Clinical Application of Growth Factors and Cytokines inWound Healing

Stephan Barrientos, MD[a]; Harold Brem, MD[a]; Olivera Stojadinovic, MD[b]; and Marjana Tomic-Canic, PhD[b]

[a] Division of Wound Healing and Regenerative Medicine, Department of Surgery, Winthrop University Hospital/ Stony Brook University School of Medicine, Mineola, NY

[b]Wound Healing and Regenerative Medicine Research Program,Department of Dermatology and Cutaneous Surgery, University of Miami Miller School of Medicine, Miami, FL

Corresponding Author:

Marjana Tomic-Canic, PhD

Department of Dermatology

University of Miami Miller School of Medicine

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1600 N.W. 10th Avenue

Miami, FL 33136

Tel: 305-243-4940

Email: MTCanic@med.miami.edu

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ABSTRACT

Wound healing is a complex and dynamic biological process that involves the coordinated efforts of multiple cell types and is executed and regulated by numerous growth factors and cytokines. There has been a drivein the past two decades to study the therapeutic effects of various growth factors in the clinical management of non-healing wounds (e.g. pressure ulcers, chronic venous ulcers, diabetic foot ulcers). For this review, we conducted anonline search of Medline and Pub Medicaland critically analyzed the literature regarding the role of growth factors and cytokines in the management of these wounds. Wefocused on currently approved therapies, emerging therapies and future research possibilities. In this reviewwe discuss four growth factors and cytokines currently being used on and off label for the healing of wounds. These include: granulocyte-macrophage colony stimulating factor (GM-CSF), platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and basic fibroblast growth factor (bFGF). While the clinical results of using growth factors and cytokines are encouraging, many studies involved a small sample size and are disparate in measured endpoints. Therefore, further research is required to provide definitive evidence of efficacy.

INTRODUCTION

Wound healing is a complex, evolutionarily conserved, multi-cellular process aimed towardepitheliumrestoration after injury. Theseprocessesrelyon a multitude of growth factors and cytokines thatexecute and regulate a complex signaling network altering the growth, differentiation, and metabolism of target cells.Duringacute wound healing, these biologically active polypeptides are readily present in the wound bed and play a role in all stages of wound healing: inflammation, formation of granulation tissue, re-epithelialization, matrix formation and re-modeling.However, *in vivo* and *in vitro* studies analyzingnon-healing acute and chronic (chronicity defined as physiologically impaired)wounds have demonstrated de-regulationofvariousgrowth factors(e.g.platelet derived growth factor (PDGF) (1), vascular endothelial growth factor (VEGF) (2) and basic fibroblast growth factor (bFGF)(3))suggesting a potential target for therapy which has led to a robust interest in using exogenous growth factors and cytokines in the clinical setting to improve clinical outcomes of non-healingwounds.

There is a critical need for new treatments to manage non-healing wounds (e.g. diabetic foot ulcers (DFUs), pressure ulcers (PUs), and chronic venous leg ulcers (VUs)) as they representa major health care burden in the United States. Studies have estimated that there are approximately 71,000 patients with DFUs who undergo limb or digit amputations each year (4). Other studies show that advanced stage PUs (stage III and IV) have a mortality rate of 68%(5) and can incur costs for the hospital as high as $124,000 per episode (6). In addition, non-healing wounds result in prolonged hospital stays (7), diminished quality of life (8) and increased likelihood of being discharged to a long-term care facility (7). With the advent of genetic engineering and advances in biological technology, the use of exogenous growth factors and cytokines in treatment of these wounds presents a potential solution to the problem.

We performed online searches of Medline and Pub Medical using the terms chronic wounds, non-healing wounds, venous ulcers, diabetic ulcers, pressure ulcers, leg ulcers, burn wounds, growth factors and cytokines. The literature regarding the potential role of growth factors in the management of non-healing wounds was reviewed. Clinical studies were critically analyzed with a focus on currently approved therapies, emerging therapies and future research possibilities for the management of non-healingwounds. In this review,we will discuss published literature concerningclinical applications of GM-CSF, PDGF, bFGF, and VEGF.

