Mechanisms of Action for Infrared Light on Tissue Healing

Selected Abstracts, Citations and Case Studies

Following are excerpts from published literature on the effects of infrared light and nitric oxide on tissue healing.

Infrared light therapy has been shown to trigger release of Nitric Oxide, a small endogenous molecule with multiple effects on body systems including fracture healing. The excerpts from published literature below highlight just some of the scientific and clinical investigations into the effects of infrared light and nitric oxide, suggesting mechanisms of action and pointing to potential clinical outcomes.

Application of infrared light to body tissues causes release of nitric oxide.

1) Studies have demonstrated infrared photochemical generation of nitric oxide by two-photon excitation of precursor molecules such as porphyrin complexes.[1]

"(The authors') laboratory has been concerned with developing strategies using photochemistry to trigger NO release from thermally stable precursors. It would be particularly desirable to utilize nearinfrared
(NIR) wavelengths for in vivo photochemical activation owing to the 800-1100 nm spectral window of greatest light penetration in mammalian tissue. In this context, we describe the successful photochemical generation of NO by two-photon excitation (TPE) of such a precursor by the use of NIR light.

In (the authors') experiments, NO was shown to be generated via Two Photon Excitation (TPE) of the porphyrin complex, PPIX-RSE ({í-S,í-S¢-protoporphyrin-IX-bis(2- thioethyl)diester]-tetranitrosyl-diiron) . Measurements of NO release from PPIX-RSE solutions were made by using a nitric oxide electrochemical sensor (Amino-700 from Innovative Instruments).

Solutions of PPIX-RSE (4.1 íM) in distilled, aerated THF were irradiated with the NIR laser under conditions identical to those for TPE fluorescence measurements. Exposure to the NIR laser varied from 1 to 3 min. Aliquots (50 íL) were withdrawn from the irradiated solutions and injected into a beaker containing 10 mL of deionized water in which the sensor was immersed. (The diluted PPIX-RSE concentration would then be _20 nM.). Upon injection of the photolysis solution, immediate signal increases indicating NO generation were seen. The first three injections were of PPIX-RSE solutions that had irradiated at 810 nm for 1, 1, and 3 min, respectively, and the last two were from PPIX-RSE solutions that had not been irradiated (to evaluate the NO produced from thermal decomposition). The solutions subjected to TPE excitation liberated NO, while the controls did not." *Influence of Differing Wavelengths on Biochemical Response*

2) Abergel and coworkers found that the irradiation of fibroblasts in culture either at 633 nm or at 904 nm stimulated the synthesis of collagen.[2]

3) The absorption spectrum of human tissue influences the depth of penetration of light. The figure at right shows the absorption spectrum of the human hand, suggesting that near infrared wavelengths (650nm – 900nm) attain deeper penetration than visible red (600nm – 649 nm). Combined with the photobiomodulatory response of 900 nm wavelengths, this suggests near infrared is suitable for eliciting photobio responses from internal tissues.[3]

Other studies of Infrared Depth of Penetration

4) The development of near-infrared fluorescent contrast agents and imaging techniques depends on the deep penetration of excitation light through several centimeters of tissue and the sensitive collection of the re-emitted fluorescence. In this contribution, the sensitivity and depth penetration of various fluorescence-enhanced imaging studies is surveyed and compared with current studies using continuous wave (CW) and frequency-domain photon migration (FDPM) measurements with planar wave illumination of modulated excitation light at 100 MHz and area collection of reemitted fluorescent light using a previously developed modulated intensified charge-- coupled device camera system. Fluorescence was generated from nanomolar to micromolar solutions of indocyanine green (ICG) in a 100 IiL volume submerged at 1-4 cm depths in a 1% Liposyn(R) solution to mimic tissue scattering properties. Enhanced depth penetration and sensitivity are achieved with optimal filter rejection of excitation light, and FDPM rejection of background light is not achieved using CW methods. We show the ability to detect as few as 100 fmol of ICG from area illumination of 785 nm light (5.5 mW/cm$_2$) and FDPM area collection of 830 nm fluorescent light generated from 3 cm below the phantom surface.

