Selected Abstracts, Citations and Case Studies: The Effects of Infrared Light on Bone Healing

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

In-Vitro Evidence for fracture healing and bone engraftment with Infrared treatment

Ion Exchange and Bone Mineralization

1) 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 and adenosine triphosphatase.[1] This may contribute accelerated uptake of ions in the mineralization process of fracture healing.

2) (Results of previous studies demonstrating infrared light stimulation of bone growth) have been attributed to the general effects of low level light therapy (infrared laser light) and its ability to increase the rates of healing through mitochondrial ATP production and alteration in the cellular lipid bi-layer. Additional hypotheses 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.[2]

Role of Nitric Oxide in Bone Formation

3) Nitric oxide (NO) has been implicated in the local regulation of bone metabolism. Aguirre, et. al. demonstrated the contribution made by specific NO synthase (NOS) enzymes. They observed that endothelial NOS gene knockout mice (eNOS –/–) have marked abnormalities in bone formation. Histomorphometric analysis of eNOS –/– femurs showed bone volume and bone formation rate was reduced by up to 45% ($P < 0.01$) and 52% ($P < 0.01$), respectively. Reduction in bone formation and volume was not related to increased osteoclast numbers or activity but rather to dysfunctional osteoblasts. Osteoblast numbers and mineralizing activity were reduced in eNOS –/– mice. In vitro, osteoblasts from calvarial explants showed retarded proliferation and differentiation (alkaline phosphatase activity and mineral deposition) that could be restored by exogenous administration of a NO donor.[3]

4) Bone metabolism involves the balance between formation and resorption. In this process, known as bone remodeling, mineralized bone is continuously resorbed by osteoclasts and new bone is formed by osteoblasts. Nitric Oxide produces rapid osteoclast detachment and contraction in-vitro, and this effect is accompanied by a profound inhibition of bone resorption. Inhibition of NO synthase (resulting in absence of NO) in normal rats is followed by increased bone resorption reflected by a marked loss in bone mineral density. Further investigation shows that inhibition of the inducible Nitric Oxide Synthase (iNOS) is accompanied by marked enhancement of bone resorption. However, nonselective NOS inhibition results in decreased resorption. The authors detected the presence of constitutive calcium-sensitive NOS isoform (cNOS) in rat osteoclasts and human preosteoclast cell lines. Thus osteoclast function may require intermittent calcium-stimulated increases in NO production by cNOS [demineralizing forces] against a basal inhibitory background activity of the iNOS isoform [bone building forces]. Bone resorption depends on both osteoclast precursor replication and on the activity of mature osteoclast cells. Addition of an NO donor results in depressed replication in human preosteoclast lines. Taken together, these results strongly suggest that NO maintains a central control of bone resorption by exerting a powerful tonic restraint of osteoclast numbers and activity. Since NO also influences behavior of the osteoblast, the bone-forming cell, in-vitro, a similar effect in-vivo might imply a general influence on bone remodeling.[4]

Lymphatic Circulation and Bone

5) Dr. Tony Pohl of the Royal Adelaide Hospital in South Australia, has provided a new theory that postulates that the majority of fluid transfer and exchange within living bone is predominantly influenced by the lymphatic circulation.[5] Pohl postulates that the majority of fluid transfer and exchange within the living bone is predominantly influenced by the lymphatic rather than the vascular circulation. This is justified through studies on bone fluid input and output levels that have demonstrated that the venous and arterial aspect of circulation alone cannot account for the demonstrated levels of output nor the presence of free radical molecules which exceed those of the vascular input. Furthermore, the diameter of large (globular) proteins within the bone exceed the diameter of the vessels that form the terminal aspects of the circulatory system making it impossible for them to have been delivered via this system. Infrared light is well documented and known as having effects that influence the lymphatic circulation and wound healing process.

