



A biochemical approach to wound healing through the use of modalities

William J. Ennis, DO*, Claudia Lee, MPT, Patricio Meneses, PhD

Comprehensive Wound and Disease Management Program, St James Hospital and Healthcare Centers, Olympia Fields Campus, Olympia Fields, IL 60461, USA

Abstract Wound healing is a complex pathway that is energy dependent. Nonhealing wounds frequently require the use of physical modalities to achieve healing. There is much debate over which treatment modality to use, with varying clinical results in the literature. This review paper describes a common biochemical pathway that helps the clinician understand, at a molecular level, how the transference of energy to a wound can result in positive clinical results. The mechanisms of action for ultraviolet light, electrical stimulation, and ultrasound are reviewed along with a proposed biochemical roadmap. An emphasis on protein biochemistry is supported with an extensive review of the literature.

© 2007 Elsevier Inc. All rights reserved.

Introduction

Over the past 10 to 15 years, the approach to a patient with a chronic wound has evolved from pure observation and topical dressing selection, into the appreciation of a complex micro-environment with numerous interdependent biochemical pathways. As the knowledge base continues to grow, so has the level of sophistication and detail with which diagnostic and treatment options are available in wound care. In the 1980s the concept of moist wound healing, originally described in the 1960s, was brought into a clinical reality with the release of moisture retentive dressings.¹ The literature has previously focused on the general work-up of a patient with a leg ulcer, diabetic foot ulcer, or venous ulcer on a systemic or “macro” level.^{2,3} As health care evolved into managed care and the creation of

integrated health care networks, this larger macro environment had an impact on the patient’s treatment options, access to care, and even clinical outcomes.⁴ Landmark papers which analyzed chronic wound fluid opened the door for clinicians to start appreciating the “micro-environment” in which wound healing occurred.^{5,6} The importance of energy for wound healing is not new and the connection to wound healing began with the use of ³¹P NMR spectroscopy.⁷ This technique allowed for the quantification of the actual energy available for a wound to heal. The technique, however, was cumbersome and the equipment costly and not readily available.

Further understanding of the energy needs for healing can be gained by understanding local tissue perfusion. Oxygen content, delivery, and subsequent use are all commonly evaluated at the organ level (Swan-Ganz catheter) in the hospital intensive care unit. Recently, investigators have found correlations between the microcirculation and overall patient survival in septic shock even when macro-vascular status was restored.⁸ The skin derives the majority of its energy requirements from the glycolytic pathway rather than

* Corresponding author. Tel.: +1 708 747 4000x2040; fax: +1 708 679 2089.

E-mail address: w.ennis@comcast.net (W.J. Ennis).

from the Krebs cycle.⁹ Oxygen delivery and the creation of reactive oxygen species (ROS) can, however, impact healing through signal transduction mechanisms.¹⁰

New technology in both diagnostics and therapeutics simply increases the scope of possible options for the chronic wound patient. The macro-environment (history, physical exam, and basic laboratory testing) still needs to be evaluated at the start of any patient evaluation.¹¹ It is now, however, possible to continue the work-up, down to the biochemical level. The advent of new therapeutic options has made manipulation of this micro-environment possible. Physical therapy modalities share in common the delivery of energy to target tissues. In this paper, we will attempt to describe the biochemical implications for the use of these treatment options for nonhealing wounds. In closing, a proposed theoretical mechanism of action is described along with potential future research projects that might be able to prove the theoretical model proposed.

Ultraviolet light C

Many skin care companies advertise that they have products which will protect you from the dangerous effects of ultraviolet (UV) radiation both UVA and UVB, a component of sunlight that is associated with aging and skin cancer. Ultraviolet light in the C-band wavelength, however, is a form of radiant energy recognized in the past 2 centuries for its germicidal and wound healing effects.¹² Physical therapists have used UV light C as a therapeutic modality for wound healing for many years; however, the physician community has been slow to adopt this technology. Ultraviolet light in the C-band wavelength has also enjoyed broad adoption in major basic science laboratories as a means for sterilization for many medical devices.¹³

The 3 previously mentioned bands of UV radiation differ in their ability to penetrate the skin. Varying biologic effects are correlated with the depth of penetration. It should be pointed out that there are more than 1 classification system to identify the specific bands of UV energy; the following description is adopted from the World Health Organization.¹² Ultraviolet light A for example has the longest wavelength (320-400 nm) and penetrates to the level of the upper dermis in human skin. Ultraviolet light in the B-band (280-320 nm) only penetrates down to the stratum basale. Ultraviolet light C (200-280 nm), which has therapeutic wound care implications, however, only reaches the upper layers of the epidermis.¹²

Ultraviolet radiation exposure to the skin produces erythema, epidermal hyperplasia, increased blood flow in the microcirculation, and has a bactericidal effect.¹⁴⁻¹⁶ The induced erythema initiates the first phase of healing (inflammatory phase) by creating an inflammatory response via the mechanism of vasodilatation. This may be partially explained by the effects of UV light on the arachidonic acid pathway.¹⁷ Kaiser et al¹⁸ used a porcine model to

demonstrate that UV radiation stimulates the production and release of interleukin 1 by keratinocytes. Interleukin 1 enhances wound epithelialization via keratinocyte chemotaxis and proliferation as well as the proliferation of fibroblasts.¹⁹

Increased cell permeability occurs which results in increased intercellular edema at the prickle cell level causing a separation of the upper and lower layers of the epidermis.²⁰ The upper layer is sloughed off, or debrided, with an accumulation of phagocytic white blood cells in the local blood vessels. Growth factors are released from epidermal cells exposed to UV irradiation which further augments the healing cascade.²¹ In addition, UV light exposure induces cellular proliferation in the stratum corneum.²² This proliferation/thickening of the skin is a protective mechanism against further sunlight damage. Research has shown that UV radiation can also cause structural DNA changes and increased RNA activity for continued transcription and repair.²³ Morykwas and Mark²⁴ examined the effects of UV light C exposure on dermal fibroblasts measuring fibronectin production and wound bed lattice contraction. Fifteen newborn foreskin fibroblast cultures were established and treated with UV light C at 254 nm. Results indicated that those fibroblasts exposed to UV light C had a decreased amount of fibronectin bound to cell surfaces (mean, 14%) and an increased amount of fibronectin released into the medium (mean, 42%). In addition, collagen lattices constructed with irradiated fibroblasts in this study contracted significantly faster as measured at 7 days.

The authors theorized that fibronectin release is one mechanism whereby UV irradiation leads to increased healing via wound contraction. These results are comparable with those obtained in a 1985 trial.²⁵ Fibronectin promotes cell migration and helps regulate cell growth and gene expression. Aging fibroblasts have alterations in fibronectin levels and extracellular matrix communication making it difficult to distinguish the contribution of aging to a study using UV irradiation.²⁶ Morykwas used neonatal fibroblasts to overcome this issue.

There are a growing number of organisms resistant to currently available antibiotics.²⁷ In addition, many wound care patients have comorbid illnesses which impair local tissue perfusion thereby affecting antibiotic delivery. This clinical scenario creates a need in the industry for effective topical therapies for treating wound infections and managing wound bed bioburden which is known to negatively impact wound healing. Ideally, this should be achieved without further impacting the resistance patterns for the involved bacteria.