5) Light in the visible range is partially absorbed by naturally abundant fluorochromes, including

hemoglobin. Photons in the infrared region of the electromagnetic spectrum are partially absorbed by water. The near-infrared region of the electromagnetic spectrum provides a window of opportunity with greater tissue penetration. The fluorochromes reported on in this review typically fluoresce in the 700–800-nm range, wavelengths that allow for tissue penetration on the order of 10–15 cm.[4]

Absorption of light *versus* wavelength. Given the decreased absorption of light in the near-infrared (NIR) region compared with visible light (400–650 nm) and infrared light (>900 nm), tissue penetration of NIR photons may be up to 10–15 cm.

Effects of light Generating Sources: Laser vs Light Emitting Diodes

1) Dr. Kendric C. Smith at the Department of Radiation Oncology, Stanford University School of Medicine, concludes in an article entitled The Photobiological Effect of Low Level Laser Radiation Therapy (Laser Therapy, Vol. 3, No. 1, Jan - Mar 1991) that, "1. Lasers are just convenient machines that produce radiation. 2. It is the radiation that produces the photobiological and/or photophysical effects and therapeutic gains, not the machines. 3. Radiation must be absorbed to produce a chemical or physical change, which results in a biological response." [5]

2) In a study entitled Low-Energy Laser Therapy: Controversies and New Research Findings, Jeffrey R. Basford, M.D. of the Mayo Clinic's Department of Physical Medicine and Rehabilitation, suggests that the coherent aspect of laser may not be the source of its therapeutic effect. He states "firstly, the stimulating effects (from therapeutic light) are reported following irradiation with non-laser sources and secondly, tissue scattering, as well as fiber optic delivery systems used in many experiments rapidly degrade coherency. Thus any effects produced by low-energy lasers may be due to the effects of light in general and not to the unique properties of lasers. In this view, laser therapy is really a form of light therapy, and lasers are important in that they are convenient sources of intense light at wavelengths that stimulate specific physiological functions.[6]

Etiology of Acute Injury: The Body's Healing Process

From the moment a bone breaks or a ligament tears, the body goes to work to repair the damage. Here's what happens at each stage of the healing process:

1. At the moment of injury: Chemicals are released from damaged cells, triggering the inflammation process. Blood vessels at the injury site become dilated; blood flow increases to carry nutrients to the site of tissue damage.
2. Within hours of injury: White blood cells (leukocytes) travel down the bloodstream to the injury site where they begin to tear down and remove damaged tissue (via lymphatic drainage), allowing other specialized cells to start developing scar tissue. Cell proliferation and modeling occurs at this point.
3. Within days of injury: Scar tissue is formed on the skin or inside the body as the proliferation and modeling continue. The amount of scarring may be proportional to the amount of swelling, inflammation, or bleeding within. In the next few weeks, the damaged area will regain a great deal of strength as scar tissue continues to form.
4. Within a month of injury: Scar tissue may start to shrink, bringing damaged, torn, or separated tissues back together. However, it may be several months or more before the injury is completely healed.

Pathology of Pain

Pain is a universal experience. Nearly one-third of people will experience chronic pain at some point in their lives. Chronic pain affects 50 million Americans, according to the Joint Commission on Accreditation of Healthcare Organizations (JCAHO). This costs the country $125 billion or more each year in treatment, disability compensation and lost productivity.

Pain is the major reason for a patient to seek healthcare. For this reason JCAHO now lists pain as the “Fifth Vital Sign,” along with pulse, blood pressure, temperature and respiration rate. Yet only an estimated one in four Americans with chronic pain receives proper treatment.

Clinical pain results from the stimulation of nerve endings, or nociceptors. This stimulation is most often caused by traumatic injury or disease affecting peripheral tissues. Pain signals are then sent along to the spinal cord and up to the brain where it is consciously recognized. Examples include skin cuts, bruises, arthritis and bone fractures. Pain can also arise from direct injury or disease of the nerves leading to the spinal cord, to the spinal cord itself or to the brain. This is called neuropathic disease. Examples include carpal-tunnel syndrome, migraines, and fibromyalgia.