6) There is also a hypothetical potential that the presence of infrared light 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 infrared light therapy.[6]

7) Infrared light, with its known general effects and specific direct effects on the lymphatic system, would act to stimulate mitochondria ATP that increases cellular and circulatory motility as well as directly influencing lymphatic flow. It also promotes increased permeability in interstitial tissue and facial layers (Gabel 1995) reducing stagnation and blockage. These actions would assist the increase in lymphatic flow and consequently the circulation within the affected bone.[7]

Osteoblast Proliferation Increases Bone Formation

8) The effect of laser irradiation on osteoblastic cells has been reported by Yamamoto (2001) and Guzzardella (2002): The effect of low-power laser irradiation on the proliferation activity of HeLa cells was investigated. The cells were irradiated by a 830 nm semiconductor BTL-10 laser in a continuous or pulsed mode at an energy density ranging from 2 to 99 J/cm2 (power output, 72 to 360 mW). The irradiated cells were incubated and their proliferation activity was assessed by the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay at 24, 48, 72 and 96 h. In comparison with the control populations, the irradiated cells showed a significant increase in proliferation, regardless of the energy density used, at 72 and 96 h but not at 24 and 48 h. In addition, the stimulation of proliferation was related to the mode of irradiation. The cells irradiated in the pulsed mode (5 000 Hz) showed a higher proliferation activity than the cells treated by continuous laser light. It is concluded that low-power lasers stimulate HeLa cell proliferation.[8]

9) The aim of (another) study was to investigate the effect of low-level laser irradiation on proliferation and differentiation of a human osteoblast cell line. Cultured osteoblast cells were irradiated using He-Ne laser irradiation (632 nm; 10 mW power output). A significant 31-58% increase in cell survival (MTT assay) and higher cell count in the once-irradiated as compared to nonirradiated cells was monitored. Differentiation and maturation of the cells was followed by osteogenic markers: alkaline phosphatase (ALP), osteopontin (OP), and bone sialoprotein (BSP). A two-fold enhancement of ALP activity and expression of OP and BSP was much higher in the irradiated cells as compared to non-irradiated osteoblasts.[9]

10) Phototherapy increased the proliferation rate of cells independently of dexamethasone presence. Adhesion and osteonectin synthesis were not significantly influenced by laser and/or dexamethasone. Based on the conditions of this study investigators concluded that phototherapy acts as a proliferative stimulus on osteoblast-like cells, even under the influence of dexamethasone. Thus, the suggestion that phototherapy can be of importance as co-adjuvant in bone clinical manipulation in order to accelerate bone regeneration.[10]

11) Osteoblasts from 3-day-old Wistar rat calvaria were irradiated using a low-power Gallium-Aluminium-Arsenide (Ga-Al-As) diode laser under various conditions. Bone formation, osteoblast differentiation, and cell proliferation were evaluated. The greatest bone formation with laser irradiation was observed the first week after seeding and with an irradiation energy of 3.75 J/cm2. Laser irradiation induced an increase in ALP activity 7 days after seeding, and an increased number of cells beginning 4 days afterwards. Results demonstrate that low-power laser irradiation initially depresses the cell cycle of osteoblastic cells, and then stimulates cell proliferation, resulting in increased bone formation. [11]

Effects on Osteoblast Gene Expression

12) Effect Of Low-Level Laser Irradiation On Osteoglycin Gene Expression In Osteoblasts: This study investigated the stimulatory effect of infrared light on bone formation during the early proliferation stage of cultured osteoblastic cells. . Among those genes that showed at least a twofold increased expression, the osteoglycin/mimecan gene was upregulated 2.3-fold at 2 h after LLLI. 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.[12]

