Autologous Platelet Grafting Procedure – A New Approach to Healing Chronic Wounds and Comparison between Current Therapies

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Diabetes Mellitus is a major public health problem, affecting about 5% of the US population. Diabetes and its complications are the third leading cause of death in this country. It is estimated that about 60% of all non-traumatic amputations are performed on diabetics. A diabetic foot complication is the most common reason a diabetic patient is admitted to the hospital. This frequently ends with amputation. Amputation is extremely costly. The average amputation on the lower extremity is about $40,000 per wound. Annually in this country, that averages $7 to $9,000,000 per year. About $10 billion is spent annually on the care of chronic wounds in general. This figure is increasing each year due to the increasing incidence of diabetes mellitus. It has been shown that the longer a wound is present, the greater the chance of amputation.[3,27,18] The emotional and social cost to the patient and family is immeasurable, however, the resultant impairment and disability can be catastrophic. After a single lower extremity amputation in the diabetic, there is a 50% probability of developing a serious lesion on the contralateral foot within 2 years.[11] The 3-year survival rate in diabetics with a lower extremity amputation is 50%.[21]

The pathophysiology of diabetic foot ulceration has been extensively studied. There is a triad of major contributing factors: peripheral neuropathy, peripheral vascular disease, and abnormal biomechanical stresses.[20] Neuropathy is the most implicated causative factor. Boulton found in a review of several studies that neuropathy was a factor in 90% of more than 600 ulcerations.[7] When neuropathy occurs, loss of protective sensation ensues, leading to abnormal stresses and increased risk of ulceration. Peripheral vascular disease also plays a vital role. Persons with diabetes have a higher incidence of vascular disease than the non-diabetic population, particularly in the vessels below the knee. Diabetics are known to be at risk for both macro- and microvascular disease. With reduced blood flow, healing of any wound is compromised.

Patients with diabetes who participate in a comprehensive multidisciplinary approach to foot sequela seem to have lower incidences of foot complications.[9,19] Studies have shown that primary healing with traditional means is more cost effective than healing with amputation.[2] Amputation is not a definitive end all therapy. Many problems have been thoroughly described following amputation.[11,21] Traditional primary therapies typically include off-loading the wound, sharp debridement of necrotic tissue, control of infection through parenteral and oral antibiotics, revascularization for ischemia, and protective dressings. While these traditional therapies have stood the test of time and have proven very beneficial, many wounds fail to respond to these therapies and persist even with adequate blood flow. In recent years, there have been alternative therapies described including hyperbaric oxygen, electrical stimulation, and topically applied growth factors. These treatments are just beginning to be thoroughly studied and some show exciting potential.

Topically applied growth factors represent a new approach to treating chronic wounds. It has been shown that many of the discovered growth factors exist in the wound space.[5] Platelets are known to contain high concentrations of different growth factors and are extremely important in the wound healing process. The entire wound healing process is beyond the scope of this article, however, activation of the platelet by endothelial injury initiates the wound healing process. When platelets are activated, their α-granules are released, resulting in an increased concentration of growth factors in the wound milieu. There is increasing evidence that the platelet cell membranes themselves also play a crucial role in wound healing through their receptor sites.

Growth factors have in recent years received much attention in the literature. They are found in a wide array of cells. The α-granule in the platelets is known to contain high concentrations of many endogenous growth factors including platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), transforming growth factor beta (TGF-β), epidermal growth factor (EGF), and insulin-like growth factor (IGF).[4] Table 1 gives an overview of some of the more extensively studied growth factors and their involvement in wound healing. There are many more, both discovered and undiscovered. The platelet is

an extremely important cell in wound healing because it initiates and plays a major role in the wound healing process.[14,15]

The first discovered growth factor was EGF in 1962 by Cohen.[8] It wasn't until 1989 before clinical trials with EGF were attempted to demonstrate enhanced wound healing.[6] Studies did demonstrate that EGF can accelerate epidermal regeneration and enhance healing of chronic wounds.

IGF-1 is another well-studied growth factor found in high concentration in platelets. IGF-1 has several functions: (1) chemotaxis for vascular endothelial cells into the wound which results in angiogenesis, and (2) promotes differentiation of several cell lines including chondroblasts, myoblasts, osteoblastes, and hematopoetic cells.[23]

TGF-β is a member of the newest family of proteins discovered. Two major sources of this protein are the platelet and macrophage. TGF-β causes chemotactic attraction and activation of monocytes, macrophages, and fibroblasts. The activated fibroblasts enhance the formation of extracellular matrix and collagen and also stimulate the cells' ability to contract the provisional wound matrix.[22]