Light Therapies and Tissue Healing

One of the beneficial characteristics of light therapy is that it has the ability to promote and enhance healing, not just treat symptoms. The irradiation by infrared light triggers the natural repair mechanisms carried out by the body. Several of the mechanisms of action for light therapy that work to alleviate pain and inflammation also play an important role in tissue healing.

Wound healing progresses through stages of inflammation, proliferation, remodeling and maturation. Light therapy has been demonstrated to impact each of these phases in beneficial ways. Light therapy can provide the following beneficial impacts in both open surface wounds and closed connective or soft tissue injuries:

1. Enhanced leukocyte infiltration. Light therapy stimulates activity involving neutrophils, monocytes and lymphocytes. These white blood cells play key roles in clearing out damaged cells.
2. Increased macrophage activity. Light therapy accelerates macrophage activity in phagocytosis, growth factor secretion and stimulation of collagen synthesis.
3. Increased neovascularization. The significant angiogenesis that occurs with laser therapy promotes revascularization with subsequent improvement in perfusion and oxygenation. Endothelial cell regeneration is accelerate[7]
4. Increased fibroblast proliferation. Light therapy stimulation increases fibroblast numbers and fibroblast-mediated collagen production.[viii]
5. Keratinocyte proliferation. The beneficial synthesis activities and growth factor ability of keratinocytes are enhanced by proliferation secondary to light therapy.[ix]
6. Early epithelialization. Laser-stimulated acceleration of epithelial cell regeneration speeds up wound healing, minimizes scarring, and reduces infection opportunities.
7. Growth factor increases. Two to five fold increases in growth-phase-specific DNA synthesis in normal fibroblasts, muscle cells, osteoblasts and mucosal epithelial cells irradiated with IR light are reported. Increases in vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF-2) secondary to infrared light irradiation have also been reported.
8. Enhanced cell proliferation and differentiation. Infrared-induced increases in Nitric Oxide, ATP and other compounds that stimulate higher activity in cell proliferation and differentiation into mature cells. Increased numbers of myofibroblasts, myofibrils, myotubules etc., as well as bone cell proliferation, have been clinically documented after light therapy. Satellite cells, the precursor cells in the process of muscle regeneration, show significant increase in proliferation when irradiated with light therapy.[x,xi,xii]
9. Greater healed wound tensile strength. In both soft tissue and connective tissue injuries, light therapy can increase the final tensile strength of the healed tissue. By increasing the amount of collagen production/synthesis and by increasing the intra and inter-molecular hydrogen bonding in the collagen molecules, laser therapy contributes to improved tensile strength.[xiii,xiv,xv,xvi] The preceding effects combine to achieve an accelerated healing rate. The time from onset of injury to mature healed wound is reduced.[xvii]

Light Therapies and Soft Tissue Healing

Numerous studies have been conducted which demonstrate the effectiveness of infrared light on soft tissue healing. LED produced in vitro increases of cell growth of 140-200% in mousederived fibroblasts, rat-derived osteoblasts, and rat-derived skeletal muscle cells, and increases in growth of 155-171% of normal human epithelial cells. Wound size decreased up to 36% in conjunction with HBO in ischemic rat models.[xviii]

A clinical study was performed on 74 patients with injuries to the following anatomic locations: ankle and knee, bilaterally, Achilles tendon; epicondylus; shoulder; wrist; interphalangeal joints of hands, unilaterally. All patients had had surgical procedure prior to infrared light. Comparison of the healing process between two groups of patients obtained the following results: wound healing was significantly accelerated (25%-35%) in the group of patients treated with infrared light.[xix] In another study, forty-seven soccer players with second degree ankle sprains were selected at random and divided into three groups: treatment with the conventional initial treatment (RICE, rest, ice, compression, and elevation), treatment with the RICE method plus placebo laser, and the third group treated with the RICE method plus an 820-nm GaA1As diode laser. The laser + RICE group showed statistically significantly edema reduction compared to controls at 24, 48 and72 hours.[xx] Finally, a review of 9 separate

placebo controlled trials measuring pain and range of motion scores in tendinopathies showed an average 32% improvement in treated over untreated.[xxi]