There is a growing body of literature examining the antimicrobial effects of UVC irradiation at 254 nm. Conner-Kerr et al²⁸ conducted an *in vitro* study demonstrating the antimicrobial effects of UV light C using a 254-nm wavelength cold quartz generator with a 90% output of UV energy. The lamp was placed 1 in from the wound

surface during treatment. Kill rates for methicillin-resistant *Staphylococcus aureus* were 99.9% at 5 seconds and 100% at 90 seconds. Kill rates for vancomycin-resistant *Enterococcus faecalis* were 99.9% at 5 seconds and 100% at 45 seconds. They proposed a further evaluation of in vivo kill rates at shorter than the recommended 72 to 180 seconds based on the results of their study. A comparative study by Sullivan and Conner-Kerr²⁹ described a 3-second exposure time for prokaryotic organisms (*Pseudomonas aeruginosa* and *Mycobacterium abscessus*), vs longer exposure times of UV light C for the eukaryotic organisms *Candida albicans* (15 seconds) and *Aspergillus fumigatus* (30 seconds) to obtain 99.9% kill rates. Sullivan and Conner-Kerr proposed that prokaryotic cells (bacteria) are preferentially injured compared with eukaryotic cells because their genetic material is located freely within the cytoplasm in comparison to eukaryotes in which the genetic material is surrounded by the nuclear membrane. In another study, Sullivan et al³⁰ determined that kill rates for *Streptococcus pyogenes* (group A Streptococcus), a common causative organism for necrotizing fasciitis, were 99.9% at 4 seconds using UV light C, consistent with their prior data on methicillin-resistant *S aureus* and vancomycin-resistant *E faecalis*. The results, however, also indicated that 100% killing of group A Streptococcus was never achieved even with treatment times lasting up to 180 seconds in pure culture. Thai et al³¹ conducted a prospective, 1-group, pre-post treatment study to evaluate the effects of UV light C on chronic wounds as well as to establish test reliability using the semiquantitative swab technique. Subjects (n = 22) were recruited from nursing homes and inpatient/outpatient settings who had chronic wounds exhibiting at least 2 signs of infection and critical colonization with bacteria. Findings suggested that the semiquantitative swab technique is a reliable and reproducible test ($\kappa = 0.92$) for assessing the identification and quantification of bacteria on the superficial layer of chronic wounds. There was a statistically significant ($P < .0001$) reduction in wound bioburden after a single 180-second treatment of UV light C. The semiquantitative swabs showed the greatest reduction in semiquantitative swab scores in wounds colonized with *Pseudomonas aeruginosa* and wounds colonized with only 1 species of bacteria. Contrary to these findings, another published multiple case report showed that 180 seconds of UVC treatment was required to kill methicillin-resistant *S aureus*.³² This study also reported that longer treatment times with repeated exposures were required to kill bacteria located in deeper compartments of the wound.

There have been a few human clinical trials using UV therapy. Unfortunately, it is difficult to draw strong conclusions or compare the papers as different wavelengths were used at various treatment times and distances from the wound surface. Wills et al³³ demonstrated the effectiveness of UV light (combination of UVA, UVB, and UVC) in the treatment of pressure sores in a randomized controlled trial. Sixteen patients with superficial pressure sores (<5 mm

deep) were treated 2 times per week compared to control patients who received the same light; however, a mica cap was left over the quartz window effectively blocking all UV radiation. In the UV-treated group, mean time to healing was 6.3 weeks, whereas mean time to healing was 8.4 weeks for the placebo group ($P < .02$). Healthy staff members established a minimal erythema dosage³⁴ of 2 seconds. The treatment dosage was 2.5 minimum erythema dosage twice weekly. Each dose of UV was increased by 50% (to maintain second-degree erythema) so patients who completed the 8 weeks of treatment would have received up to 7 minutes and 30 seconds. The authors stated that most of the wounds were infected on enrollment, but there was no standardized definition of infection and culture techniques were not described.

Nussbaum et al³⁵ examined the effects of UV light C combined with ultrasound (US) therapy on pressure ulcers in a spinal cord-injured population. Twenty patients with 22 wounds were randomly assigned to either laser light therapy, UV light C combined with US, or standard of care which consisted of wound products that maintain a moist environment. Treatment parameters for UVC were based on wound appearances using erythema dosages with E₁ for granular wounds and E₄ for heavily infected areas. Ultrasound was applied at 3 MHz and at 0.2 W/cm². Ultrasound and UV therapy were performed 5 days a week on an alternating day basis. Some patients therefore received 3 US therapies 1 week and only 2 the following week. Laser light at 820-nm wavelength was applied 3 times a week with a final energy density of 4 J/cm² in a 35-second treatment time. The results indicated that a combination of UV and US treatment was more effective on wound healing than nursing care alone or laser light therapy ($P = .32$). The fact that US was combined with UV therapy, however, makes it difficult to arrive at any meaningful interpretation of the results.

Electrical stimulation

The concept of a skin “battery” and the potential implications for wound healing have been known since the early 1980s.^{36,37} The use of electrical stimulation for wound healing uses this concept and allows the clinician to deliver exogenous electrical signals into wound tissue thereby mimicking the underlying natural bioelectrical response to injury.³⁸ Over the years, there have been a large amount of clinical trials and animal experiments which have elucidated numerous mechanisms of action for the positive wound healing responses of electrical stimulation.

Preclinical

Using human fibroblast cell cultures, Bourguignon and Bourguignon³⁹ used high-voltage, pulsed, galvanic stimulation and demonstrated an increase in DNA and protein synthesis. Interestingly, however, at voltages above 250 V, protein and DNA synthesis were inhibited, implying that, not unlike medicines, energy-based therapies need to be

studied in dose-escalating trials. In a subsequent work, the above authors identified immediate increased levels of intracellular calcium with subsequent increases in insulin receptor sites on the cell membrane of human fibroblasts *in vitro*.⁴⁰ Adenosine triphosphate levels and thymidine incorporation have also been shown *in vitro* to be accelerated with the use of electrical stimulation.^{41,42} The use of sodium and calcium channel blockers has mitigated the effects of electrical stimulation during *in vitro* studies, further supporting the theory that membrane depolarization and ion shifts are at least partially responsible for the changes noted with electrical therapy.^{43,44} The movement of cells toward an electrical field is known as galvanotaxis.⁴⁵ Various cells important for wound healing can be attracted into the wound bed through the use of electrical fields.^{46,47} Another area of intense research has been the potential antimicrobial effects of electrical stimulation. It has become increasingly clear to wound care practitioners that managing the bioburden is a critical component of wound bed preparation.⁴⁸ An excellent review of all of the antibacterial studies focused on the bacteriostatic or bacteriocidal actions of electrical stimulation can be found in a recent paper by Kloth.⁴⁹ Although a definitive mechanism of action remains elusive, temperature and pH changes do not appear to be the cause of the observed effects.⁵⁰ Many wound care products have incorporated silver ions to achieve a level of bioburden control.⁵¹ The addition of an electrical current to a silver dressing may have enhanced effects and requires further evaluation.^{52,53}

