ELECTROMAGNETIC LINKAGES IN SOFT TISSUE WOUND HEALING

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In: Bioelectromagnetic Medicine, 2004 ed. PJ Rosch, MS Markov, Decker, ISBN 0-8247-4700-3
Chapter 30 pp 461-483

I. Introduction

The concept underlying electric current therapy (ET) or electromagnetic field therapy (EMFT) for soft tissue wound healing is that the fields and/or currents beneficially affect functional aspects of cells and processes involved in tissue repair. For soft tissue wound healing, the most relevant application is for patients with wounds that are chronic, non- or slow-healing, or otherwise recalcitrant to standard therapy. The rationale for its use has multiple historical bases, most notably its therapeutic efficacy in bone healing. Extensions to soft tissue wound, or ulcer, healing have evolved with their own plausible rationales, which in part stem from the body's natural bioelectric system(1, 2) and early observed relationships between electrical events and wound repair(3). At the macroscopic level, naturally occurring current loops of about 10 μA have been measured in the legs of humans(4), and at the microscopic level, membrane function is largely determined by intrinsic electrical processes. Because dermal wounds interrupt normal transepithilial potentials at injury sites, the electric field and injury current that develop are postulated to play an important role in the healing process (5, 6). Central to this concept is the fact that cells involved in wound healing are electrically charged, so that endogenous bioelectricity may facilitate cellular migration to the wound area, and might be involved with angiogenesis(7) and other wound healing processes. The extension of this concept is that if wound healing becomes stalled, external electrical stimulation may mimic one or more of the bioelectric effects and help to trigger a renewed healing progression. It has also been suggested that externally applied EMF may interact directly with the wound currents or with related signal transduction processes(8), thereby restimulating retarded or arrested wound healing. Wound healing acceleration via direct currents in the range of 200-800 μA, applied via a portable unit, may be an example of such a process (9). Research indicating potential benefits of both electric current and electromagnetic fields on a variety of cellular or other processes involved in wound repair are available: Reviews (8, 10) indicate effects that include edema reduction, neutrophil and macrophage attraction, growth factor receptor upregulation, fibroblast and granulation tissue proliferation, epidermal cell migration, and increased blood flow, all of which are important for wound healing. However, many findings are only suggestive of beneficial outcomes for clinical wounds, and require verification in a clinical setting. Such studies in humans are made difficult by the complexity of the wound healing process itself and by logistical and practical aspects of clinically based wound research. In spite of these intrinsic difficulties, clinical research with ET and EMFT continues, with a number of promising findings and an increasing amount of direct and indirect evidence of benefits.

To date, wound studies have typically involved skin ulcers caused by arterial or venous dysfunction, diabetes related ulcers, pressure ulcers and surgical and burn wounds. Human testing has provided some evidence that some therapies help to trigger the healing of "stalled" soft tissue ulcers or wounds. Human studies showing a positive benefit of EMFT range from those on a single subject with multiple experimental wounds(11) through (a limited number of) randomized controlled trials. Many experiments on animals have also shown positive connections between ET or EMFT and wound healing. But most, if not all, of these are based on wound models that diverge in one or more important aspects from human "chronic" wounds, those in which repair is stalled and difficult to manage. Yet these are precisely the types of wounds that would most likely benefit from such adjunctive treatments. However,

many EMFT-related effects on cells, tissues and processes involved in tissue repair have been convincingly shown to occur, as will subsequently be described. A meta-analysis of studies on combined chronic wound types and therapies revealed that ET was associated with an overall weekly healing rate of 22% compared with 9% for controls (12). Extrapolation of these figures would indicate that those with ET would heal fully in less than five weeks compared to about 10 weeks without stimulation. In another meta-analysis, which included 613 wound patients, a significant favorable effect of ET or EMFT was concluded (13). However, many published clinical reports do not meet the rigorous inclusion criteria associated with the high level of confidence required to validate medical efficacy. Experimental protocols that control and adequately characterize patient, wound, and treatment variables are logistically difficult and very expensive of both time and money. However, the importance of this has been emphasized (14) and is being increasingly recognized, so it is likely that more well designed "randomized, controlled clinical trials" will be forthcoming.

The scientific case for an electrical or electromagnetic connection in soft tissue wound repair processes is neither complete nor fully validated. But based on many specific clinical, experimental and cellular observations, a clear linkage between EMFT and wound healing is strongly suggested. In July of 2002, the accumulated background of information led the U.S. Centers for Medicare and Medicaid Services to finally approve coverage for electrical stimulation as adjunctive therapy for stage III and stage IV pressure ulcers, arterial ulcers, diabetic ulcers, and venous ulcers, providing that improvement had not occurred after 30 days of standard wound treatment. Despite this implicit acknowledgement of efficacy, much is yet to be learned about the factors involved, mechanisms of action, specific targets, optimal dosing and patterns and temporal strategies for treatment. These aspects require further targeted research and exploration.

II. Overview of the Wound Healing Process

Normal Healing is characterized by three broad phases: inflammation, proliferation, and remodeling. These normally proceed in a well-ordered, functionally overlapping sequence, the outcome of which depends upon interactions among many cell types, growth factors and processes. Vascular, (platelets, macrophages, mast, neutrophils, monocytes, endothelial, and smooth muscle), epidermal (keratinocytes, melanocytes, and Langerhans cells) and dermal (fibroblasts and myofibroblasts) cells are involved (15). As part of the repair process, cells release and/or interact with many components including structural proteins, growth factors, cytokines(16), chemokines (17), adhesion molecules(18), nitric oxide(19), trace elements(20) and proteases. Any participant or interaction could, in theory, be a target for adjunctive electromagnetic field-related therapy.