Osteoclast Inhibition Prevents Bone Mineral Resorption

13) The osteoclast is unique in its ability to resorb bone, and excessive osteoclastic activity has been implicated in osteoporosis, Paget disease of bone, rheumatoid arthritis, and the growth of metastases in bone. The activity of this cell is controlled by the main circulating inhibitor, calcitonin, in association with locally produced modulators. The authors showed that nitric oxide (NO) may be an important member of the latter group. NO is produced by the vascular endothelium and nervous system and is involved in both neurotransmission and the regulation of blood pressure. However, results showed that the autocoid is also a potent inhibitor of osteoclast function. NO (30 microM) produced a decrease to approximately 50% of the original osteoclast spread area. Similar effects were also produced by 3-morpholinosydnonimine or sodium nitroprusside, reagents that spontaneously release NO. These shape changes were associated with a reduction of bone resorption after a 24-hr incubation of isolated osteoclasts on devitalized bone slices. NO is thought to act by stimulating guanylate cyclase, with a consequent increase in cyclic GMP, but a different mode of action is likely in the osteoclast since dibutyryl or 8-bromo cyclic GMP have no effect. It should be noted that calcitonin can produce similar changes in shape and activity but is associated with an increase in osteoclast intracellular calcium and cessation of membrane movement; neither of these is produced by NO, suggesting that its mode of action is different. The abundance of NO-producing endothelial cells in bone marrow and their proximity to osteoclasts suggests that marrow endothelial cells may play a physiological role in the regulation of osteoclastic activity.[13]

Bone Engraftment on Synthetic Materials

14) Human fetal osteoblast cells (hFOB) were used in an in vitro test to examine changes in cell adhesion on a magnesia-partially stabilised zirconia (MgO-PSZ) bioceramic after CO_2 laser treatment. The in vitro cell evaluation revealed a more favourable cell response on the CO_2 laser-treated MgO-PSZ than on the untreated sample. After 24-h cell incubation,

no cell was observed on the MgO-PSZ, whereas a few cells attached on the CO_2 laser-treatedMgO-PSZ and showed well spread and good attachment. Moreover, the cell coverage density indicating cell proliferation generally increases with CO_2 laser power densities applied in the experiments.[14]

15) Mesenchymal stem cells (MSCs) seeded on three-dimensional (3D) coralline (Porites lutea) biomatrices were irradiated with low-level laser irradiation (LLLI). The consequent phenotype modulation and development of MSCs towards ossified tissue was studied in this combined 3D biomatrix/LLLI system and in a control group, which was similarly grown, but was not treated by LLLI. The results obtained from the irradiated samples showed enhanced tissue formation, appearance of phosphorous peaks and calcium and phosphate incorporation to newly formed tissue. Findings of cell and tissue parameters up to 28 days of culture revealed higher ossification levels in irradiated samples compared with the control group.[15]

Animal Studies of Infrared Light and Bone Healing

1) Nicolau and colleagues (2002) from Brazil demonstrated the positive effect of infrared light therapy 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).[16]

2) 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.[17]

3) 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.[18]

Differential Localization, Expression and Activity Of Nitric Oxide Synthases Observed In Fracture Repair

4) To localize the sites of expression compared with those in normal bone investigators made standardised, stabilised, unilateral tibial fractures in male Wistar rats. Immunohistochemistry showed increased expression of endothelial NOS (eNOS) to be strongest in the cortical blood vessels and in osteocytes in the early phase of fracture repair. Significantly elevated calcium-dependent NOS activity was observed at day 1 after fracture. Inducible NOS (iNOS) was localized principally in endosteal osteoblasts and was also seen in chondroblasts especially in the second week of fracture healing. Western blotting showed a reduction in iNOS during the early healing period. Significantly reduced calcium-independent NOS activity was also seen. No neuronal NOS was seen in either fracture or normal tissue. Increased eNOS in bone blood vessels is likely to mediate the increased blood flow recognized during fracture healing. eNOS expression in osteocytes may occur in response to changes in either mechanical or local fluid shear stress. The finding that eNOS is increased and iNOS reduced in early healing of fractures may be important in their successful repair.[19]

Human Case Studies of Infrared Light and Bone Healing

1) As far as is known, the first attempt at treating bone fracture with infrared light was reported by Shugaharov and Voronkov. In 1974 they used low level laser radiation (infrared wavelengths) on fracture sites observing intramedullar osteosynthesis.[20]