Clinical

We have used the use of electrical stimulation as a skin graft “salvage” procedure for many years. When a graft demonstrates partial take, selective “spot debridement” with a 3-mm curette is performed to remove the bio-film that is produced. Daily electrical stimulation with an initial day of negative active polarity followed by positive polarity stimulation is performed for 2 weeks. This protocol results in rapid keratinocyte migration and the filling of graft interstices. There are numerous reports in the literature describing skin graft survival, incisions, and flaps with the use of adjunctive electrical stimulation.⁵⁴⁻⁵⁹

It has now been well established that electrical stimulation can enhance the formation and release of vascular endothelial growth factor (VEGF) and is thereby a form of therapeutic angiogenesis.^{60,61} Electric fields reoriented, elongated, and enhanced the migration of endothelial cells in culture.⁶² In addition, there was a differential response with microvascular endothelial cells migrating toward the cathode and macrovascular endothelial cells migrating toward the anode.⁶³ It is still unknown how cells can sense and transduce electric signals *in vivo*. Increasing perfusion at the microvascular level using noninvasive modalities is important for patients with nonreconstructable vascular disease and nonhealing wounds. A prospective, randomized trial of high-voltage pulsed electrotherapy vs a sham device

demonstrated that wound area decreased and microcirculation improved as measured by trans-cutaneous oxygen levels.⁶⁴ Researchers have attempted to identify vasodilators, such as vasoactive intestinal polypeptide, that might account for the increases in blood flow noted in ischemic patients after electrical stimulation.⁶⁵ There have been several reports of increased tissue perfusion and wound healing in diabetic ulcer patients.^{66,67} One report demonstrated a decrease in trans-cutaneous oxygen values after electrical stimulation, but this could be due to increased tissue metabolism and in this trial there was no concomitant laser Doppler performed.⁶⁸ Another clinical application in which electrical stimulation has had positive effects on perfusion is the healing of pressure ulcers in spinal cord-injured patients.⁶⁹ It is known that skin below the level of cord injury is at increased risk for breakdown secondary to a loss of sympathetic vasomotor control.⁷⁰ Spinal cord-injured patients were treated with electrical stimulation or sham, and increases in transcutaneous oxygen values were noted post therapy.⁷¹ In this study, there were differences noted when patients were in the supine vs prone position as well as with changes in the treatment voltages. Patients with stage 4 pressure ulcers without spinal cord injury were treated with electrical stimulation or standard of care.⁷² The active treatment group demonstrated accelerated healing. In another study, 47 patients with 50 wounds of various etiology were randomized to active electrical stimulation vs sham.⁷³ After 4 weeks of therapy, the active group demonstrated a wound size of 44% of the original vs 67% of original in the control group ($P = .02$).

Edema control is another important aspect in the overall treatment of chronic wounds, in particular venous ulcerations of the lower leg. Electrical stimulation has had various results depending on the animal model selected.⁷⁴ With the use of a hamster cheek pouch, electrical stimulation was followed by a histamine infusion.⁷⁵ Fluorescein-labeled dextran served as a tracer for plasma proteins. Those animals pretreated with electrical stimulation had decreased amount of leakage giving support to the theory that electrical stimulation reduces edema by decreasing the “leakiness” of the microcirculation. Taylor et al⁷⁶ furthered this work by varying the polarity of the electrode and noted less edema with cathodal therapy.

Ultrasound

Ultrasound is defined as a mechanical vibration transmitted at a frequency above the upper limit of human hearing (>20 kHz).⁷⁷ Recently, within the wound healing community, there has been an increased interest in both diagnostic and therapeutic US. High-frequency US (20-40 MHz) devices are able to assess the peri-wound skin, wound bed, and the underlying soft tissue components.^{78,79} Therapeutic US has been used in sports medicine, physical therapy, and psychiatry for years, but wound care clinicians are only recently becoming aware of the potential benefits for treating

recalcitrant wounds. Information concerning the bioacoustic effects of US continues to evolve from animal, plant, human, cellular, and epidemiologic studies.⁸⁰ One of the main mechanisms of action for US is achieved through the process of cavitation.⁸¹ Cavitation involves the production and vibration of micron-sized bubbles within the coupling medium and fluids within the tissues. As the bubbles collect and condense, they are compressed before moving on to the next area. The movement and compression of the bubbles can cause changes in the cellular activities of the tissues subjected to US.⁷⁷ Microstreaming is defined as the movement of fluids along the acoustic boundaries as a result of the mechanical pressure wave associated with the US beam.⁸² The combination of cavitation and microstreaming, which are more likely to occur with kilohertz US, provides a mechanical energy capable of altering cell membrane activity.⁸³ A new hypothesis known as the frequency resonance theory has been proposed which carries the above concepts to the protein and genetic level.⁸⁴ Mechanical energy from an US wave is absorbed by individual protein molecules resulting in conformational changes. Signal-transduction pathways are also stimulated from the US-generated mechanical energy that results in a broad range of cellular effects. There are multiple, well-documented cellular effects of US therapy which have direct implications for wound healing. Leukocyte adhesion, growth factor production, collagen production, increased angiogenesis, increased macrophage responsiveness, increased fibrinolysis, and increases in nitric oxide (NO) are all examples of US-induced cellular effects.⁸⁵⁻⁹¹ Historically, megahertz-range US has been studied *in vitro* and to treat peri-wound tissue clinically. There has been a recent shift toward the use of low-frequency US in the kilohertz range to achieve vascular vasodilatation, bone healing, and with the use of cytotoxic chemicals that are sonosensitizers, even for the treatment of malignant cells.⁹²⁻⁹⁴ MIST (MIST™ Celleration Inc., Eden Prairie, MN, USA) US therapy is a newly Food and Drug Administration–cleared low-frequency US therapy which has been shown to enhance angiogenesis and collagen deposition in a diabetic mouse model.⁹⁵ In a recently completed randomized, controlled, double-blinded, sham study of diabetic foot ulcers in human subjects, MIST therapy achieved statistically significant greater healing outcomes than Sham therapy (40.7% vs 14.3%).⁹⁶ This multi-center trial had more than 25 inclusion and exclusion criteria and required the investigators to use a prescribed dressing regimen throughout the study to maintain the rigid criteria for a randomized controlled trial. Dogs and pigs with occluded coronaries were treated with low-frequency, catheter-based US for up to 1 hour.⁹² Significant increase in flow was noted, but these were completely abated with the use of L-nitro arginine methyl ester a known NO synthase (NOS) inhibitor. In this study, catheter-based, 27-kHz pulsed US was used with a maximal intensity of 1.4 W/cm², directly applied to heart muscle. This article describes the release and/or formation of NO as a possible mechanism of action for low-frequency US therapy.

Discussion

In this paper, we have described several physical therapy modalities with an emphasis on their biochemical impact on wound healing. At a very basic level, all of these therapies end up delivering energy, although in different initial forms, to the wound bed. Much has been written in the alternative medical community concerning energy medicine, homeopathy, and acupuncture, all of which deal with diagnosing and treating patients using energy in various forms.⁹⁷ This type of complimentary therapy is gaining acceptance in the general public and slowly with health care practitioners.⁹⁸

There are many other modalities including low-level laser and near-infrared light therapy, which deliver energy and have been shown to improve healing.^{99,100} We have not attempted to provide a complete review of all energy-based modalities that might positively impact wound healing, but have tried to review some of the common therapeutic options used by clinicians. More importantly, it is our belief that there is an underlying universal mechanism of action responsible for the cellular effects seen with these treatments.