In terms of the sequence and functional aspects of wound healing events, the initial inflammatory process serves to limit blood loss (via clotting), to promote entry of antibodies and fibrin into interstitial spaces (via increased vascular permeability), and to deliver needed blood flow to the affected area via vasodilation. This initial hyperemia increases oxygen delivery, which supports the antibacterial, (and other), actions of accumulating neutrophils. Activated macrophages, attracted to the wound area by chemotactic and/or galvanotactic signals associated with inflammation, release substances important for angiogenesis, for the development of granulation tissue, and for the proliferation of fibroblasts and epithelial cells. Angiogenesis involves endothelial cell proliferation and new capillary formation that serves to supply ischemic regions of the wound, and represents an important phase of granulation tissue development. It is stimulated by angiogenic factors released from macrophages in response to low oxygen in the wound (21), and by growth factors from fibroblasts and endothelial cells. Nitric oxide, present in the wound (22), affects macrophage and fibroblast functions and also affects the keratinocytes (23)). Fibroblasts migrate to the wound site and subsequently proliferate. Collagen synthesis is triggered by fibroblast-stimulating growth factors from macrophages, and continues at a rate that is

linked to the adequacy of local blood flow to deliver oxygen and nutrients for protein synthesis. These nutrients include amino acids and interestingly, ferrous iron. Epithelialization of dermal open wounds depends on an initial migration of epithelial cells, triggered by epidermal growth factor released from both macrophages and platelets, and subsequently on the proliferation of epithelial cells. Epithelialization can take days to months depending on wound related factors, and depends on keratinocyte proliferation, migration, stratification, and differentiation (24), and on features of the extracellular matrix (25). Wound closure occurs as a result of active contractile forces developed within myofibroblasts, which in turn depends on adequate blood flow to provide energy substrates. Wound closure does not end the wound healing process, and wound remodeling may continue for months to years. In the remodeling phase, wound strength is increased via collagen crosslinking, excess collagen within the wound is eliminated, and many of the capillaries developed during early wound healing are resorbed.

Retarded Wound Healing may be due to many factors. A wound is an added "metabolic organ," and appropriate progression of healing depends on the abilities of the body as a whole to supply the demands of this "temporary organ." Delay of wound healing beyond about three months is a criterion sometimes used to characterize a wound as "chronic" or "non-healing," the causes of which may be of systemic or local origin. Some of the factors that impede wound healing include infection, inadequate blood flow, tissue hypoxia (due to inadequate O_2 delivery or to increased O_2 demand by white blood cells or other exudate components), and inadequate nutrient availability to support tissue building metabolic processes. Certain conditions such as diabetes have further implications: Hyperglycemia and impaired insulin signaling may directly impair keratinocyte glucose utilization thereby altering both proliferation and differentiation (26). Inhibition of nitric oxide production diminishes wound-healing activities of fibroblasts and keratinocytes and causes delay in wound healing (27, 28). Deficiencies in wound concentrations of platelet activating factor are associated with impaired healing of chronic venous ulcers (29). Given the variety of causes for retarded wound healing, no "most important" electromagnetic target has been defined. However, because blood supply plays a major role in many of the processes, increases in blood flow and/or oxygen supply are often intrinsic targets.

III. EMF Methods and Strategies for Wound Healing

Therapeutic approaches using ET (direct skin contact using electrodes) and EMFT (non-contact) may be divided into two broad categories; (a) those applied at the wound site and (b) those applied remote from the wound site. Included in category (a) are electric currents and fields, generated in variety of ways, with a range of excitation patterns, in which the wound itself is directly exposed to the currents or fields. In the case of ET, an electrode may be placed directly in the wound bed or the wound may be in the path of electrode pairs that straddle the wound. Included in category (b) is electrostimulation (ET or EMFT) of either nerves or tissue regions that functionally connect with, and potentially alter, wound site processes, either directly or via reflex effects. Both categories have been reviewed as they relate to different wound conditions (30, 31).

Another useful broad distinction between devices that has been used for wound healing is whether they are mainly electric or mainly electromagnetic. In electromagnetic devices, no electrodes are needed and target tissues are exposed to electric and magnetic fields and their associated induced currents. Among electromagnetic devices, all use time varying or pulsed excitation, some of which modulate a carrier frequency, commonly 27.12 MHz. A further distinction among pulsed radio frequency devices is made with respect to their potential tissue heating effects which is related to the energy they deliver to the tissue. Commercially available EMF devices usually specify device average or peak power but these do *not* specify the energy or field strengths delivered to target tissues. Pulse width and shape generated

by most commercial devices is fixed (65--95 μsec), with the power per pulse usually controlled by varying pulse amplitude. Total power is adjusted by varying the pulse repetition frequency, which, for "nonthermal" devices, typically ranges between 80 and 600 pps (Diapulse and Sofpulse). Devices that function in nonthermal and thermal ranges may allow both variable pulse width and rates (Magnatherm, 700--7000 pps), whereas other devices provide no control features (Regenesis). Tissue thermal effects are thought to be minimized by use of low duty cycles, on the assumption that heating due to high power single, short pulses, will be dissipated during a much longer off-time between successive pulses. In general, for ET or EMFT, the parameter variants include generated power, excitation frequency, pulse width, repetition rate and duty cycle, carrier frequency, current magnitude, and magnetic field intensity. In addition there are variants with respect to specific features of the excitation patterns, *i.e*., whether stimulation is continuous or pulsed, galvanic or frequency modulated, biphasic or monophasic, symmetrical or asymmetrical, sinusoidal or not, and whether high voltage or low voltage stimulation is used(30-32). It is partly because of this wide range of physical excitation parameters that it has been impossible to correlate specific features with wound healing efficacy. However, it has been argued that the use of pulse radio frequency EMF (PREMF), with its inductive coupling to tissue, provides for a more uniform and predictable electromagnetic field signal in the target tissue than is currently achieved with surface contact electrodes(33). Thus, the tissue dose is more reliably characterized. It has also been argued that, because of the large spectral range of PREMF, there are more possibilities for coupling of the field to produce effects in a wider range of possible (but as yet unspecified) biological processes. More detailed technical descriptions may be found in several sources(30-34).