Granulocyte-macrophage Colony Stimulating Factor

GM-CSF is a cytokine found to be present inthe wound bed after acute injury(9,10). GM-CSF has been shown to have important biological effects onwound healing *in vivo*including: promotion of myofibroblast differentiation andwound contracture facilitation (11); simulation oflocal recruitment of inflammatory cells(12,13); mediation ofepidermal proliferation(14); andLangerhans cell recruitment (15). In addition, GM-CSFalso has the ability to stimulate proliferation and differentiation of hematopoietic progenitor cells making it an effective immune-stimulator(16-21). GM-CSF gene knockout animal models show impaired wound healing with reduced neutrophil and macrophage recruitment and reduced vascularization in wounds(22).While systemic administration of GM-CSF has no effect on wound healing, local applicationhas been shown to enhance wound healing in animal models (23-28).

In 1991, the Food and Drug Administration (FDA) approved Sargramostin (Leukine), an injectable recombinant human- GM-CSF (rh-GM-CSF) as an immune-stimulator following chemotherapy and bone marrow transplantation(29). The FDA specifically approved its use in the following circumstances: following induction of chemotherapy in acute myelogenous leukemia (AML); in mobilization and following transplantation of autologous peripheral blood

progenitor cells; in myeloid reconstitution after allogenic bone marrow transplantation, in bone marrow transplantation failure or engraftment delay (16-21). Sargramostin comes in a liquid or lyophilized form that requires reconstitution with sterile water and can be administered via subcutaneous injection or intravenous infusion. Dosing depends on the intended therapeutic outcome. Side-effects include slight temperature elevations, swelling, redness and/or discomfort at the site of injection. Sargramostin iscommercially available along with its analog Molgramostim (Leucomax)(29).

In two randomized controlled studies of chronic VUs,positive effect of rh-GM-CSF was observed (30, 31).In one study, a single 400 mcgdose of rh-GM-CSF (Leucomax) injection was administered perilesionally in four equal doses in the four quadrants of the wound. Treated patients fared much better than placebo controls prompting early termination of the study. Of the rh-GM-CSF treated patients, 50% completely healed by week 8 as compared to 11% of placebos (30). In a second double-blind, randomized, placebo-controlled study, chronic VU patients were randomized to placebo or 200 or 400 mcg of rh-GM-GSF (Leucomax). Perilesional injections of the drug were administered in four weekly treatment episodes. Again, there was significant improvement in the healing of wounds in the treatment group. There was no appreciable difference between using 200 or 400 mcg of rh-GM-CSF (31). The side effect profile in these patients was minimal; with some patients complaining of pain, malaise or discomfort at the injection site. Smaller non-randomized studies of topicalrh-GM-CSF(Leucomax) at concentration of 0.5 to 1.0 mcg/cm^2 applied three times a weekshowed beneficial effects in patients with chronic VUs. A 90% of wounds had complete healing with an average healing time of 19 weeks with no sideeffects. In addition, there was no re-ulceration of the healed ulcer observed after 40 months (32).

Efficacy studies of rh-GM-CSF on PUshave yielded diverseresults. Robson et al. compared the healing response of sequentially applied rh-GM-CSF and rh-basic fibroblast growth factor (rh-bFGF) to that of each cytokine alone and to a placebo in patients withstage III or IV PUs with duration longer than 8 weeks. One treatment group received topical 2.0 mcg/cm^{2}rh-GM-CSF daily for 35 days. A second treatment group received topical5.0 mcg/cm^{2}rh-bFGF for 35 days. A final group received 2.0mcg/cm^{2}rh-GM-CSF for 10 days, followed by 25 days of topical 5.0 mcg/cm^{2}rh-bFGF. Wounds treated with cytokines had greater closure than those in the placebo group. Patients treated with rh-bFGF alone did the best, followed by the rh- GM-CSF/rh-bGFG group. In this study, topical rh-GM-CSF showedno effect when compared to placebo(33).Conversely, several case reports have shown improvement in PU healing with the use of rh-GM-CSF. El Saghir et al used diluted GM-CSF and injected it locally around a stage IV PU every 2-3 days for 2 weeks and then weekly for 4 weeks. Firm granulation tissue growth was noted within a few days. The ulcer showed 85% healing within 2 weeks and 100% by 2 months(34).While there are currently no randomized controlled trials studying the effect of rh-GM-CSF on DFU healing rate, there was one randomized controlled study conducted by Gough Et al. of diabetic patients withinfected foot ulcers receiving subcutaneous injection of 2.5 mcg/kg granulocyte-colony stimulating factor (G-CSF)(Filgrastim). In conjunction with antibiotic and insulin therapy, patients receiving G-CSFexperienced earlier eradication of pathogens, shorter hospitalization, shorter duration of antibiotics, and no need for amputation, as compared to placebo. There were no side-effects reported in this study (35).Diabetic patients commonly fail to develop an appropriate neutrophil response to infection(36);therefore improved outcomesin this study were attributed to the potential of G-CSF to increase superoxide