After reviewing many published articles, we have designed a biochemical pathway that might explain the relationship between energy therapy and the cellular events that lead to the clinical results obtained with these modalities. There are several key cells in the early stages of the healing process of an open wound. Neutrophils, vascular smooth muscle cells, endothelial cells, fibroblasts, and macrophages all arrive at the wound bed during the initial response after skin injury. All of these cells produce NO at certain points along the healing continuum.¹⁰ These cells also contain niacin adenine dinucleotide phosphate oxidase, an enzyme at the cell membrane level. In the presence of niacin adenine dinucleotide phosphate, oxygen (O₂), and NO, niacin adenine dinucleotide phosphate oxidase is capable of synthesizing ROS represented principally by hydrogen peroxide (H₂O₂).¹⁰ For this redox mechanism to proceed, energy is required. The ROS in an open wound seems to be involved via the regulation or modulation of all stages of the healing process.¹⁰ A chronic wound has been defined as one that fails to proceed through an orderly and timely process to produce anatomic and functional integrity, or one that proceeds through the repair process without establishing a sustained anatomic and functional result.¹⁰¹ There is little agreement in the literature concerning time frames for the healing of various wound etiologies. Some authors feel that with a consistent clinical work-up and treatment protocol, wounds of various etiologies will heal along with a similar time course.¹⁰² If the inflammatory phase persists beyond its certain physiologic limits, therefore, a chronic wound is generated. Under these conditions, ROS production continues from macrophages, fibroblasts, and endothelial cells, which keep damaging the area and perpetuating the inflammatory state. This situation has been called “oxidative stress,” and it correlates with the “stunned wound,” a cellular physiology described in the

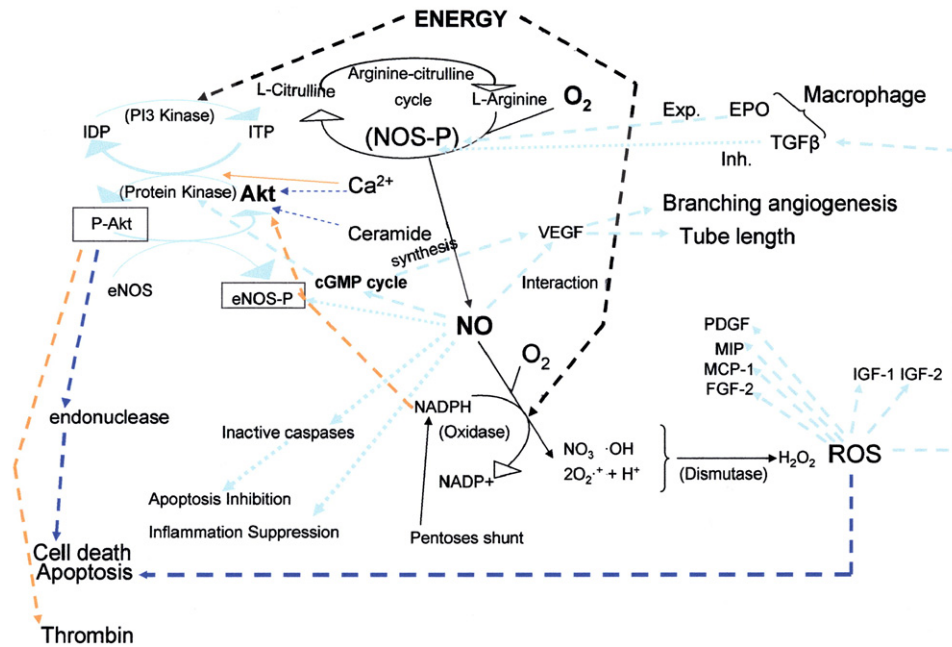


Fig. 1 Solid arrow indicates reaction; dashed arrow, stimulus or expression; dotted arrow, inhibition or negative feedback.

cardiac literature known as the stunned myocardium.^{102,103} Many disease states and in fact the process of aging have been related to the level of oxidative stress within the organism.^{104,105}

Nitric oxide synthesis begins with the oxidation of the substrate, L-arginine, in the presence of an enzyme, NOS.¹⁰⁶ Nitric oxide synthase is composed of the protein calmodulin (CaM), flavin, tetrahydrobiopterin (BH₄), and a reduced thiol.¹⁰⁷ Calmodulin can either be free or membrane attached and requires the addition of calcium.¹⁰⁸ Niacin adenine dinucleotide phosphate is also required as a cofactor. The end products of this reaction include L-citrulline and NO. There are 3 forms of NOS. Two of these are constitutively expressed (nNOS neuronal, eNOS endothelial). Neuronal NOS is located in the cytosol of neurons, skeletal muscle, pancreas, and the kidneys.¹⁰⁹ Endothelial NOS is primarily membrane bound and plays a crucial role in regulating blood vessel physiology.¹¹⁰ Inducible NOS, however, is not expressed in resting state cells but is responsible for the larger release of NO.¹¹¹ The resulting L-citrulline can resynthesize arginine by the transference of an amino group from aspartate. This reaction is known as the arginine-citrulline cycle. As a result of this cycle, arginine is never depleted and oxygen becomes the limiting factor under normal conditions. When high concentrations of arginine accumulate, the action of a second enzyme, arginase, catalyzes the arginine into ornithine and urea.¹¹² This use of arginine has been implicated to downregulate NO synthesis via substrate depletion.¹¹³ It is necessary to consider that NOS has a K_m of 2 to 20 $\mu\text{mol/L}$, whereas arginase has a K_m of 2 to 20 $\mu\text{mol/L}$, which means that NOS requires a concentration of substrate 1000 times less than arginase.¹¹⁴

Angiogenesis, the creation of new blood vessels, is a multi-step complex component of the wound healing process.¹¹⁵ Nitric oxide, and therefore NOS, is an important mediator of the angiogenesis process.^{116,117} There are several biochemical processes by which this modulation of NOS can occur. Within endothelial cells, NO has a positive feedback effect over cyclic GMP. This activates via phosphorylation, a serine/threonine kinase (akt), which in turn activates (also by phosphorylation) the NOS enzyme.¹¹⁸ Erythropoietin, acting as a cytokine, is also a stimulus for the expression of NOS enzyme, whereas the transforming growth factor β (TGF β) is an inhibitor of this enzyme when macrophages are present in the granulation tissue.^{119,120} It is also known that VEGF mediates angiogenesis.¹²¹ Nitric oxide and VEGF may interact to produce pro-angiogenic responses.¹²² There are 2 types of receptors that define the role of VEGF in endothelial cells, VEGFR-1 and VEGFR-2. VEGFR-1 activates NOS and NO synthesis and promotes vascular connections through branching, whereas VEGFR-2 promotes tube length between branches.³⁴ When NO concentrations are high VEGFR-2 is inhibited via cGMP, but if NO concentrations are low VEGFR-2 is stimulated.¹²³