Possibly the most important and most studied growth factors is PDGF. It was discovered in 1974 and is ubiquitous in the body. It is known to be released by platelet α-granules during wound healing, and stimulate the proliferation of many cells, including connective tissue cells. In fact, thus far, high affinity cell surface receptors specific for PDGF have only been demonstrated on connective tissue cells. When released, PDGF is chemotactic for monocytes, neutrophils, and fibroblasts. In an animal model, it was shown that upon stimulation by PDGF, monocytes and fibroblasts release their own PDGF, thus creating a positive autocrine feedback loop.[13] Other functions of PDGF include effects on cell growth, cellular migration, metabolic effects, and modulation of cell membrane receptors.[1] It exists as a cationic glycoprotein of approximately 30,000 M_r. With reduction of disulfide bonds, multiple protein species are produced of 14,000 to 17,000 M_r.[25] With sequential analysis, two distinct bands were seen. These chains are termed α and β chains. Both chains appear to possess mitogenic activity, but less than the parent molecule.

Knowledge of growth factors and their function is far from complete. Many of the known functions were learned through *in vitro* study. *In vivo* study is much more complex due to the inability to control the environment. Further complexing matters is the fact that the same growth factor, depending on the presence or absence of other peptides, may display either stimulatory or inhibitory activity within the same cell.[12] Also, a particular growth factor can alter the binding affinity of another growth factor receptor.

Becaplermin gel (Regranex®) 0.01% is a recombinant DNA form of PDGF, consisting of a homodimer of two β-chains. It has been shown to improve the healing rate in pressure and diabetic ulcers. It is currently only FDA approved for use in diabetic foot ulcers. A recent, large, multi-centered phase III trial involving decubitus ulcers was recently stopped, apparently because the healing rates using becaplermin was not increased compared to placebo. Steed[26] reports on results from becaplermin gel therapy. In his study, 118 patients with full-thickness, lower extremity diabetic ulcers were randomized to receive either becaplermin gel 30 μg/g or placebo gel once daily. Study length was resurfacing of the ulcer or 20 weeks, whichever occurred first. 48% of patients receiving becaplermin achieved complete wound healing while 25% of the placebo group did. Also, the median reduction in wound area was statistically significant at 98.8% reduction vs. 82.1%, treatment group vs. placebo respectively.

Wieman *et. al.*[28] reported on the safety and efficacy of becaplermin in a phase III randomized double-blind study. He included 382 patients with type 1 or type 2 diabetes with chronic ulcerations of at least 8 weeks' duration. Patients were divided into three groups who received: (1) becaplermin 30 μg/g, (2) bacaplermin 100 μg/g, or (3) placebo gel. Patients applied moist saline-soaked gauze dressings twice daily and applied the gel at the evening dressing change. Good wound care including sharp debridement of necrotic tissue was administered to all patients. End point was complete resolution of the ulcer or 20 weeks, whichever came first. 50% of patients receiving the 100 μg/g dose of becaplermin gel had complete closure of their

wound compared to 35% of the placebo group. The 30 μg/g becaplermin group did not show statistical difference from the placebo group.

Rees[24] and associates reported on 124 adults with pressure ulcers. They were treated with becaplermin gel 100 μg/g, 300 μg/g, or placebo gel. There was no statistical difference between the two different doses. Endpoint was complete healing, > 90% wound volume reduction, or 16 weeks. 23% of ulcers were completely healed by 16 weeks utilizing becaplermin 300 μg/g. This compared to 19% and 0% for the 100 μg/g and placebo respectively. 59% of ulcers treated with the 300 μg/g dose and 58% of the 100 μg/g achieved ≥ 90% healing. However, 29% of the placebo group achieved ≥ 90% healing.

Procuren®, or Thrombin-Induced Platelet Releasate (TIPR) is a solution of growth factors, manufactured through an autologous process by Curative Health Services, Inc. A patient's blood is harvested and the platelets separated. The platelets are then treated with thrombin causing platelet activation and the release of their granular contents. The specific growth factors that have been identified in Procuren® are PDGF, TGF-β, and basic FGF. The solution containing the growth factors are then diluted in a buffered solution, processed, and packaged for patient use.

There have been several studies utilizing TIPR. In studies, it is called platelet-derived wound healing formula, or PDWHF. Knighton *et. al.*[16] has published data on his experience with PDWHF. In a non-randomized trial involving 49 patients with wounds of various etiologies, successful re-epithelization was obtained in 90% of the patients. The average time to 100% re-epithelization was 7.5 weeks, with a range of 1-22 weeks. Knighton again reported on results from a double-blind, crossover, placebo-controlled study.[17] A total of 32 patients were randomized into a treatment group and control group. After 8 weeks, the control group was crossed over to treatment. 17 out of 21 wounds in the treatment group achieved 100% epithelization in an average of 8.6 weeks. 2 out of 13 wounds in the placebo group healed. The remainder were crossed over and treated. All 11 remaining wounds healed in an average of 7.1 weeks. There were several problems identified by Knighton: the study sample was small, and after randomization, the control group wounds had a higher total wound score due to a higher infection score. Glover *et. al.*[10] reported on a 4 year multi-center retrospective study involving 3830 patients. They looked at patients receiving comprehensive wound care + TIPR compared to comprehensive wound care alone. There was a 43% higher healing rate in patients with diabetes. Patients with pressure ulcers demonstrated a 53% higher healing rate, and patients with arterial insufficiency had a 36% higher healing rate.