Light Therapies and Bone Healing

Studies of bone healing response to infrared light show acceleration of osteoblast formation as well as calcium salt deposition under the influence of infrared light.[xxii,xxiii] Studies have demonstrated that bone growth factors are stimulated by IR light. Osteoglycin is a small leucine-rich proteoglycan (SLRP) of the extracellular matrix which was previously called the osteoinductive factor. SLRP are abundantly contained in the bone matrix, cartilage cells and connective tissues, and are thought to regulate cell proliferation, differentiation and adhesion in close association with collagen and many other growth factors. In osteoblastic cells the osteoglycin/mimecan gene was upregulated 2.3-fold at 2 h after exposure to infrared light.[xxiv] Nicolau and colleagues (2002) from Brazil demonstrated the positive effect of LLLT on the stimulation of bone in mice with latent promotion of bone remodulation at injury sites without changes in bone architecture, increased bone volume and increased osteoblast surface through increased resorption and formation of bone with higher apposition rates. A positive effect on bony implants has been demonstrated by Dörtbudak (2002) and Guzzardella (2003).[xxv]

An animal trial of 4 weeks' duration was conducted on osseous defects of 2.7 mm diameter made in each parietal bone of 20 rats (20 additional rats received placebo treatment). A GaAlAs diode laser was applied immediately after surgery and then daily for 6 consecutive days. Five rats from each group were killed on day 14 and the remainder on day 28 postoperatively. At both time points the tissue samples from the experimental animals contained significantly more calcium, phosphorus, and protein than the controls. Similarly, histological analyses disclosed more pronounced angiogenesis and connective tissue formation, and more advanced bone formation in the experimental group than in the controls.[xxvi]

The effect of HeNe laser on the healing of tibial bone fractures in rats: 63 J (35mW) was given transcutaneously daily over the fracture area. After 4 weeks the tibia was removed and tested at tension up to failure. The maximal load at failure and the structural stiffness of the tibia were found to be elevated significantly in the irradiated group, whereas the extension maximal load was reduced. In addition, gross non-union was found in four fractures in the control group, compared to none in the irradiated group.[xxvii]

Pain Reduction from Light Therapy

The pain reduction effects of light therapy have been extensively researched and documented in numerous clinical studies and medical papers. While much remains to learn with respect to the various mechanisms through which light therapy achieves pain reduction, there is a wealth of knowledge currently available to demonstrate the effectiveness of laser therapy in this regard. Because the pain amelioration capabilities of light therapy are accomplished via the combination of local and systemic actions —utilizing enzymatic, chemical and physical interventions — the process is very complex. However, there is a preponderance of medical evidence that justifies a conclusion that effective pain reductions and accelerated tissue healing can be achieved via light therapy. Following are processes and events that are promoted by light therapy.

Mechanisms of Action for Light Therapies

Mechanisms of Action: Nitric Oxide

One of the most important mechanisms of action for light therapy is release of nitric oxide. A naturally occurring chemical in the body, nitric oxide is a key signaling molecule which can set off a number of beneficial effects. Most notably, it has a critical role in promoting blood flow to tissues. It also indirectly inhibits inflammation processes, thus reducing inflammation. In acute inflammatory responses, such as sudden injury, large increases in nitric oxide levels can play a role in increased pain. However, within the nervous tissues smaller levels of nitric oxide release, as stimulated by light therapies, can paradoxically have pain reducing effects. This was demonstrated in an animal model by Mrowiec et al in which they showed that an inhibitor of nitric oxide signaling blocks the analgesic effect of low power laser on intact rats.

The benefits of light therapy are that they reduce the discomfort of pain and inflammation while promoting blood flow and the body’s tissue repair mechanisms.