The final phases of healing epithelialization and remodeling lead to the end result of the repair process. Programmed cell death or apoptosis is a more recently appreciated process that is also critical in wound healing.¹²⁴ Calcium ions (Ca²⁺), ceramides, and ROS are all involved in the signaling for cell apoptosis. Ca²⁺ stimulates receptors at the membrane level, which stimulates inositol triphosphate. Inositol triphosphate stimulates Ca²⁺ release from the endoplasmic reticulum, which subsequently activates protein kinase Akt and protein kinase C.¹²⁴ These 2 enzymes stimulate endonuclease activation destroying the cell nucleus

and producing cell death.¹²⁵ Ceramides, a component of sphingolipids and a second messenger, produce apoptosis through the activation of protein kinase Akt.¹²⁴ Reactive oxygen species are also capable of directly producing apoptosis.¹²⁴ It is important to note that NO can influence both cell growth and apoptosis depending on the environment. Through the inactivation of caspases, NO can inhibit the apoptotic process.¹²⁶ Given a different environment, for example, the low concentrations of transforming growth factor β seen under a muscle flap which leads to enhanced NO levels, apoptosis is stimulated.¹²⁷

All the modalities used in wound healing are, by mechanical or electrical methods, introducing energy to the affected area. The energy needs to activate certain processes in the cell to trigger the aforementioned sequence of reactions. It is obvious from the complexity of this pathway (which is in no way complete) that it would be impossible to assume that one treatment or modality can generate positive results in all clinical scenarios. Many of the processes we have described can either stimulate or inhibit a step along the pathway depending on the environment, the internal cellular interpretation, cell type, and maturity.

Ultraviolet C

Ultraviolet C radiation can modify cellular apoptosis through tyrosine phosphorylation of growth factor receptors or the production of ROS.^{128,129} Apoptosis is also influenced by caspase activation which also occurs with UVC irradiation.¹³⁰ Therefore, through the processes of protein kinase phosphorylation, the production of ROS, and cell signaling, UVC irradiation can lead to cellular changes described in the proposed pathway (Fig. 1).

Electrical stimulation

Electrical stimulation results in tyrosine kinase activation, growth factor receptor modification, and molecular changes in signaling pathways that result in cell migration.¹³¹⁻¹³³

Ultrasound

Biochemical changes are also possible when using US as an energy source to stimulate healing. Nitric oxide synthase activity has been shown to increase in cultured vascular endothelial cells.¹³⁴ The increase in NOS may be due to increases in intracellular calcium, which ultimately result in increased NO.¹³⁵ Both endothelial and neuronal NOS are calcium dependent. On the other hand, iNOS contains calmodulin tightly bound to each subunit of the enzyme and is permanently activated. iNOS is calcium independent.¹¹¹ Apoptosis is affected by ROS generation and intracellular calcium ion changes.^{136,137} Receptor tyrosine kinases and integrins can serve as mechanosensors to transduce mechanical stimuli into chemical signals.¹³⁸ Extracellular signal-regulated kinase and c-Jun terminal kinase can be induced by shear stress and are therefore potential markers for the cellular stress induced by the use of US.^{139,140}

Conclusions

Healing a wound is an energy requiring process that often requires adjunctive therapies in addition to moist wound care products to achieve success. These modalities all deliver energy to the wound with resulting biochemical results. Previously, the authors evaluated energy levels by using P-31 NMR spectroscopy to assess adenosine triphosphate levels.¹⁴¹ Recently, the authors have used the use of MIST US to deliver energy to the wound bed of chronic nonhealing wounds with good results.⁹⁶ Given the complexity and interrelatedness of the pathways described in this paper, it is our belief that the future direction of our research should focus on the protein level. After establishing a “library” of protein profiles for various wound etiologies, it is our intention to use MIST US and other modalities and reevaluate the protein profile using mass spectroscopy. It is our hope to identify “diagnostic protein profiles” which would help the clinician to decide which modality to use, for how long, and in what sequence to achieve healing in recalcitrant chronic wounds.

References

1. Winter GD. Formation of the scab and the rate of epithelization of superficial wounds in the skin of the young domestic pig. *Nature* 1962;193:293-4.
2. Ennis WJ, Meneses P. Leg ulcers: a practical approach to the leg ulcer patient. *Ostomy Wound Manage* 1995;41(7A Suppl):52S-62S [discussion 63S].
3. Boulton AJ, Meneses P, Ennis WJ. Diabetic foot ulcers: a framework for prevention and care. *Wound Repair Regen* 1999;7:7-16.
4. Ennis WJ, Meneses P. Managing wounds in a managed care environment: the integration concept. *Ostomy Wound Manage*, 1998;44:22-6, 28-31, 34-6, passim.
5. Trengove NJ, et al. Analysis of the acute and chronic wound environments: the role of proteases and their inhibitors. *Wound Repair Regen* 1999;7:442-52.
6. Ulrich D, et al. Effect of chronic wound exudates and MMP-2/-9 inhibitor on angiogenesis in vitro. *Plast Reconstr Surg* 2005;116:539-545.
7. Ennis WJ, Claudia Lee MPT, Meneses P. 31-P NMR Spectroscopy: a powerful tool for wound analysis using high energy phosphates—a preliminary study. *Wounds* 1994;6:166-73.
8. Sakr Y, et al. Persistent microcirculatory alterations are associated with organ failure and death in patients with septic shock. *Crit Care Med* 2004;32:1825-31.
9. Gupta A, Manhas N, Raghurir R. Energy metabolism during cutaneous wound healing in immunocompromised and aged rats. *Mol Cell Biochem* 2004;259:9-14.
10. Sen CK. The general case for redox control of wound repair. *Wound Repair Regen* 2003;11:431-8.
11. Ennis WJ. Comprehensive wound assessment and treatment system. In: Kirsner R, Falabella A, editors. *Wound healing*. Boca Raton: Taylor and Francis; 2005. p. 59-68.
12. Conner-Kerr T. Ultraviolet light and wound healing. In: Sussman BJB, C. BJB editors. *Wound care*. Gaithersburg (Md): Aspen; 2001. p. 580-95.
13. Kubey W, Holmes CJ. In vitro studies on the microbicidal effectiveness of a xenon-based ultraviolet light device for continuous ambulatory peritoneal dialysis connections. *Blood Purif* 1991;9:102-8.