IV. Clinical Findings of EMF-Therapy for Specific Wound Types

A. Venous Ulcers

Venous ulcers occur on the lower extremities and are the most common chronic skin wounds in humans. Venous disease increase with age and results in venous ulcers in about 0.3% of the adult population (35). Venous reflux and venous hypertension due to incompetence of deep and communicating vein valves and thrombosis of deep vein segments are linked to the development of venous ulcers. The evolution of skin ulcers from venous hypertension is not fully understood, but contributory factors probably include inflammatory processes, intercellular and vascular adhesion molecule upregulation (36), protein rich edema, leukocyte trapping, oxygen deprivation, and microcirculatory deficits (37-40). Microangiopathy due to venous hypertension may have several manifestations that include abnormally dilated and tortuous capillaries, loss of some functional capillaries, microvascular thrombosis, increased capillary permeability and transcapillary fluid efflux, tissue edema and altered function of microlymphatics. Compression therapy has been reported to help normalize capillary numbers and size (41) and to tend to normalize the abnormally elevated limb blood flow (42). Healing has been reported to occur only after aspects of the dermal microangiopathy have improved (43). Increased activation of platelets, monocytes and neutrophils leading to microvascular aggregation (44) and microvascular entrapment of neutrophils (45) has been shown.

In a review of randomized controlled trials (RCT) (46), only three eligible studies were identified (47-49). The reviewers state that there is “currently no reliable evidence of benefit of electromagnetic therapy” in the healing of venous leg ulcers. Although this conclusion may be warranted for one of the reviewed studies, (too few subjects and mixed results), one may question judgments based on the other studies. One was double-blind and compared sham vs. active pulsed EMF therapy (75 Hz, peak field of 2.8 mT) in 37 patients (19 sham) for 90 days (47). At the beginning of the study, ulcers had been present for an average of 30 months in the actively treated group, versus 23 months in the controls. Stimulation was applied with an enclosed coil placed over the wound by patients, at home, for 3--4 hours per day. After 90 days, of the actively treated ulcers, 12 were healed, as compared to six in the sham group

($p<0.02$). Further, granulation tissue, not present prior to active treatment, was present in all patients actively treated by day 15, whereas only seven of the sham group showed new granulation tissue.

The other study (48) was a prospective, randomized, double-blind, placebo-controlled multicenter investigation of 27 patients with recalcitrant venous ulcers (mean duration of 39--47 weeks). Patients were treated for 8-weeks at home for three hours per day with a wearable portable EMF device (22 Gauss, bidirectional pulse of 3.5 msec, 25% duty cycle). All received compression bandaging and daily 3-hour leg elevation. At week 8 the active group (N=17) had a 47.7% decrease in wound surface area vs. a 42.3% increase for placebo ($P < 0.0002$). The investigators' global evaluations indicated that 50% of ulcers in the active group had healed or were markedly improved, vs. 0% in the placebo group. Other studies have directly indicated a beneficial effect of EMF therapy for venous ulcer healing. In one of these, twin 100 volt pulses (0.1 msec, 100Hz) were applied directly to the ulcers, which resulted in healing rates that were superior to standard therapy alone(50). In another study, EMF patterns were first tailored to interact with human monocytes, as judged by an in vitro assay, and then the EMF pattern was used as the sole treatment for patients with predominantly venous ulcers(51). Results, which were assessed with each patient's long standing and non-responding ulcer as a control, were suggestive of a beneficial action of the stimulation.

B. Arterial-Ischemic Ulcers

The main predisposing condition for arterial-ischemic ulceration is advanced peripheral artery disease affecting arteries supplying the lower leg and foot. These ulcers, which can be particularly painful and difficult to treat, frequently occur in areas subject to pressure or trauma such as between the toes or at the malleolus or posterior heel regions. Adjunctive therapies for ischemic ulcers for which standard available medical approaches have failed are sorely needed. In this author’s opinion they could become one of the most important targets of EMF therapy in the future. Recent pilot work (52) using high voltage pulsed currents to treat ischemic ulcers in six diabetic patients with very poor initial microcirculation, suggests that treatment can raise local oxygen levels sufficiently to save some legs from amputation.

Because the main impediment to wound healing in this condition is inadequate blood flow, effective EMFT would be expected to affect blood flow. There is substantial evidence that this is indeed a realizable goal. Although effects of EMFT on blood circulation are dealt with in detail elsewhere in this book, it is useful here to examine certain aspects that may be tightly linked to wound healing potential. Early work using PREMF addressed the issue of augmenting blood flow to ischemic regions via reflex effects. PREMF (27.12 MHz) was applied to the epigastric region using low duty cycle excitation in normal persons(53) and in persons with peripheral arterial disease(54). The idea was that the use of short but intense pulses could deliver useful therapeutic excitation without causing significant tissue heating. The physiological strategy of targeting a remote site (epigastric area), rather than the ischemic region itself (foot or ulcer), is to reflexively increase blood flow to the ischemic region without imposing added metabolic demand via local heating on the distant (ischemic) region. Results on 20 normal subjects showed a dose-dependent increase in foot perfusion as judged by toe-volume plethysmography and toe temperature, which rose an average of 2^{o} C, without a significant core temperature elevation(53). A series of 12 similar PREMF treatments (65 μsec, 600 pps) given over a period of two weeks to 18 patients with intermittent claudication, also resulted in an increase in toe temperature ($>3.0^{o}$ C), with no significant increase in core temperature(54). Although the duration of toe temperature elevation was short-lived after each 20 minute stimulation was ended, the cumulative effects appeared to be sustained, as measured by increased pain-free walking distance at the end of the two week treatment sequence. A toe temperature increase was also noted in normal subjects but to a lesser degree than in patients.