Mechanisms of Action in Healing:

Mobilization of endogenous chemical or protein signals for adult stem cell engraftment

Recent work has shed light on the underlying mechanisms of tissue repair within the body. Conboy[xxviii] et al, investigated the influence of systemic factors on aged progenitor cells of peripheral tissues such as muscle and liver. They conducted an experiment wherein they established parabiotic pairings (that is, a shared circulatory system) between young and old mice (heterochronic parabioses), exposing old mice to factors present in young serum. Notably, heterochronic parabiosis restored the activation of Notch signaling as well as the proliferation and regenerative capacity of aged satellite cells. The exposure of satellite cells from old mice to young serum enhanced the expression of the Notch ligand (Delta), increased Notch activation, and enhanced proliferation in vitro.

In another line of research, Gold[xxix] et al elucidated the role of a protein signaling molecule, calreticulin (CRT), an intracellular protein with functional significance in the transport of protein through the endoplasmic reticulum. It has more recently been recognized as a critical regulator of extracellular functions, particularly, in mediating cellular migration and as a requirement for the uptake and clearance of dead cells. Dr. Leslie Gold and her team discovered that CRT has remarkable effects on the acceleration of the rate and quality of tissue repair in experiments that involved both the application of CRT to different animal models of skin injury and in vitro assays commonly used to assess wound repair.

Minimized scarring is observed with infrared light treatment. Some of these signaling mechanisms may underlie the efficacy of infrared light in accelerating wound healing, as seen in clinical trials.

Mechanisms of Action: Lymphatic Drainage

There is also a hypothetical potential that the presence of IR by increasing lymphatic circulation does so by virtue of an increase in the diameter of the lymphatic vessels, not just by increased flow rates within the vessel at an unchanged diameter. This diameter increase, if definitively present, would also explain the presence of large diameter protein cells within the normal bone circulation that cannot be attributed to the vascular circulation and would additionally explain a facilitated process for removal of debris and larger protein cells passing out of traumatized areas that is additionally stimulated by the use of IR.[xxx]

Mechanisms of Action: Effects on Nerves

Studies of the effectiveness of light therapy on a number of chronic pain conditions suggest that it may have activity on specific nerve fibers involved in “slow conduction” of pain signals. Human and animal studies have found elevated levels of endorphins (small proteins which block pain signals in nerves) in response to light therapy.

Mechanisms of Action: ATP – The energy source for cells in the body

Light affects the mitochondrial respiratory chain by changing the electric potential of cell membranes and, consequently, their selective permeability for sodium, potassium and calcium ions, or by increasing the activity of certain enzymes such as cytochrome oxidase or adenosine triphosphatase.[xxxi]

Clinical results have been attributed to the general effects of infrared light therapy and its ability to increase the rates of healing through mitochondrial ATP production and alteration in the cellular lipid bi-layer. Additional hypothesis includes the subsequent capacity of irradiated cells to alter their ion exchange rate and thus influence the catalytic effects of the specific enzymes and substrates. These in turn initiate and promote the healing process completing the cascading cycle of events.[xxxii]

Studies of cultured cells show that levels of Adenosine Triphosphate (ATP) are raised upon exposure to specific wavelengths of infrared light. ATP is the final fuel into which all food is ultimately transformed. It is the energy currency for the body’s cells. The body’s self repair of injured tissues requires enhanced amounts of ATP. Studies of rats exposed to infrared light show increased ATP levels in their brains. In addition to serving as the energy currency for cells, ATP can serve as a neurotransmitter. After being broken down to adenosine, it binds to adenosine receptors that block pain signals in the nervous system.

Mechanisms of Action: Acetylcholine, Bradykinin and Ions

Two more neurotransmitter chemicals involved in pain are acetylcholine and bradykinin. These two are affected by light therapy. Nerve tissue membranes are threaded through with

ion channels. It is now conventional wisdom that acetylcholine and nicotine act at these receptors to alter electrochemical properties at a variety of synapses, which can in turn affect the release of several other neurotransmitters. When activated by acetylcholine they allow selected ions to flow across the cell membrane, suppressing pain responses. Infrared light exposure increases acetylcholine levels. In contrast, bradykinin levels are suppressed by infrared light, reducing signaling of pain in the central nervous system. It is likely that several or all of these mechanisms contribute to the analgesic activity of light therapy.
[1]