2) Gatev studied the effect of stimulating repair of fractures with He-Ne laser. The majority of patients had fractures of the distal radius treated with a plaster cast. On the 5th to 8th day after injury a hole was cut out of the cast over the fracture site and laser radiation applied at 632 nm, 2 mW/cm^2. Evaluations were made based on radiographic evidence and clinical assessment. Results showed statistically significant differences [$p<0.001$] from the control group in favor of light treated fractures.[21]

3) A 1990 case study looked at a non-union long bone fracture refractive to treatment over a period of 8 months. A 24 year-old patient was treated conservatively for displaced fracture of the diaphyses of both bones of the right forearm. When secondary displacement occurred the fracture was operated on with use of a compression plate for the radius and a single Rush Rod for the ulna. Eight months after the injury the radiological and clinical examination showed signs of delayed union of both fractures. A diode laser emitting 890 nm wavelength near infrared light with average output of 3 mW and an energy deposition of 1.8 Joules/cm^2 was applied 3 times per week. After 4 weeks of treatment the signs of callus formation appeared. After another 5 weeks the radiogram showed complete remodeling of the ulnar bone and union in the radius. No side effects were observed.[22]

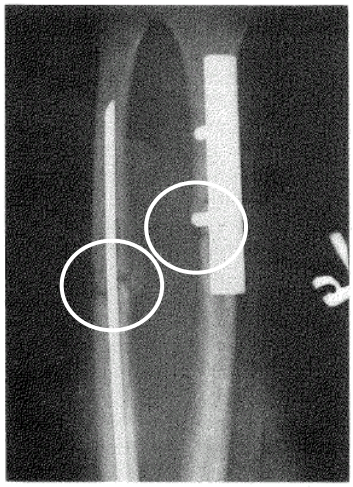


Figure 1. Radiogram demonstrating delayed union of both forearm bones

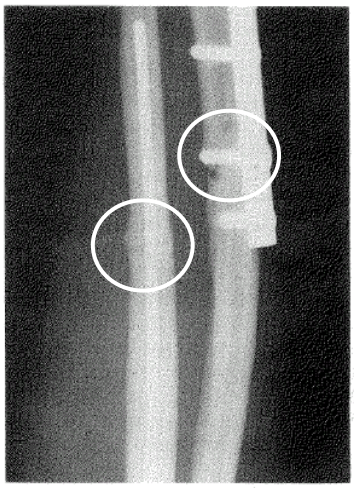
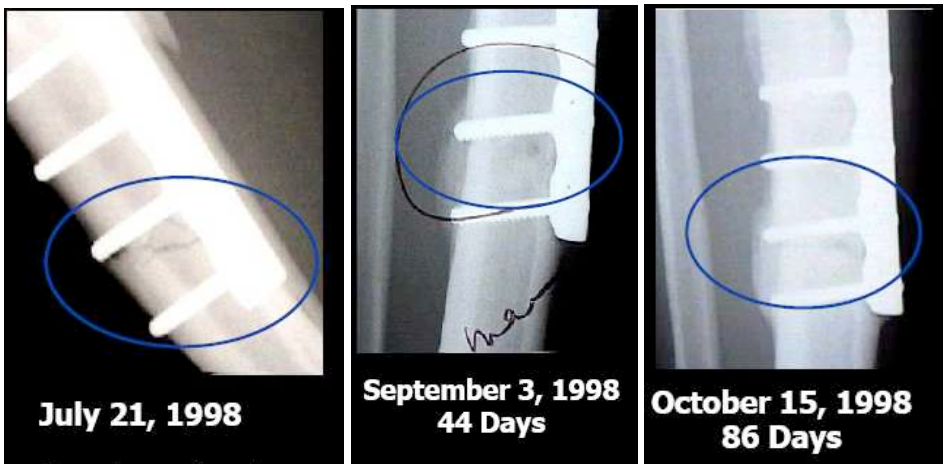