More direct measures of blood flow have used laser-Doppler perfusion monitoring (55-57) which permits skin blood flow to be directly monitored before, during and after EMF exposures. PREMF (65 μsec, 600 pps, one Gauss) was applied 1.5 cm above open foot ulcers in diabetic patients. Results showed an EMF-treatment related increase in periulcer blood perfusion. Based on the observed flow patterns, these authors judged that the increase was predominantly caused by an increase in the number of capillaries with active blood flow. This flow feature was consistent with an EMF-field-related capillary recruitment process(57) that may have reflected precapillary vasodilation. Similar microcirculatory flow increases were reported for forearm skin of normal persons (58) and for persons with post-mastectomy arm lymphedema (59), but interestingly, no effects of a static magnetic field (500 Gauss) were observed in the hands (60) or forearms of normal subjects(61).

To date there have been no reported clinical trials using PREMF directly for ischemic ulcers, but other forms of electrical stimulation have produced similar changes in blood flow. A particularly promising approach is epidural spinal cord electrical stimulation (ESES), which appears to benefit patients with severe lower extremity ischemia secondary to atherosclerotic disease. This therapeutic approach requires implanted electrodes at the T10--T11 level and usually the use of an implanted pulse generator. This therapy significantly increased microscopically measured blood velocity in capillaries and density of skin capillaries in the foot(62). In patients with rest pain and ischemic ulcers, this technique resulted in immediate pain reduction, and in most patients was accompanied by microscopically verified increases in capillary blood velocity and density, and a significant increase in post-occlusive microvascular hyperemia (63). In more than half of these patients, the ulcers subsequently healed, resulting in significant limb salvage. Other studies using ESES have shown similar limb salvage rates and ulcer healing potential (64, 65). In patients with and without ulcers, the degree of therapeutic success tends to correspond to an increase in transcutaneous oxygen tension (66-68), (which, is itself dependent on blood flow increases in the foot).

C. Diabetes-Related Ulcers

Persons with diabetes are more susceptible to developing skin ulcers due to neuropathy, ischemia and poor glycemic control. The higher likelihood of peripheral arterial disease and the presence of microvascular deficits increase the chances of ischemia, tissue to breakdown, and ulcer formation. Ulcers in diabetic patients are generally more difficult to heal for reasons that include reduced blood flow and wound oxygenation, deficits in wound cell function, and infection. Recent work has shown that it takes much less local pressure to reduce skin blood flow in regions of bony prominence in persons with diabetes(69). When sensory neuropathy is present, normal pressure/pain signals are diminished or absent, thereby removing warning of developing tissue injury. Most of these types of ulcers develop on the foot, with plantar ulcers often associated with neuropathy. Effective therapy should include elimination of elevated foot pressures combined with standard wound care, but this is not always adequate to effect wound healing. Statistics suggest that about 15% of persons with diabetes will get a foot ulcer(70), with an annual incidence rate of 2.2%(71). In this population, non-healing ulcers account for 54,000 extremity amputations per year(72), and an annual amputation incidence rate between 0.5--0.8% (number of amputations per patient-year)(73).

Pulsed-galvanic electric stimulation (50 volts, 100 μs), delivered through a conductive stocking for 8 hours every night, was used as an adjunct to standard care for healing diabetic foot ulcers in 40 patients(74). The study was of a randomized, double-blind, placebo-controlled pilot trial design, with half of the patients receiving ET and half receiving sham treatment. All received standard wound care including off-loading with removable cast walkers. Patients were followed for 12 weeks or until healing, whichever occurred first. Considering only patients who were protocol compliant, 71% of those actively treated healed, compared with 29% in the sham treatment group ($p=.038$). From these data the authors concluded that the ET improved wound healing. A different ET regime was employed to treat the ulcers

of a group of 80 diabetic patients. Daily treatment included a biphasic stimulation pattern consisting of either asymmetric or symmetric square-wave pulses at amplitudes set to activate intact peripheral nerves in the skin. Controls consisted of groups that received either very low levels of stimulation current, or no electrical stimulation. Average healing rates, measured weekly as changes in ulcer perimeter, were significantly greater than in controls only when the asymmetric treatment was used (75). In a group of 64 diabetic patients with chronic ulcers, electrical nerve stimulation was used therapeutically for 20 minutes twice daily for 12 weeks(76). The excitation parameters in this study consisted of an 80 Hz pulse train with a one msec pulse width and an intensity sufficient to evoke strong paresthesias. All patients received standard treatment with half also receiving either sham or active electrical stimulation. At 12 weeks, the active treatment group was reported to have significantly reduced ulcer area and more healed ulcers ($p < 0.05$).

In many ways, plantar ulcers in persons with leprosy resemble diabetic ulcers. In a pilot, randomized, double-blind, controlled clinical trial (77), 40 leprosy patients with plantar ulcers received standard treatment and half of them (EMF group) received exposure to pulsed sinusoidal magnetic fields (0.95 to 1.05 Hz, 2400 nT) for four weeks. Outcome measures were the calculated ulcer volume recorded on the day of admission and at the end of treatment. In the control group, mean ulcer volume at entry was 2843 mm^3 which was reduced to 1478 mm^3 at the end of treatment ($P = 0.03$); corresponding values in the EMF-treated group were 2428 $mm^3$and 337 mm^3 ($P < 0.001$). These data indicate that the EMF therapy caused a significantly more rapid healing of plantar ulcers in these leprosy patients.

D. Pressure Ulcers

Pressure (decubitus) ulcers result from sustained or inadequately relieved pressure, most frequently on bony prominences such as the heel and sacral region. These ulcers represent an important clinical, humanitarian and economic problem with an average prevalence in acute care facilities of 10.1%(78) and a reported incidence in persons age 65 and older of 0.18 to 3.36 per 100-person years depending on age(79). Ulcer development depends on many factors including age, nutritional status, mobility, skin irritations and general health status(80-82), but a final common pathway is associated with blood flow changes within pressure-loaded tissue (83-88). Some experimental evidence suggests that both ischemia and ischemia-reperfusion injuries are involved(89).The clinical stages of pressure ulceration range from non-blanching erythema (Stage I) through full-thickness skin loss with extensive destruction and tissue necrosis involving muscle or bone (stage IV).