Autologous platelet grafting has recently been implemented in the treatment of chronic wounds. It is rich in growth factors however differs substantially from TIPR or any previous growth factor therapy. The process involves collecting blood from a patient and pheresing it to obtain a platelet-rich concentrate. A semi-solid graft is then constructed by activating the platelet concentrate with a series of reagents. This entire process takes about 20 minutes. After standard wound preparation, the graft is applied and the wound dressed. This dressing is left intact for 5-7 days. Depending on wound progression, this process may be repeated at 2 week intervals. This process differs from Procuren® in several ways:

(1) The native growth factors are not separated from the resultant supernatant. Not only that, with the autologous platelet graft procedure, a patient benefits from every growth factor made by the body, both unknown and known, and in high concentrations.

(2) The resultant platelet graft is not diluted thus much more concentrated. It also is not stored, processed, buffered, or frozen. Some studies suggest that the biology of the growth factors are altered when frozen and stored for long periods of time as with Procuren®.

(3) The autologous platelet graft is left in place 5 days thereby negating daily dressing changes. This decreases cost.

(4) The procedure is performed and directed by the physician at bedside, thus

negating costly processing, freezing, storing, and the need for the patient to directly apply a product. This eliminates the potential for patient to patient cross contamination and the need for expensive viral screenings.

The current process described compares extremely favorable to Regranex® (becaplermin gel). Becaplermin gel is a DNA sequenced gel that contains ββ chains of PDGF. Studies have demonstrated that most of the PDGF (75% of it) isolated from human platelets and in wound fluid exists as an αα homodimer molecule[29]. Further study has demonstrated that wounds increase the synthesis of the αα homodimer molecule when they are treated with becaplermin gel[30] in both chronic and acute wounds, however, PDGF-αα production in chronic wounds was substantially delayed. In treating wounds using autologous platelet gel, the wound is subjected to high concentrations of the native PDGF-αα, other forms of PDGF, and all other known and unknown growth factors in proportionate concentrations.

Autologous platelet gel, unlike Regranex® or Procuren®, contains the platelet cell membrane which is proving to be vitally important in the wound healing process. The cell membrane contains cellular receptors that bind cytokines and growth factors which are responsible for additional chemotactic activity as well as participating in the coagulum matrix.

Our early experience with autologous platelet grafting has been extremely positive. We have not had complications to date. Any treatment that can reduce healing times of diabetic ulcers would greatly benefit the patient. The potential savings to the health care system and the benefit to the patient in reducing risk of amputation would be enormous. Our early data shows average healing time to closure is 4 to 6 weeks irrespective of wound size. We have had ulcers fully close (100% epithelialization) in as little as 7 days. Obviously further study is needed involving randomized patients, a larger sample size, and blinded investigators. Should additional studies continue to repeat our early data, the benefits will be immeasurable.

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TABLE ONE (1)

Growth Factor	Function
PDGF, Il-1	Neutrolphil chemotaxis
PDGF, TGF-β, IL-1	Macrophage chemotaxis
EGF, PDGF, TGF-β	Fibroblast chemotaxis
EGF, PDGF, IGF, TBF-β TGF-α, IL-1, TNF-α	
EGF, acidic & basic FGF TGF-β, TGF-α, TNF-α	Angiogenesis, endothelial cell chemotaxis, mitogenesis
EGF, basic FGF, TGF-α, TGF-β	Epithelialization
EGF, basic FGF, PDGF	Collagen synthesis
TGF-β, IL-1, TNF-α, basic FGF, PDGF, TGF-β	Fibronectin synthesis
basic FGF, PDGF, TGF-β, IL-1	Proteoglycan synthesis
basic FGF, TGF-β	Wound contraction
EGF, PDGF, TGF-β, IL-1, TNF-α	Scar remodeling, collagenase stimulation

PDGF – Platelet Derived Growth Factor, IL – Interleukin, TGF- Transforming Growth Factor, EGF – Epidermal Growth Factor, IGF – Insulin-Like Growth Factor, TNF – Tumor Necrosis Factor, FGF – Fibroblast Growth Factor.

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