production(35). In a small cohort study of patients with chronic leg ulcers, topical rh-GM-CSF (Leucomax) resulted in complete healingafter 1 month of treatment (37).

The therapeutic effects of topically applied rh-GM-CSF hydrogel on deep partial-thickness burns were confirmed by healing rates in two recent randomized controlled studies.With a dose of 100 mcg/m^2/day ofrh-GM-CSF hydrogel for the treatment of the burn wound, complete healing of was 100 mcg/m^2/day. By 28 days all wounds in the treatment group was achieved within 28 days. Therapeutic effects were more obvious in the 14 day time period, followed by 20 days, and 28 days, implying that rh-GM-CSF hydrogel should be applied as early as possible to attain the best therapeutic effect. There were no reported side effects (38, 39).

Biopsiesobtained from non-healing VUs before and after treatment with intra-dermal injections of GM-CSF were analyzed in an attempt to understand the molecular basis of GM-CSF. An increase in blood vessel density and vascular endothelial growth factor (VEGF) was found in the wound bed specimens afterGM-CSF administration.This suggested that GM-CSF treatment leadsto increased vascularization and inflammatory cell-derived VEGF may act as an angiogenic mediator in the healing of these ulcers(40).

Given the literature on the healing effects of rh-GM-CSF and mild side-effect profile, it is routine practice in our clinic to apply Leukine to most physiologically impaired ulcersas an adjunct to debridement and other standard of care therapies when they prove to be refractory to standard treatment. In addition, we have usedLeukine in combination with other growth factors for refractory wounds of all types with variable success. To date, we use a dose of 500µg locally up to two times per week with only one complication recorded in over a thousand of patients treated (41). Larger high quality randomized controlled studies are needed to support the therapeutic efficacy of the rh-GM-CSF for all non-healing wounds.

Platelet Derived Growth Factor

Platelet derived growth factor (PDGF) plays a role in each stage of the wound healing process.PDGF is released from degranulating platelets upon injury and is present in wound fluid (1, 42).PDGFstimulates mitogenicity and chemotaxis of neutrophils, macrophages, fibroblasts, and smooth muscle cells to the wound site aidingtheinitiation ofthe inflammatory response(43).*In vivo* studies demonstrate that PDGF is important in recruiting pericytes and smooth muscle cells to capillaries thus increasing the structural integrity of these vessels(44,45).During the epithelialization phase of wound healing, PDGF up-regulates the production of insulin growth factor 1 (IGF-1) and thrombospondin-1 (46).Furthermore, IGF-1 has been shown to increase keratinocyte motilityand thrombospondin-1, which protectsproteolytic degradation of PDGF and promotesfibroblast growth in vitro in a dose-dependent manner(47).PDGF also enhances proliferation of fibroblasts and consequently the production of ECM(48), induces the myofibroblast phenotype in these cells and stimulates fibroblasts to construct collagen matrices (49).PDGF is decreased in chronic wounds due to itssusceptibilityto the proteolytic environment found in the chronic wound.(50, 51)

In 1997, the FDA approved Becaplermin (Regranex) for the treatment of DFUs that extend into the subcutaneous tissue or beyond and have adequate blood supply (52).Becaplermin is a recombinant human-PDGF (rh-PDGF- BB) that has been shown to accelerate wound closure in DFUsin randomized clinical trials(53-56).In a multicenter double-blind placebo-controlled trial in patients with type 1or type 2 diabetes and chronic ulcers of at least 8 week duration, 100ug/g of Becaplermin significantly increased the incidence of complete wound closure by 43% and decreased the time to achieve complete wound closure by 32% when compared to placebo (54). The treatment with Becaplermin is expensive and requires frequent dressing changes. A

combined analysis of four randomized controlled trials of rh-PDGF in the treatment of DFUs has shown that 100mcg/gBecaplermin gel used once daily is effective in improving healing(57).The efficacy of a drug in controlled trials does not equate to its effectiveness in actual clinical practice. Margolis et al. set out to determine the effectiveness of Becaplermin gel in "real world" situations. Retrospective analysis of patients with neuropathic foot ulcers showed an increase in healing rates by 32% with the use of Becaplermin gel versus the control group. Similarly,Becaplermin was linked to a significant reduction in amputations (58).Data from this study was consistent with data from the randomized controlled trials.