Examining available literature-based randomized controlled trials led reviewers to the judgment that there is insufficient data from too few clinical trials to conclude that electromagnetic therapy to treat pressure sores is beneficial (90, 91). In spite of this conclusion, data from these reviewed studies, and others not included, do provide interesting and strongly suggestive findings of potential benefits of PREMF therapy. One small study (92) used PREMF (27.12 MHz) on patients with long-standing pressure ulcers and found significant improvement over standard treatment alone. Another study (93) was randomized and double-blind and used similar PREMF therapy or sham to treat a total of 30 spinal cord injured patients who had either stage II or III pressure ulcers. Wounds were treated for 30 minutes, twice daily, for 12 weeks, or until healed. The authors indicate that, after controlling for the baseline status of the pressure ulcers, PREMF treatment was independently associated with a significantly shorter median time to complete healing. An additional study (94) using the same PREMF method to treat patients with either stage II or stage III long-standing pressure ulcers, also reported improved healing. PREMF (20 and 110 pps) was also reported to trigger healing progress in five elderly males with trochanteric or sacral pressure ulcers(95). Similar positive results of pulsed ET (300--600 μA) were reported in a double-blind placebo controlled study of long-standing stage II and III pressure ulcers in which healing rates were significantly improved with active treatment(96). High voltage pulsed galvanic stimulation (200 volts, 100 pps) was used to treat 17 persons with spinal cord injury for 20 days. One

electrode was placed on the ulcer and one on the thigh. The reported outcome was a greater reduction in ulcer area as compared to a placebo group(97). In an extensive study of 150 persons with spinal cord injury, the use of pulsed biphasic ET (0.25 msec, 40 Hz, 15--25 ma), with electrodes applied across pressure ulcers, resulted in significantly faster healing(98).

Based on available clinical data, it appears to this author that a strong, if not conclusive, case is made for a beneficial effect of electromagnetic therapy for pressure ulcers. In fact, the National Pressure Ulcer Advisory Committee (NPUAC) has included electrotherapy as an adjunctive therapy for pressure ulcers that have failed to heal by other means. Further, aside from treatment benefits, there is some experimental evidence that ET of the gluteal muscles may have preventative effects related to beneficial buttock shape changes(99) and by increasing muscle thickness and blood flow(100, 101). High voltage pulsed galvanic treatment (75 volts, 10 Hz) of 29 persons with spinal cord injuries resulted in a 35% increase in sacral skin oxygen tension (102). Further studies of the role of EMF stimulation as a potential preventative modality would thus appear warranted.

V. Potential Physiological Targets of EMF Wound Therapy and Mechanisms

The mechanisms by which externally applied EMFs alter cellular properties and biological processes to effect improved wound healing are unknown, although there are many theories that describe how EMF interactions may occur at cellular and subcellular levels. Whatever the specific mechanisms turn out to be, it is this author's opinion that clinical efficacy depends on determining the proper therapeutic parameters and timing to optimally modulate cellular features and their interacting processes within the context of the wound healing cascade. Specific targets for any of the postulated mechanisms could theoretically be any of the cells, or functions, involved in the wound healing process. In the following subsections the focus is only on some of the main relevant experimental cellular targets and findings.

A. Endothelial Cells

A role of EMF stimulation on the growth rate of endothelial cells was suggested by studies in which partially denuded cell layers reacted to an external field in a manner similar to in vivo angiogenisis, but with an accelerated rate as compared to non-exposed cells(103). Other in vitro studies, in which cells derived from human umbilical vein and bovine aorta were subjected to repetitive five msec pulse bursts from a Helmholtz device at 15 Hz, produced corresponding results(104). In these studies the calculated electric field at the center of the tissue culture dish containing the cells was 1.3 mV/cm and the measured magnetic field was about one Gauss. By examining the rate at which the cells transformed from a monolayer configuration to tubular structures, after cell-layer wounding, the authors determined that vascularization rate was increased in the presence of the EMF stimulation.

Studies on human umbilical vein cells showed that endothelial cell migration to a wounded area is accelerated if cell cultures are exposed to a sawtooth pulse train (2 mT peak, 25 Hz) (105).These results were demonstrated in the presence of growth factor and an induced electric field (0.04 -- 0.11 volts/m) perpendicular to the wound edges. Further evidence of an angiogenesis-electrical connection stems from studies on skeletal muscle in which chronic stimulation of rat muscles resulted in an increase in blood vessel density, thought by the authors to involve both angiotensin and vascular endothelial growth factor pathways(106). Various pulsed EMF waveform patterns applied to the rabbit ear chamber(107), also suggested an EMF-affected increase in vessel growth, but results were highly selective and limited to a specific excitation pattern. Evidence that vascular smooth muscle relaxation may be induced by EMF exposure due to endothelial cell mediated processes is provided by studies of rings of bovine aorta(108). The rings, which were initially contracted with phenylephrine, were found to relax when exposed to the effluent from bovine endothelial cells treated with a pulsating electric field (one five second pulse train every 30 minutes, pulse width 0.1 msec, 30 volts, 100 -- 500 mA). Threshold levels for relaxation were

found to be between 0.5 to 1 Hz with a maximum relaxation at 16 Hz. The authors concluded in part that the EMF-induced endothelium-dependent relaxation was due to nitric oxide released from endothelial cells. Taken together, these findings offer strong evidence for an endothelial cell-electric connection which may affect both angiogenisis within the wound bed and vasoactive changes that mediate blood flow delivery. Whether or not these are related to enhancement of nitric oxide release, alone or in combination with other factors, represents an important research question.