14. Eaglstein WH, Weinstein GD. Prostaglandin and DNA synthesis in human skin: possible relationship to ultraviolet light effects. *J Invest Dermatol* 1975;64:386-9.
15. Agin PP, et al. Changes in epidermal forward scattering absorption after UVA or UVA-UVB irradiation. *J Invest Dermatol* 1981;76:174-7.
16. Taylor GJ, Bannister GC, Leeming JP. Wound disinfection with ultraviolet radiation. *J Hosp Infect* 1995;30:85-93.
17. Camp RD, et al. Irradiation of human skin by short wavelength ultraviolet radiation (100-290 nm) (u.v.C): increased concentrations of arachidonic acid and prostaglandins E₂ and F₂alpha. *Br J Clin Pharmacol* 1978;6:145-8.
18. Kaiser MR, Davis SC, Mertz PM. Effect of ultraviolet radiation induced inflammation on epidermal wound healing. *Wound Rep Regen* 1995;3:311-5.
19. Sauder DN, et al. Interleukin-1 enhances epidermal wound healing. *Lymphokine Res* 1990;9:465-73.
20. Holtz F. Pharmacology of ultra-violet radiation. *Br J Phys Med* 1952;15:201-5.
21. James LC, et al. Transforming growth factor alpha: in vivo release by normal human skin following UV irradiation and abrasion. *Skin Pharmacol* 1991;4:61-4.
22. Sauder DN, Stanulis-Praeger BM, Gilchrist BA. Autocrine growth stimulation of human keratinocytes by epidermal cell-derived thymocyte-activating factor: implications for skin aging. *Arch Dermatol Res* 1988;280:71-6.
23. Hadden CT. Postreplication repair of ultraviolet-irradiated transforming deoxyribonucleic acid in *Bacillus subtilis*. *J Bacteriol* 1981;145:434-41.
24. Morykwas MJ, Mark MW. Effects of ultraviolet light on fibroblast fibronectin production and lattice contraction. *Wounds* 1998;10:111-7.
25. Oka H. Changes in quantity of fibronectin from human skin fibroblasts with cellular aging. *Ann Plast Surg* 1985;14:248-57.
26. Pieraggi MT, Julian M, Bouissou H. Fibroblast changes in cutaneous ageing. *Virchows Arch A Pathol Anat Histopathol* 1984;402:275-87.
27. Wise R. The relentless rise of resistance? *J Antimicrob Chemother* 2004;54:306-10.
28. Conner-Kerr TA, et al. The effects of ultraviolet radiation on antibiotic-resistant bacteria in vitro. *Ostomy Wound Manage* 1998;44:50-6.
29. Sullivan PK, Conner-Kerr TA. A comparative study of the effects of UVC irradiation on select prokaryotic and eucaryotic wound pathogens. *Ostomy Wound Manage* 2000;46:28-34.
30. Sullivan PK, Conner-Kerr TA, Smith ST. The effects of UVC irradiation on group A streptococcus in vitro. *Ostomy Wound Manage*, 1999;45:50-4, 56-8.
31. Thai TP, et al. Effect of ultraviolet light C on bacterial colonization in chronic wounds. *Ostomy Wound Manage* 2005;51:32-45.
32. Thai TP, et al. Ultraviolet light C in the treatment of chronic wounds with MRSA: a case study. *Ostomy Wound Manage* 2002;48:52-60.
33. Wills EE, Anderson T, Beattie BL, Scott A. A randomized placebo-controlled trial of ultraviolet light in the treatment of superficial pressure sores. *J Am Ger Soc* 1983;31:130-3.
34. Bussolati B, et al. Vascular endothelial growth factor receptor-1 modulates vascular endothelial growth factor-mediated angiogenesis via nitric oxide. *Am J Pathol* 2001;159:993-1008.
35. Nussbaum EL, Biemann I, Mustard B. Comparison of ultrasound/ultraviolet-C and laser for treatment of pressure ulcers in patients with spinal cord injury. *Phys Ther* 1994;74:812-23 [discussion 824-5].
36. Foulds IS, Barker AT. Human skin battery potentials and their possible role in wound healing. *Br J Dermatol* 1983;109:515-22.
37. Jaffe LF, Vanable Jr JW. Electric fields and wound healing. *Clin Dermatol* 1984;2:34-44.
38. Kloth LC, McCulloch JM. Promotion of wound healing with electrical stimulation. *Adv Wound Care* 1996;9:42-5.
39. Bourguignon GJ, Bourguignon LY. Electric stimulation of protein and DNA synthesis in human fibroblasts. *FASEB J* 1987;1:398-402.
40. Bourguignon GJ, Jy W, Bourguignon LY. Electric stimulation of human fibroblasts causes an increase in Ca²⁺ influx and the exposure of additional insulin receptors. *J Cell Physiol* 1989;140:379-85.
41. Cheng K, Goldman RJ. Electric fields and proliferation in a dermal wound model: cell cycle kinetics. *Bioelectromagnetics* 1998;19:68-74.
42. Cheng N, et al. The effects of electric currents on ATP generation, protein synthesis, and membrane transport of rat skin. *Clin Orthop Relat Res* 1982;264-72.
43. Bedlack Jr RS, Wei M, Loew LM. Localized membrane depolarizations and localized calcium influx during electric field-guided neurite growth. *Neuron* 1992;9:393-403.
44. Eltinge EM, Cragoe Jr EJ, Vanable Jr JW. Effects of amiloride analogues on adult *Notophthalmus viridescens* limb stump currents. *Comp Biochem Physiol A* 1986;84:39-44.
45. Mycielska ME, Djamgoz MB. Cellular mechanisms of direct-current electric field effects: galvanotaxis and metastatic disease. *J Cell Sci* 2004;117(Pt 9):1631-9.
46. Erickson CA, Nuccitelli R. Embryonic fibroblast motility and orientation can be influenced by physiological electric fields. *J Cell Biol* 1984;98:296-307.
47. Sheridan DM, Isseroff RR, Nuccitelli R. Imposition of a physiologic DC electric field alters the migratory response of human keratinocytes on extracellular matrix molecules. *J Invest Dermatol* 1996;106:642-6.
48. Schultz GS, et al. Wound bed preparation: a systematic approach to wound management. *Wound Repair Regen* 2003;11(Suppl 1):S1-S28.
49. Kloth LC. Electrical stimulation for wound healing: a review of evidence from in vitro studies, animal experiments, and clinical trials. *Int J Low Extrem Wounds* 2005;4:23-44.
50. Szuminsky NJ, et al. Effect of narrow, pulsed high voltages on bacterial viability. *Phys Ther* 1994;74:660-7.
51. Ip M, et al. Antimicrobial activities of silver dressings: an in vitro comparison. *J Med Microbiol* 2006;55(Pt 1):59-63.
52. Spadaro JA, Chase SE, Webster DA. Bacterial inhibition by electrical activation of percutaneous silver implants. *J Biomed Mater Res* 1986;20:565-77.
53. Deitch EA, et al. Silver nylon cloth: in vitro and in vivo evaluation of antimicrobial activity. *J Trauma* 1987;27:301-4.
54. Brown M, Gogia PP. Effects of high voltage stimulation on cutaneous wound healing in rabbits. *Phys Ther* 1987;67:662-7.
55. Bach S, et al. The effect of electrical current on healing skin incision. An experimental study. *Eur J Surg* 1991;157:171-4.
56. Im MJ, Lee WP, Hoopes JE. Effect of electrical stimulation on survival of skin flaps in pigs. *Phys Ther* 1990;70:37-40.
57. Politis MJ, Zanakis MF, Miller JE. Enhanced survival of full-thickness skin grafts following the application of DC electrical fields. *Plast Reconstr Surg* 1989;84:267-72.
58. Chu CS, et al. Multiple graft harvestings from deep partial-thickness scald wounds healed under the influence of weak direct current. *J Trauma* 1990;30:1044-9 [discussion 1049-50].
59. Kjartansson J, et al. Transcutaneous electrical nerve stimulation (TENS) increases survival of ischaemic musculocutaneous flaps. *Acta Physiol Scand* 1988;134:95-9.
60. Kanno S, et al. Establishment of a simple and practical procedure applicable to therapeutic angiogenesis. *Circulation* 1999;99:2682-7.
61. Patterson C, Runge MS. Therapeutic angiogenesis: the new electrophysiology? *Circulation* 1999;99:2614-6.
62. Zhao M, et al. Electrical stimulation directly induces pre-angiogenic responses in vascular endothelial cells by signaling through VEGF receptors. *J Cell Sci* 2004;117(Pt 3):397-405.
63. Bai H, et al. DC electric fields induce distinct preangiogenic responses in microvascular and macrovascular cells. *Arterioscler Thromb Vasc Biol* 2004;24:1234-9.
64. Goldman R, et al. Electrotherapy promotes healing and microcirculation of infrapopliteal ischemic wounds: a prospective pilot study. *Adv Skin Wound Care* 2004;17:284-94.