Commonly observed adverse reactions to Becaplermin topical application include erythematous rashes and aburning sensation at the site of application(55).More serious adverse eventssuch as osteomyelitis and cellulitisare reported less frequently (56).Data prompting the FDA to release a warning of malignancy risk associated with Becaplermin use remains anecdotal (57).A20-month follow up study from two randomized controlled trials revealed an increased risk of malignancy with Becaplermin. In the Becaplermin group, the frequency of new cancer was 3% compared with 1% in the control group. In a larger patient database study, the incidence of cancer or cancer related mortality was not increased with Becaplermin treatment compared with the control group(52). However, patients who had been treated with more than three tubes of Becaplerminshowedincreased cancer risk(51). In both studies, there was no association with any particular type of malignancy and all cancers were remote from the treatment site (51, 52). This led to an announcement by the FDA in 2008 warning of the malignancy risk associated with Becaplermin (52).

Randomized controlled trials conducted on patients with advanced stage PUs (stage III and IV) demonstrate improved healing outcomes with the application of topical rh-PDGF(59-

60).In a double blind study, patients with advanced stage PUs were randomized to receive placebo, or 100mcg/g or 300mcg/g of Regranex gel once daily until theyachievedcomplete healing or for 16 weeks. Use of the gel at 100mcg/g and 300mcg/g significantly increased the incidence of complete and ≥ 90% healing and reduced the median relative ulcer volume at endpoint compared with placebo. The adverse reactions described in this study included rash, erythema and fever; however, these events were attributed to the underlying disorder and age of the patients (60).

Topical application of PDGF to VUs has been attempted with minimal efficacy (61).The reasons for failed efficacy may be due to problems in growth factor penetration into the wound, without which the growth factor cannot reach its target cell. This is further complicated by the fact that growth factor application often requires daily treatmentthat interrupts the use of standard limb compression (61).The use of gene transfer to introduce PDGF into animal wounds demonstrated superior results to topical application (62-66). In one study peri-ulcer injection of a replication-incomplete adenoviral construct expressing PDGF was injected into patients with chronic VUs. The injection was well tolerated with no reports of erythema or pain. 73% of patients showedevidence of wound healing after 4 weeks; however, this percentage dropped to 47% at 24 weeks of care. It was noted that patients did not consistently wear limb compression during this time, suggesting that unless a patient wears their compression dressing they are unlikely to heal(62).

In the clinical setting there is evidence that rh-PDGF is effective in the healing of DFUs that extend beyond the level of the subcutaneous tissue (55). However, given the cost and its variability in number needed to treat to achieve healing, it is a reasonable choice in those wounds which have not healed after optimal conservative treatment. In addition, there is also benefit to

using rh-PDGF in patients with advanced stage PUs. Venous ulcers have had less success with treatment with topical rh-PDGF, however, the advent of new gene therapies as vehicles for growth factor administration have promising results. Larger randomized controlled trials are needed to test its efficacy in PUs and VUs. In addition, further studies are needed to test the efficacy of rh-PDGF in the treatment of ischemic DFUs, and those that do not extend beyond the dermis.

Fibroblast Growth Factor

Within the fibroblast growth factor family(FGF), FGF-2, FGF-7 and FGF-10 have been shown to be integral in cutaneous wound healing. FGFs are produced by keratinocytes, fibroblasts, endothelial cells, smooth muscle cells, chondrocytes, and mast cells (67-72).FGF-2 or basic FGF (bFGF) is increased in acute wounds and plays a role in granulation tissue formation, re-epithelialization and tissue remodeling (73).*In vitro* studies have demonstrated that FGF-2 regulates the synthesis and deposition of various EMC components, increases keratinocyte motility during re-epithelialization (74-76), promotes the migration of fibroblasts and stimulates them to produce collagenase(77). Levels of FGF-2 are decreased in chronic wounds (50).