B. Fibroblasts

Sinusoidal currents (300 Hz) applied for 15 minutes to rat incision wounds was reported to improve microcirculation and to stimulate proliferation and differentiation of fibroblasts (109). Sinusoidal magnetic fields (0.06--0.7 mT, 50, 60 and 100 Hz) increased chick embryo fibroblast proliferation (26--31%) with excitation frequency or intensity when the other was held constant (110). However, treatment of rat incision wounds with PEMF of the type and intensity used for bone healing failed to produce significant increases in soft tissue fibroblast counts or improvement in wound closure (111). More recent work on normal human fibroblasts exposed to 50 Hz, 20 or 500 mT for 1 or 4 days failed to show any significant effects on measured fibroblast parameters (112).

High voltage pulsed galvanic stimulation (HVPGS) of cultured human fibroblasts showed that increases in protein and DNA synthesis could be demonstrated, but only for specific combinations of voltage (50-75 volts) and pulse rates (100/s) (113). HVPGS also increased the rate of fibroblast formation and wound contraction in a pig burn wound model(114). When human dermal fibroblasts in a type I collagen dermal matrix were exposed to electric fields ranging from 18 to 1,000 mV/m at frequencies of 10 and 100 Hz, only a narrow amplitude window between 37 and 50 mV/m at 10 Hz yielded increases in cell proliferation, which, at the reported maximum (41 mV/m), resulted in a 70% increase in total DNA (115, 116). Fibroblast proliferation and collagen synthesis were also demonstrated in a tendon explant model when exposed for four days to 1--Hz, 1--ms duration pulses (peak 7 A/m^2, average 7 mA/m^2). Exposures to lower (1.8 mA/m^2) or higher (10 mA/m^2) current densities had either no effect or an inhibitory effect on fibroplasia (117). Dermal fibroblast growth into a collagen sponge matrix was found to be increased in the presence of direct currents between 20 and 100 μA, with maximum effects near the cathode at a current of 100 μA (118). Experimental surgical abdominal wounds in rats, when treated with an implanted stimulator (bipolar pulses, 0.87 Hz, 25 μA), showed earlier fibroblast formation and collagen deposition, and more rapid maturation and longitudinal alignment of the collagen fibers, which resulted in stronger scars (119). In rabbits, patellar ligament healing, with increased capillary and fibroblast densities and more mature longitudinally oriented collagen fibers, occurred earlier with pulsed (10 Hz, 25 μsec) EMF therapy. The most consistent results were obtained at a field strength of 50 Gauss(120). Recent work has indicated that when cultured fibroblasts are exposed to PREMF (27.1 MHz, 32 mW/cm^2, 15 minutes) there is a significant enhancement in cell proliferation(121, 122). Taken together these findings suggest that EMF affects aspects of fibroblast activities that are important to wound healing. However, the forms and patterns of excitation needed to consistently affect the fibroplasia features must to be further elucidated, an aspect that likely depends on a better understanding of the mechanisms involved.

Regarding possible mechanisms, it has been proposed that EMF stimulation of fibroblasts induces transmembrane currents that open voltage-controlled calcium channels causing ATP re-synthesis, activation of protein kinase mechanisms to synthesize cell protein, and DNA replication for mitotic cell division(123). Sinusoidal EMF exposure (20 Hz, 8 mT) of human skin fibroblasts (124) has been shown to change cellular calcium oscillation activity within 40 min, with responses (increase or decrease in dynamics) depending on a cell's differentiation state. It has been hypothesized that modulation of

proliferation and differentiation phases is triggered by immediate but transient increases in cAMP-dependent protein kinase activity (125).

Based on stimulation experiments (10--100 Hz, 0--130 $\mu A/cm^2$) with human dermal fibroblasts in a collagen matrix, an amplitude and frequency windowing process that may predict fibroblast proliferation conditions has been proposed (126). The proposed ion-interference mechanism considers the effects of induced electric gradients on protein-bound substrate ions. Tissue cultures of human foreskin fibroblasts, when exposed to 2 V/cm fields at either 1 or 10 Hz, demonstrated a six-fold increase in internal calcium, but excitation at 100 Hz had no significant effect(127). The fact that the internal calcium increase depended on external calcium concentration, and was blunted by a calcium channel entry blocker, suggested that the stimulation-induced calcium increase was due to increased calcium influx via voltage-gated calcium channels. Since the channel-gating process may be initiated by a membrane depolarization of 30--40 mv(128), it has been argued that the coupling with the applied external oscillatory field may be due to forced vibrational effects on free ions on either side of the plasma membrane, which in turn alter transmembrane potentials sufficiently to open voltage-gated channels(129). However such an oscillatory mechanism would not directly explain the fact that DC fields (10 V/cm) cause an even greater calcium increase(127). The fact that the kinetics of the calcium entry process saturate after about 30 minutes of continued field exposure may provide initial guidelines for durations of electrotherapy treatments.

In view of the many linkages between EMF stimulation and verified selective modulations of intracellular calcium, it would seem to this author that the search for optimal stimulation parameters to selectively control calcium fluxes is narrowing, and represents an exciting and useful research target. However, it is important to bear in mind that although calcium entry into fibroblasts is associated with fibroblast stimulation(130, 131), calcium entry effects on vascular caliber, and thus on blood flow, depends on the specific cell type experiencing the field-induced calcium influx effect. Increased calcium entry into vascular smooth muscle promotes vasoconstriction and blood flow reduction, whereas calcium increase in endothelial cells promotes synthesis of nitric oxide(132-134), which normally produces vasodilation and a blood flow increase.