65. Kaada B, Helle KB. In search of mediators of skin vasodilation induced by transcutaneous nerve stimulation: IV. In vitro bioassay of the vasoinhibitory activity of sera from patients suffering from peripheral ischaemia. *Gen Pharmacol* 1984;15:115-22.
66. Baker KG, Robertson VJ, Duck FA. A review of therapeutic ultrasound: biophysical effects. *Phys Ther* 2001;81:1351-8.
67. Peters EJ, et al. The benefit of electrical stimulation to enhance perfusion in persons with diabetes mellitus. *J Foot Ankle Surg* 1998;37:396-400 [discussion 447-8].
68. Gilcreast DM, et al. Effect of electrical stimulation on foot skin perfusion in persons with or at risk for diabetic foot ulcers. *Wound Repair Regen* 1998;6:434-41.
69. Griffin JW, et al. Efficacy of high voltage pulsed current for healing of pressure ulcers in patients with spinal cord injury. *Phys Ther* 1991;71:433-42 [discussion 442-4].
70. Bogie KM, Nuseibeh I, Bader DL. Transcutaneous gas tensions in the sacrum during the acute phase of spinal cord injury. *Proc Inst Mech Eng [H]* 1992;206:1-6.
71. Mawson AR, et al. Effect of high voltage pulsed galvanic stimulation on sacral transcutaneous oxygen tension levels in the spinal cord injured. *Paraplegia* 1993;31:311-9.
72. Kloth LC, Feedar JA. Acceleration of wound healing with high voltage, monophasic, pulsed current. *Phys Ther* 1988;68:503-8.
73. Feedar J, Kloth L, Gentzkow G. Chronic dermal ulcer healing enhanced with monophasic pulsed electrical stimulation. *Phys Ther* 1992;72:539.
74. Thornton RM, Mendel FC, Fish DR. Effects of electrical stimulation on edema formation in different strains of rats. *Phys Ther* 1998;78:386-94.
75. Reed BV. Effect of high voltage pulsed electrical stimulation on microvascular permeability to plasma proteins. A possible mechanism in minimizing edema. *Phys Ther* 1988;68:491-5.
76. Taylor K, et al. Effect of high-voltage pulsed current and alternating current on macromolecular leakage in hamster cheek pouch microcirculation. *Phys Ther* 1997;77:1729-40.
77. Sussman C, Dyson M. Therapeutic and diagnostic ultrasound. In: Batel-Jensen B, Sussman C, editors. *Wound Care*. 2nd edition. Gaithersburg (Md): Aspen; 2001.
78. Wendelken ME, Markowitz L, Patel M, Alvarez O. Objective, noninvasive wound assessment using B-mode ultrasonography. *Wounds* 2003;15:351-360.
79. Chen L, et al. The use of high-frequency diagnostic ultrasound to investigate the effect of hormone replacement therapy on skin thickness. *Skin Res Technol* 2001;7:95-7.
80. Kremkau K. Bioeffects of ultrasound. In: Kremkau K, editor. *Diagnostic ultrasound; principles and instruments*. Philadelphia (Pa): WB Saunders; 2002. p. 319-47.
81. Webster DF, et al. The role of cavitation in the in vitro stimulation of protein synthesis in human fibroblasts by ultrasound. *Ultrasound Med Biol* 1978;4:343-51.
82. Dijkmans PA, et al. Microbubbles and ultrasound: from diagnosis to therapy. *Eur J Echocardiogr* 2004;5:245-56.
83. Dinno MA, et al. The significance of membrane changes in the safe and effective use of therapeutic and diagnostic ultrasound. *Phys Med Biol* 1989;34:1543-52.
84. Johns LD. Nonthermal effects of therapeutic ultrasound: the frequency resonance hypothesis. *J Athl Train* 2002;37:293-9.
85. Young SR, Dyson M. The effect of therapeutic ultrasound on angiogenesis. *Ultrasound Med Biol* 1990;16:261-9.
86. Maxwell L, et al. The augmentation of leucocyte adhesion to endothelium by therapeutic ultrasound. *Ultrasound Med Biol* 1994;20:383-90.
87. Ito M, et al. Effects of ultrasound and 1,25-dihydroxyvitamin D₃ on growth factor secretion in co-cultures of osteoblasts and endothelial cells. *Ultrasound Med Biol* 2000;26:161-6.
88. Doan N, et al. In vitro effects of therapeutic ultrasound on cell proliferation, protein synthesis, and cytokine production by human fibroblasts, osteoblasts, and monocytes. *J Oral Maxillofac Surg* 1999;57:409-19 [discussion 420].
89. Young SR, Dyson M. Macrophage responsiveness to therapeutic ultrasound. *Ultrasound Med Biol* 1990;16:809-16.
90. Francis CW. Ultrasound-enhanced thrombolysis. *Echocardiography* 2001;18:239-46.
91. Reher P, et al. Ultrasound stimulates nitric oxide and prostaglandin E₂ production by human osteoblasts. *Bone* 2002;31:236-41.
92. Steffen W, et al. Catheter-delivered high intensity, low frequency ultrasound induces vasodilation in vivo. *Eur Heart J* 1994;15:369-76.
93. Yang KH, et al. Exposure to low-intensity ultrasound increases aggrecan gene expression in a rat femur fracture model. *J Orthop Res* 1996;14:802-9.
94. Yu T, Wang Z, Mason TJ. A review of research into the uses of low level ultrasound in cancer therapy. *Ultrason Sonochem* 2004;11:95-103.
95. Thawer HA, Houghton PE. Effects of ultrasound delivered through a mist of saline to wounds in mice with diabetes mellitus. *J Wound Care* 2004;13:171-6.
96. Ennis WJ, Formann P, Mozen N, Massey J, Conner-Kerr T, Meneses P. Ultrasound therapy for recalcitrant diabetic foot ulcers: results of a randomized, double-blind, controlled, multicenter trial. *Ostomy Wound Manage* 2005;51:24-39.
97. Gerber R. *Vibrational medicine for the 21st century*. New York: Harper Collins; 2000.
98. DiNucci EM. Energy healing: a complementary treatment for orthopaedic and other conditions. *Orthop Nurs* 2005;24:259-69.
99. Eells JT, et al. Mitochondrial signal transduction in accelerated wound and retinal healing by near-infrared light therapy. *Mitochondrion* 2004;4:559-67.
100. Posten W, et al. Low-level laser therapy for wound healing: mechanism and efficacy. *Dermatol Surg* 2005;31:334-40.
101. Lazarus GS, et al. Definitions and guidelines for assessment of wounds and evaluation of healing. *Arch Dermatol* 1994;130:489-93.
102. Ennis WJ, Meneses P. Wound healing at the local level: the stunned wound. *Ostomy Wound Manage* 2000;46(Suppl 1A):39S-48S [quiz 49S-50S].
103. Wlaschek M, Scharffetter-Kochanek K. Oxidative stress in chronic venous leg ulcers. *Wound Repair Regen* 2005;13:452-61.
104. Leite PF, et al. Redox processes underlying the vascular repair reaction. *World J Surg* 2004;28:331-6.
105. Giordano FJ. Oxygen, oxidative stress, hypoxia, and heart failure. *J Clin Invest* 2005;115:500-8.
106. Witte MB, Barbul A. Role of nitric oxide in wound repair. *Am J Surg* 2002;183:406-12.
107. Schwentker A, Billiar TR. Nitric oxide and wound repair. *Surg Clin North Am* 2003;83:521-30.
108. Cho HJ, et al. Calmodulin is a subunit of nitric oxide synthase from macrophages. *J Exp Med* 1992;176:599-604.
109. Nathan C. Nitric oxide as a secretory product of mammalian cells. *FASEB J* 1992;6:3051-64.
110. Fish JE, Mardsden PA. Endothelial nitric oxide synthase: insight into cell-specific gene regulation in the vascular endothelium. *Cell Mol Life Sci* 2006;63:144-62.
111. Morris Jr SM, Billiar TR. New insights into the regulation of inducible nitric oxide synthesis. *Am J Physiol* 1994;266(6 Pt 1):E829-39.
112. Albina JE, et al. Temporal expression of different pathways of l-arginine metabolism in healing wounds. *J Immunol* 1990;144:3877-3880.
113. Shearer JD, et al. Differential regulation of macrophage arginine metabolism: a proposed role in wound healing. *Am J Physiol* 1997;272(2 Pt 1):E181-90.
114. Pollock JS, et al. Nitric oxide synthase isoform expression in a porcine model of granulation tissue formation. *Surgery* 2001;129:341-50.