Recombinant human-bFGF (rh-bFGF) topical application has shown some promise in the management of PUs. In one randomized controlled trial, patients with PUs (stage III/IV) were randomized to receive placebo or rh-bFGF at varyingdoses (1mcg/cm^2, 5 mcg/cm^2,10 mcg/cm^2). When all subgroups were combined, comparison showed a general trend toward increased healing over time. In addition, when assessed histologically, rh-bFGF- treated wound tissuedemonstrated increased number of fibroblasts and capillaries. More patients in the treatment group achieved >70% wound closure (78).The positive effect in PUs can be seen with

statistical significance ina more recent study by Robson Et al. wherethe effects of a combination of growth factor therapies on PU healing were compared. This trial found that wounds treated withrh-bFGF had better healing ratesthan rh-bFGF and rh-GM-CSF together and rh-GM-CSF treatment alone when compared to placebo. In this study, rh-bFGF was applied at a dose of5.0 mcg/cm^2(33). In a case control study, treatment of PUs with rh-bFGF accelerated wound healing over time when compared to control with respect to volume ofexudate, ulcer depth, granulation tissue formation and epithelialization (79).A study examining the long term outcomes of use of rh-bFGF in PUs found that 84.6% of patients who received rh-FGF and achieved ≥85% healingduring the treatment phase remained healed after 1 year (80).

The effect of rh-bFGF on DFU management showed more variable effect. In arandomized controlled trial, patients with diabetic ulcers on the plantar surface of the foot were randomized to receive topically applied rh-bFGF or placebo. The wounds were Wagner grade 1-3 and more than 0.5 cm in largest diameter. A concentration of 5mcg/mL rh-bFGF or placebo was applied daily for 6 weeks and then twice a week for thefollowing 12 weeks. After the study period there was no difference between healing rates for patients receiving rh-bFGF or placebo (81).More recently,a randomized controlled clinical trial conducted by Uchi Et al. with patients suffering from non-ischemic diabetic ulcers with a Wagner grade of 2 and measuring 900 mm^2 or less were randomized to placebo or treatment with 0.001% (50 mcg) rh-bFGF and 0.01% (500mcg) rh-bFGF for up to 8 weeks. Differences in healing were significant. Ulcerstreatedwith 0.01% rh-bFGFshowed a75% or greater reduction in the area of the ulcer compared to placebo (82).

Treatment with bFGF has demonstrateda positive effect in second degree burns. In one randomized controlled trial, patients with superficial and deep second degree burns were

randomized to receive placebo or daily topical recombinant bovine bFGF (rb-bFGF). All patients treated with rb-bFGF had faster granulation tissue formation and epidermal regeneration than those in the placebo group. Superficial and deep second-degree burns treated with rb-bFGF healed in a mean of 9.9 days and 18.0 days respectively, which was significantly better than the placebo group (83).Topical oxygen therapy supplementing bFGF application accelerated deep second-degree burn healing(84). In addition, the use of bovine amnion in conjunction with topical rb-bFGF was found to decrease healing time for deep partial thickness burn wounds (85).In anobservational study in rats, treatment of deep partial thickness burn wounds using gene gunmediated delivery of the recombinant human bFGF (rh-bFGF) gene shortened complete healing time by 1.75 days and increasedhydroxyproline and collagenase levels during healing (86).

Other important members of the FGF family include FGF-7 (keratinocyte growth factor-1 (KGF-1)) and FGF-10 (keratinocyte growth factor-2 (KGF-2)). *In vitro* studies have shown that FGF-7 and FGF-10play an important role in re-epithelialization by stimulating proliferation and migration of keratinocytes(67). In addition, FGF-7 and FGF-10 increase transcription of factors involved in the detoxification of reactive oxygen species (ROS). This helps to reduce ROS-induced apoptosis of keratinocytes, preserving these cells for re-epithelialization (87).*In vitro*studies have also shown FGF-7 to be important during later stages of neovascularization when luminal spaces and basement membranes are being developed. It is a potent mitogen for vascular endothelial cells and helps in the up regulation of VEGF. It also stimulates endothelial cells to produce urokinase type plasminogen activator, a protease required for neovascularization(88).