C. Leukocytes and Macrophages

Much of the contribution of leukocytes to wound healing depends upon their activation during the inflammatory phase. This activation is associated with a respiratory burst, the release of cytokines and oxygen radicals, and an upregulation of cell surface receptors that increases adhesion between leukocytes and endothelial cells. Although entry of neutrophils into the wound area is needed for their antibacterial actions, a process perhaps initiated by electric field gradients via galvanotaxis(135), their continued entry, sustained presence, and activation may be associated with diminished local blood flow due to capillary plugging, abnormal vasoconstriction, and tissue damage associated with continued enzyme release. Evidence of such involvement in impaired healing comes from studies on genetically diabetic mice, in which the inflammatory phase is prolonged and dermal wound healing is significantly retarded(136). The sustained inflammatory phase was related to prolonged expressions of inflammatory and chemoattractant proteins that were expressed by keratinocytes and resulted in the persistence of both neutrophils and macrophages within the wound site. Under conditions in which the inflammatory phase is abnormally prolonged, actions of EMF stimulation that affect these and other features of activated leukocytes could influence the wound healing process. Of particular note is the fact that neutrophil activation is accompanied by oscillations in intracellular free calcium concentrations and membrane potentials at frequencies in the range of 0.05 to 0.1 Hz.

In a series of elegant experiments it has been shown that the intensity of these oscillations could be increased in the presence of electric fields (20 msec pulses) that were delivered during the trough of the oscillations at a rate that matched the intrinsic oscillatory frequency(137). The effect, termed metabolic

resonance, was found to occur with electric fields of 1 x 10^{-4} through 2 x 10^{3} V/m. An additional finding revealed that reactive oxygen metabolites, normally generated at the 0.05--0.1 Hz rate from migrating neutrophils, could be increased or terminated depending on the phase relationship between applied field and the intrinsic oscillatory process. Electrical stimulation has also been proposed to promote neutrophil, monocyte and macrophage migration to the wound area(138) by virtue of the interaction between their surface charge and the prevailing electric field. Selection of initial polarity (anode or cathode) placed on the wound in the case of electrode type stimulation may enhance this effect.

D. Keratinocytes

Normal wound healing depends on epithelial cell proliferation and migration to effect wound reepithelialization and closure. Normal early triggering of proliferation is in part related to secretion of granulocyte- macrophage colony stimulating factor from several cell types, including the keratinocytes themselves (139). Deficiencies in adhesion molecules, such as L-selectin and intercellular adhesion molecule-1 (ICAM-1), lead to impaired keratinocyte migration and retarded wound healing(18). Based on the fact that keratinocytes exhibit galvanotaxis (140), it has been proposed that the lateral electric field associated with a wound or injury current is an early stimulus for the initiation of the migration process of epidermal keratinocytes (141). Directed migration of keratinocytes toward a wound is endogenous, associated with wound-associated direct currents corresponding to a field of about 100 mV/mm, a process that depends on growth factors, extracellular calcium(142) and intact keratinocyte β_1-integrins(143). It is significantly reduced if protein kinase activity is inhibited(141). Thus, the field strength required to promote epithelial migration seems to depend on the constituents of the wound environment and on the ambient levels of growth factors, but in a simulated normal wound environment, migration is noted at field strengths close to those generated by the wound (144-146). It has also been observed that exposure of keratinocytes to pulsed electric fields may enhance cellular differentiation at the expense of migratory and proliferative aspects(147).

VI. Blood Flow and Edema as EMF-Related Wound Healing Targets

Blood flow as a target for EMF-related wound healing therapy can be conveniently considered in two categories: flow to the wound site and flow within the wound. In the first case, that of flow to the wound, the EMF targets are principally small arteries and arterioles feeding the wound bed site. Changes in vasoactivity of these vessels may be induced either directly, by EMF effects on vascular smooth muscle or endothelial cells, or indirectly, via neural activation, as in transcutaneous electrical nerve stimulation (TENS) (148-150) or by magnetic stimulation(151). Recent histological work indicates that skin blood vessels are innervated by sensory, sympathetic and parasympathetic fibers(152), so any of these may be suitable targets for EMF-effects. In addition, EMF-related reductions in impediments to local flow, such as by release of trapped leukocytes via EMF-related deactivation, or by increasing global blood flow to the region, as by spinal cord stimulation, may also be suitable targets. The other flow-related category relates to blood flow *within* the wound bed, which supports granulation and its functions in wound healing. This is a process that depends on angiogenesis and relative flow distribution within the wound. In this case it is unlikely that EMF exposure remote from the wound site would have benefit, unless neural (or other) pathways that selectively innervate (or effect) the wound site can be identified and appropriately stimulated.

Another point that should be considered is that, although blood flow deficits are involved in ischemic and in some diabetic ulcers, it is not necessarily true that greater blood flow means faster wound healing. Nor is it clear that greater tissue oxygenation is always good for the natural wound healing process. It may be argued that effects of blood flow on wound healing depend, at least in part, on the

timing of increases or decreases: If blood flow is too high initially it may affect the trigger for angiogenesis, and if it is sustained at too high a level it may result in increased edema. On the other hand, if flow becomes too low, it will no longer support wound metabolism and may cause a sustained inflammatory phase that inhibits healing. It is possible that the need to reverse polarity of some forms of ET to effect wound healing may reflect the need for these different requirements for blood flow(153). Of course, polarity also influences the direction of cell migration(154).