115. Li J, Zhang YP, Kirsner RS. Angiogenesis in wound repair: angiogenic growth factors and the extracellular matrix. *Microsc Res Tech* 2003;60:107-14.
116. Babaei S, Stewart DJ. Overexpression of endothelial NO synthase induces angiogenesis in a co-culture model. *Cardiovasc Res* 2002;55:190-200.
117. Ziche M, Morbidelli L. Nitric oxide and angiogenesis. *J Neurooncol* 2000;50:139-48.
118. Kawasaki K, et al. Activation of the phosphatidylinositol 3-kinase/protein kinase Akt pathway mediates nitric oxide-induced endothelial cell migration and angiogenesis. *Mol Cell Biol* 2003;23:5726-37.
119. Mitani T, et al. TGF-beta1 enhances degradation of IFN-gamma-induced iNOS protein via proteasomes in RAW 264.7 cells. *Nitric Oxide* 2005;13:78-87.
120. Haroon ZA, et al. A novel role for erythropoietin during fibrin-induced wound-healing response. *Am J Pathol* 2003;163:993-1000.
121. Yancopoulos GD, et al. Vascular-specific growth factors and blood vessel formation. *Nature* 2000;407:242-8.
122. Dulak J, et al. Nitric oxide induces the synthesis of vascular endothelial growth factor by rat vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol* 2000;20:659-66.
123. Howdieshell TR, et al. Inhibition of inducible nitric oxide synthase results in reductions in wound vascular endothelial growth factor expression, granulation tissue formation, and local perfusion. *Surgery* 2003;133:528-37.
124. Hale AJ, et al. Apoptosis: molecular regulation of cell death. *Eur J Biochem* 1996;237:884.
125. Smith CA, et al. Antibodies to CD3/T-cell receptor complex induce death by apoptosis in immature T cells in thymic cultures. *Nature* 1989;337:181-4.
126. Poliandri AH, et al. Nitric oxide protects the mitochondria of anterior pituitary cells and prevents cadmium-induced cell death by reducing oxidative stress. *Free Radic Biol Med* 2006;40:679-88.
127. Darby IA, et al. Skin flap-induced regression of granulation tissue correlates with reduced growth factor and increased metalloproteinase expression. *J Pathol* 2002;197:117-27.
128. Sachsenmaier C, et al. Involvement of growth factor receptors in the mammalian UVC response. *Cell* 1994;78:963-72.
129. Huang RP, et al. UV activates growth factor receptors via reactive oxygen intermediates. *J Cell Biol* 1996;133:211-20.
130. Takasawa R, et al. Differential apoptotic pathways in human keratinocyte HaCaT cells exposed to UVB and UVC. *Apoptosis* 2005;10:1121-30.
131. Peng HB, Baker LP, Dai Z. A role of tyrosine phosphorylation in the formation of acetylcholine receptor clusters induced by electric fields in cultured *Xenopus* muscle cells. *J Cell Biol* 1993;120:197-204.
132. Fang KS, et al. Epidermal growth factor receptor relocalization and kinase activity are necessary for directional migration of keratinocytes in DC electric fields. *J Cell Sci* 1999;112(Pt 12):1967-78.
133. Chernyavsky AI, et al. The Ras/Raf-1/MEK1/ERK signaling pathway coupled to integrin expression mediates cholinergic regulation of keratinocyte directional migration. *J Biol Chem* 2005;280:39220-8.
134. Altland OD, et al. Low-intensity ultrasound increases endothelial cell nitric oxide synthase activity and nitric oxide synthesis. *J Thromb Haemost* 2004;2:637-43.
135. Suchkova VN, et al. Ultrasound improves tissue perfusion in ischemic tissue through a nitric oxide dependent mechanism. *Thromb Haemost* 2002;88:865-70.
136. Honda H, et al. Role of intracellular calcium ions and reactive oxygen species in apoptosis induced by ultrasound. *Ultrasound Med Biol* 2004;30:683-92.
137. Abdollahi A, et al. Apoptosis signals in lymphoblasts induced by focused ultrasound. *FASEB J* 2004;18:1413-4.
138. Chen KD, et al. Mechanotransduction in response to shear stress. Roles of receptor tyrosine kinases, integrins, and Shc. *J Biol Chem* 1999;274:18393-400.
139. Li S, et al. Fluid shear stress activation of focal adhesion kinase. Linking to mitogen-activated protein kinases. *J Biol Chem* 1997;272:30455-62.
140. Bogoyevitch MA, Ketterman AJ, Sugden PH. Cellular stresses differentially activate c-Jun N-terminal protein kinases and extracellular signal-regulated protein kinases in cultured ventricular myocytes. *J Biol Chem* 1995;270:29710-7.
141. Ennis WJ, Meneses P. 31P NMR spectroscopic analysis of wound healing: the effect of hydrocolloid therapy. *Adv Wound Care* 1996;9:21-6.