Repifermin, a recombinant human KGF-2 has been used clinically with mixed results. A randomized, double blind, parallel group placebo controlled multi-center study was conducted to evaluate the safety and efficacy of topical Repifermin treatment for 12 weeks, in the healing of chronic VUs. Repifermin accelerated wound healing; with significantly more patients achieving 75% wound closure in the treatment group over placebo (89). However, in a randomized double-blind, parallel group multicenter clinical trial conducted by Robson et al, there was no significant difference in percent of VUs achieving 100% closure after 20 weeks when administering 60 $\mu g/cm^2$ or $120\mu g/cm^2$ of topical Repifermin Time to complete closurewas not statistically significant(90).

While rh-bFGF showeda potential healing effect in PUs, DFUs and second degree burns, studies have been limited to small trials. Larger randomized controlled studies are needed to confirm efficacy. There are currently no published studies analyzing the use of rh-FGF in chronic VUs. However, a clinical trial is underway looking at the effects of topical FGF-1 in the treatment of VUs.

Vascular Endothelial Growth Factor

Much like FGF, the vascular endothelial growth factor(VEGF) family is made up of multiple members (VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E) (67,91).VEGF-A has been shown to be important in wound healing by promoting the early events in angiogenesis, particularly endothelial cell migration (67, 92-94)and proliferation (67, 95-98). VEGF-A is produced by endothelial cells, keratinocytes, fibroblast smooth muscle cells, platelets, neutrophils and macrophages(67, 99-104). In animal studies, the administration of VEGF-A has been shown to restore impaired angiogenesis found in diabetic ischemic limbs (105-108).Other

in vivo experiments show that VEGF-A improves re-epithelialization of diabetic wounds associated with enhanced vessel formation(109).

Despite these improvements, it has been shown that exogenous administration of VEGF induced sustained vascular leakage and promotedthe formation of disorganized blood vessels as well as malformed lymphatic vessels(110,111). Arecombinant human-VEGF (rh-VEGF) gene carrying plasmid, VEGF165, hasbeen used in patients with diabetic and ischemic wounds. Intramuscular gene transfer of VEGF165 to patients with ischemic ulcers and or rest pain secondary to peripheral arterial disease resulted in limb salvage significantly decreasing rest pain(112).

Randomized controlled trials have beenconducted on the efficacy of topical application of rh-VEGF (Telbermin) in patients with neuropathic DFUs. In onestudy, subjects with type 1and 2diabetes mellitus were randomized to receive either topically applied Telbermin treatment (72 mcg/cm^2) or placebo to the foot ulcer surface(113). Subjects received treatment 72 mcg/cm^{2}every 42 days for up to six weeks(112).There were positive trends suggestive of potential signals of biological activity observed for incidence of complete ulcer healing (41.4% treatment vs. 26.9% placebo) and time to complete ulcer healing (32.5 days treatment vs. 43 days placebo) (112). Currently there is a phase II, double blind randomized placebo controlled study to assess the safety/efficacyof Telbermin inthepromotionof DFU healing.

While other non-healing wounds such as PUs and VUs may benefit from exogenous VEGF administration given that they have local areas of ischemia, randomized controlled trials for treatment in these wounds havenot been conducted to date.

Proteases

The milieu of the chronic wound not only contains reduced levels of many growth factor important for wound healing, but also has been shown to have an imbalance between collagenolytic activity and endogenous inhibitors. Such harsh environment of high protease activity represents a challenge in sustaining presence of growth factors and cytokines. It has been demonstrated that there is rapid degradation of exogenous growth factor when applied to chronic wounds. In one study, exogenous TGF-B and PDGF were incubated with fluid from venous stasis ulcers, pressure ulcers and acute surgical wounds. There was significant degradation of these growth factors in the chronic wounds. In particular, there was a correlation between the level of neutrophil elastase and degradation, with those wounds expressing greater levels of elastase having more degradation. Furthermore, applying an inhibitor to neutrophil elastase blocked degradation. (114)

Matrix metalloproteases, specifically MMP-2 and MMP-9 have also been shown to be elevated in chronic wounds. (115,116). One study looked at acute surgical wounds and non-healing pressure ulcers and found elevated levels of MMP-2 and MMP-9 on the order of 10-fold and 25-fold respectively in the non-healing pressure ulcers. In addition, this study demonstrated that there were elevated levels of collagenolytic activity in the non-healing pressure ulcers. (116).The abundance of activated proteases is synergized with abnormally low levels of protease inhibitors. One study showed that tissue inhibitor of metalloproteases-1 is decreased in chronic wounds.(117). Given these findings simply adding an exogenous growth factor to a chronic wound is not enough for successful healing to occur. Application of protease inhibitors in combination with wound debridement could create a microenvironment that is more suitable for growth factor activity.