Since low oxygen tension triggers angiogenesis, hyperperfusion, occurring at the wrong time, may actually inhibit healing. For example, in patients with venous ulcers, overall limb blood flow is elevated (42, 155-157) as is total peri-ulcer skin microvascular blood flow (55, 157, 158). Yet abnormally dilated pre-capillary arterioles are present (159), and there is a maldistribution of the total flow between nutritive and non-nutritive pathways (41). This maldistribution may be related to activated leukocytes that plug nutritional capillaries or other selective flow diminution processes. If leukocytes are involved, then an EMF-related reduction in neutrophil activation and adherence might be beneficial from three perspectives: reduction in local ischemia in regions served by obstructed capillaries, normalization of the effects of enzymes and free radicals released by activated leukocytes, and reduction in the edema associated with their activation. Further, in patients with venous ulcers, the arteriolar vasoconstriction normally induced by standing is significantly blunted (160). This undoubtedly contributes to the local microvascular hyperperfusion, which exacerbates hypertension within post-capillary venules and capillaries, and causes further tissue edema. Such "high perfusion microangiopathy" may also be involved in neurogenic diabetic ulcers (160). Thus the possibility of global hyperperfusion, with simultaneously reduced wound blood flow and localized tissue edema, is a plausible basis for delayed healing. This scenario suggests that an EMF-related selective *vasoconstriction* of non-nutrient circulation may be of benefit. Alternatively, an EMF-related increase in local nutritional wound blood flow, if it overcomes the relative ischemia without causing substantial edema, might favor wound healing. Normally, edema (such as occurs with venous ulcers) is controlled via compression bandaging, which, among other aspects, is thought to redistribute microcirculation and thereby to normalize the deficient nutritional capillary network (157). Therefore, EMF therapy to increase total blood flow should always be used in conjunction with standard compression bandaging.

Patients with chronic venous insufficiency, and presumably those who go on to develop venous ulcers, appear to have increased vasomotion frequency (161). This vasomotion, which is due to spontaneous changes in blood vessel diameter, manifests itself as measurable rhythmic changes in blood flow at frequencies that range from 0.05 to 0.5 Hz(162). This suggests that EMF-related effects on vasomotion(163) may also have an impact on wound blood flow and wound healing. EMF excitation may alter arteriolar vasomotion through its effect on intracellular calcium ion oscillations and other calcium signaling processes. Although not specifically studied in vascular smooth muscle cells, (the effectors for vasomotion), an EMF-related (50 Hz) reduction in total spectral power content of cytosolic calcium ion [Ca^{++}] oscillations, and specific changes in the low-frequency band (0--10^{-3} Hz), have been demonstrated in human leukemia cells(164). Effects were noted only in cells in which such oscillations were already present(165). An argument for the role of spectral power changes as a mode of cellular encoding has been made(166), although both amplitude(167) and frequency(168) may be involved in encoding and decoding. Such a process may be involved in the EMF-related effects that alter the arteriolar vasomotion that is linked to local blood flow changes. Based on these findings and other considerations, it is the author's view that the effectiveness of EMF therapy for altering blood flow to stimulate wound healing may be optimized by linking field/current parameters to rhythms of the healing process using feedback that detects and accommodates naturally occurring physiological and vascular dynamics.

Edema

Although the role of the lymphatic system in wound healing has generally received little attention, several aspects of this “orphan” component of the circulatory system may have important consequences with respect to wound healing and the role of EMF therapy. Interstitial accumulation of fluids as edema or as a protein rich lymphedema retards blood flow by reducing perfusion pressure, reducing oxygen diffusion to tissue, and acts as a breeding area for infection (169, 170). In the early phases of a wound, edema is largely due to changes in capillary permeability associated with the inflammatory phase, but damage or dysfunction of the terminal lymphatic system is also probably involved. The presence of edema is obvious under some conditions, but in others its presence is "silent," as microedema within the wound environment, and its effects on wound healing are often not considered. Further, the physical features of sustained edema may change over time due to a progressive increase in protein concentration and fibrin cross-linking. These changes further impact the wound healing process. In view of the well documented ability of EMF therapy to reduce gross edema, the question arises as to whether EMF-related effects that may reduce microedema, either directly or by its effects on lymphatic pathways, plays a role in the favorable effect of EMFT on wound healing. It has been argued that PREMF affects lymphatic channels as they do blood vessels(59). There is also evidence that lymphatic vessels near ulcers are reduced in number and have partially destroyed endothelium (171), at which site one finds vascular endothelial growth factor receptor-3 . In experimental dermal wounds.(172), the expected angiogenic derived vessels were observed to evolve into granulation tissue. But, unexpectedly, from day five after wounding and onward, blood vessels that were positive for this growth factor appeared to sprout from periwound lymphatic vessels to become part of the granulation tissue. Although blood vessels remained, the growth factor positive lymphatic vessels regressed(172). This suggests a potentially important role of the lymphatic vessels in processes involved in forming wound granulation tissue. This would be dependent on a transient lymphangiogenesis and, based on data from human wounds, an upregulation of the vascular endothelial growth factor contained therein(172). Preliminary results(59) indicate that PREMF (27.1 MHz) significantly reduces edema in patients with postmastectomy lymphedema. Since in these patients the main deficit is a dearth or absence of normal lymphatic pathways due to surgery and/or radiation, reduction in edema is most likely achieved by the development of alternate lymphatic pathways. This observation suggests the possibility that a new and potentially promising target for EMF therapy is the lymphatic vessels within and surrounding the wound area.

In conclusion, the cumulative substantial evidence from cellular and animal experiments and from human studies strongly indicate important positive linkages between forms of electromagnetic therapy and wound healing. The composite findings provide a firm underlying basis for EMF therapy when used in a thoughtful and selective manner in the treatment of certain chronic or recalcitrant wounds. However, the involved mechanisms remain at best speculative and there remain large gaps in our understanding of the specific cellular and functional targets, therapeutic dose and regimens to achieve *optimal* treatment of specific wound types. It is suggested that the complexity of the wound healing process in general, and the differential features of specific chronic wound types in particular, demand a selective approach for choosing EMF therapy parameters, timing and targets. This implies that therapeutic EMF approaches need to be based both on physical and physiological considerations, which ultimately need to be judged on the basis of therapeutic outcomes. The functional concepts and EMF targets described in this chapter in relation to deficits of specific wound types may provide a basis for continued advances in this still evolving adjunctive therapeutic modality.

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