronic wounds. Recombinant PDGF-BB has been evaluated by two multicenter, placebocontrolled, blinded efficacy studies for the treatment of uncomplicated diabetic foot ulcers. These studies demonstrate a significant enhancement of complete healing with topical use of the growth factor (18) (19). In the first efficacy study (Fig. 2), the incidence of complete healing of non-ischemic foot ulcers was 25% in the placebo-treated group and 48% in the PDGF-treated group ($p<0.01$) (18). The second efficacy study found that healing occurred in 35% of placebo-treated wounds, compared to 50% of PDGF-treated wounds, representing a 43% increase in healing ($p = 0.007$) (19). Additionally, PDGF treated wounds reduced the time to healing by 6 weeks ($p = 0.013$). In both studies, growth factor therapy was combined with excellent wound care, including moist dressings, off-loading of the affected foot and aggressive debridement. Indeed the positive effect of debridement on healing was markedly correlated with the use of PDGF, whereas the effect was not nearly as great in the placebo-treated patients (20). A higher dose of PDGF was required to demonstrate an effect on healing in the second study, a finding attributed to the need for more growth factor when wound care is less aggressive (wound clinics in the first study versus general clinics in the second study). Due to these studies, human recombinant PDGF (Regranex®) is approved by the FDA for treatment of diabetic foot ulcers in non-ischemic and noninfected extremities. It is important to note that PDGF must be used as an adjunct to good wound care. It will be ineffective if used as sole therapy.

Figure 2

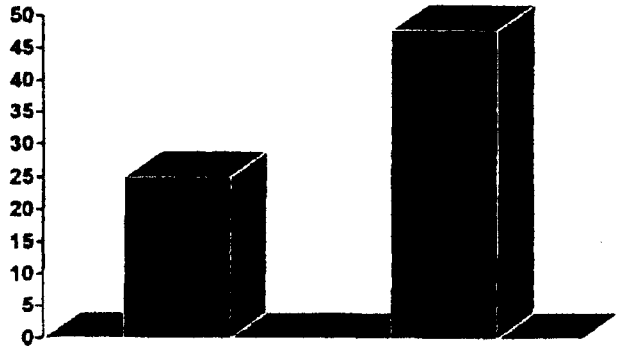

Fig. 2. Healing of diabetic foot ulcers was significantly ($p<0.01$) improved with the use of recombinant human PDGF. (adapted from data contained in Steed DL, the Diabetic Ulcer Study Group. Clinical evaluation of recombinant human platelet-derived growth factor for the treatment of lower extremity diabetic ulcers. J Vasc Surg 21 :71-81, 1995).

The healing of pressure ulcers has also been evaluated using PDGF. Early pilot studies suggested that PDGF may provide some improvement in the rate of closure of such wounds (21) (22). Recently, a multicenter, prospective,

placebo controlled double blinded study evaluated PDGF for the treatment of full thickness pressure ulcers (23). This study used complete healing and 90% healing at 16 weeks as the study endpoints. PDGF was found to result in significantly greater healing for both endpoints: 19% vs. 0% (placebo) for complete healing: 58% vs. 29% (placebo) for 90% healing (Figure3). Further studies are underway to assess the use of PDGF in pressure ulcers.

Figure 3:

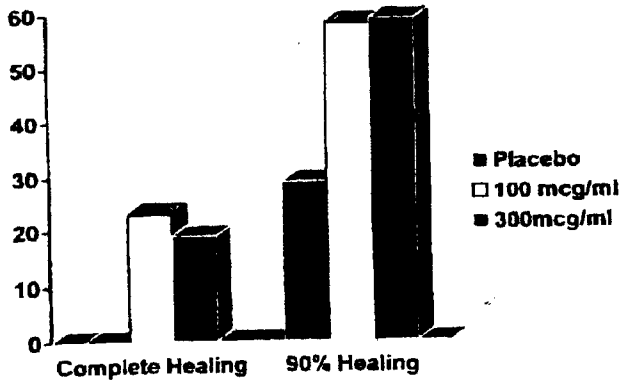

Fig. 3. The healing of stage III and IV pressure ulcers was significantly improved with recombinant human PDGF, but higher doses did not lead to further improvement in healing. (adapted from data contained in Ress RS, Robson MC, Smiell JM, Perry BH, et al. Becaplermin gel in the treatment of pressure ulcers: a phase II randomized, doubleblind, placebo-controlled study. Wound Rep Regen, 1999).

CONCLUSIONS

Growth factors are powerful regulatory molecules that control tissue repair. To date, only PDGF has been shown conclusively to be effective as a therapy for chronic wounds. Other growth factors, such as VEGF show significant promise as potential interventions for patients with problematic wounds, or failing reconstructive flaps. Present and future research will be concentrated on finding more applications for growth factors, as well as developing better delivery systems, whether it is gene therapy, special dressings, or tissue engineered wound coverings. Indeed, tissue engineering has brought to market a composite skin equivalent containing a bilayer of living keratinocytes over fibroblasts within a bovine collagen matrix (Apligraf™) (24). These living cells produce various growth factors and cytokines and it is postulated that the skin substitute may be affective by means of delivery of these regulatory proteins. Other similar products are under investigation. Therapy with other cytokines may also be forthcoming. A large body of data exists from animal models looking at bone morphogenic proteins (growth factors for bone healing) as a means of augmenting bone repair (25). Studies have shown systemic administration of growth hormone in burned children caused accelerated healing of skin graft donor Sites (26) (27). Another pilot study demonstrated reduction in hypertrophic burn scars following systemic therapy with interferon- 2b, an effect postulated to result from down regulation of transforming growth factor-ß 28

Biologic therapy with growth factors will certainly have great applications to medicine in the future. Its use in plastic surgery, as well as other fields is still in its infancy. As research continues and greater experience is accumulated, growth factors will certainly be a powerful tool for a variety of clinical entities.

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