Debridement

Prior to the application of any exogenous growth factor, the target wound should be sharply debrided. Cells grown from the non-healing edge of a wound have diminished capacity to migrate or respond to wound healing stimuli, whereas the cells derived from the adjacent, non-ulcerated area of the wound showed increased capacity to migrate and responded well to wound-healing stimuli(118). More specifically, differential expression of various growth factor receptors on the surface of cells derived from non-healing wound edges suggest a possible explanation for their diminished responsiveness to wound-healing signals (118).

The goal of operative debridement is to remove hyperkeratotic tissue, necrotic tissue, functionally abnormal and infected tissue; all of which inhibit wound healing (119-121).In this manner, the remaining tissue, although physiologically impaired, can then better respond to exogenous topical treatment (122).Debridement of chronic wounds isa safe and effective technique.The indications for debridement include: 1) removal of asource of sepsis, defined as systemic inflammatory response syndrome in the presence of infection, 2) removal of local infection to decrease bacterial burden to reduce the probability of resistance to antibiotic treatment, and to obtain accurate cultures, 3) collection of deep cultures taken after debridement from the tissue left behind to evaluate persistent infection and requirement for systemic antibiotic treatment, and 4) stimulate the wound bed to promote healing and prepare for a skin graft, flap,topical application of growth factors or cell therapy(122).

In summary, growth factors and cytokines are essential for the regulation and coordination of wound healing and have been demonstrated to be insufficiently present or dysfunctional in the non-healing wound.(113, 123-125)This underscores the importance of evaluating the functionality of the signaling pathways in patients prior to proceeding with clinical testing. For example, a possible aberrant localization of the respective receptors and

presence of essential signaling molecules in patients' tissueshould be assessed prior to clinical trials to minimize potential costs and further justify use of particular growth factor.

Administration of exogenous growth factors and cytokines has shown promise in improving healing results in wounds. The four growth factors that have shown the greatest potentialin randomized controlled trialsinclude GM-CSF, PDGF, bFGF and VEGF. Current studies to date are small, and have disparate endpoints and modes of growth factor and cytokine administration. Larger randomized controlled trials are needed to supportefficacy, side effect profiles, and long term outcomes.All data should be interpreted with caution and any off-label use of these products for the management of wounds should be used in conjunction with the standard of care for non-healing wounds. Areas for future study include optimal delivery methods for growth factors and use of different combinations of growth factors and other adjuvant therapiesin addition to debridement.

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Table 1- Efficacious Growth Factors and Cytokines in the Management of Non-Healing Wounds *Based on RCTs*

Growth factor/ Cytokine	**Generic Name**	**Brand Name**	**Administration**	**Wound Type**	**Dosage/Frequency**
rh- GM-CSF	Sargramostin Molgramostim	*Leukine* *Leucomax*	Topical or subcutaneous injection	Chronic Venous Ulcers	400mcg perilesional injection/ single dose
Rh-G-CSF	Filgrastim			Second degree burns	200-400mcg perilesional injection/ weekly
				Diabetic foot ulcers (deep beyond subcutaneous tissue)	100mcg/m^2 topical / daily 5mcg/kg perilesional injection/ initial dose 2.5mcg/kg perilesional injection/ subsequent doses daily
rh-PDGF- BB	Becaplermin	*Regranex*	Topical	Diabetic foot ulcers (deep beyond subcutaneous	100mcg/m^2 topical/ daily

				tissue) Pressure ulcers (stage III/IV)	100mcg/m^2 topical/ daily
rh-VEGF	Telbermin	N/A	Topical	Diabetic foot ulcers	720mcg/^{2} topical/ every 48 hours
rh-bFGF	N/A	N/A	Topical	Pressure ulcers (stage III/IV)	500mcg/m^2 topical/ daily

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