Figure 2. Radiogram demonstrating healed bones after laser treatment

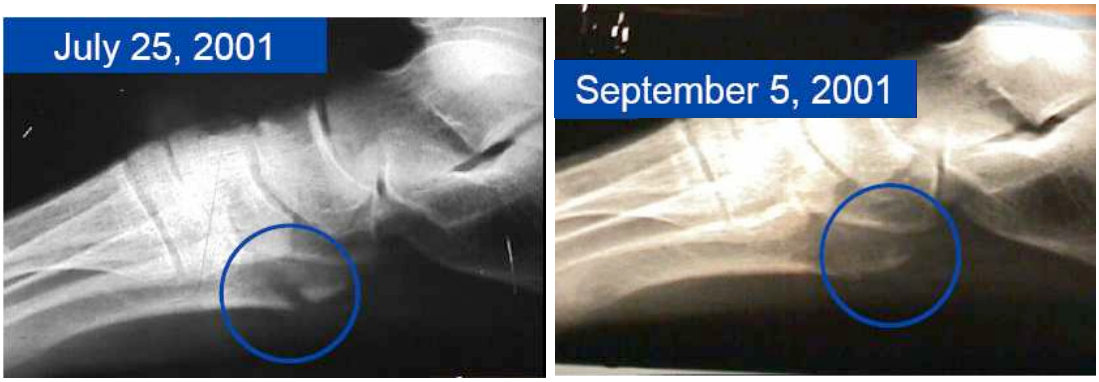
4) A 15 year old male athlete presented with an avulsion fracture with involvement of the inferior aspect of Anterior Superior Iliac Spine. ASIS injury was non weight bearing. Patient was taking 3200 mg ibuprofen daily. Normal prognosis is 4-6 weeks non weight bearing followed by 6 weeks of rehab and additional 10 weeks before return to sport (running). Protocol followed for this case: initiated daily infrared light treatments, 890 nm, 20 Joules/cm^2, 20 min treatments. Rehab begun on third visit. Discontinued ibuprofen after third treatment. Discharged from treatments after 24 visits and orthopedist released patient at 100% to return to running. Total time reduced from 22 weeks (normal prognosis) to 5 weeks.[23]

5) An 18 year old high school athlete presented with a non union tibial fracture. The patient had previously fractured the same site, taking 15 months to heal. Re-fracture was fixed with a compression plate. After 2 years the patient still showed edema and pain with

radiographic evidence on non union. Once daily treatments (5 days per week) were initiated with infrared light, 890 nm, 20 Joules/cm^2, 20 min per treatment. After 44 days radiographic analysis showed pannus formation over the set screws. After 86 days radiograph showed complete fracture healing.[24] Note pannus formation over screws.



6) A patient presented with a non union 5th metatarsal fracture of the left foot. The patient was treated conservatively with immobilization and non weight bearing. After 3 months no progress was evident from radiographic and clinical assessments. Daily treatments (5 days per week) were initiated with infrared light, 890 nm, 20 Joules/cm^2, 20 min per treatment. After three weeks radiographic and clinical assessment showed complete healing.[25]



Ultrasound: Improved Bone Healing Through Enhanced Circulation

(NOTE: Ultrasound has been shown to effect nitric oxide release, which would reasonably be expected, as past evidence has shown that shear-force on vascular endothelia elicits NO release. Nitric oxide release results in increased circulation and lymphatic drainage and has been shown to be one of the effects of infrared light on tissues. Hence, some of the proposed mechanisms of action for ultrasound on bone healing relate to the actions of infrared light.)

1) The *mechanisms by which therapeutic ultrasound can be effective for fracture repair includes nitric oxide (NO) pathways* and prostaglandin (PGE2) (Reher et al 2002, Warden et al 2001, Kobubu et al 1999).

2) A multicenter, prospective, randomized, double-blind, placebo-controlled clinical trial was conducted to test the efficacy of a specifically programmed, low-intensity, non-thermal, pulsed ultrasound medical device for shortening the time to radiographic healing of dorsally angulated fractures. Sixty patients (sixty-one fractures) were enrolled in the study within seven days after the fracture. The patients used either an active ultrasound device (thirty fractures) or a placebo device (thirty-one fractures) daily for twenty minutes at home for ten weeks. Clinical examination was performed and radiographs were made at one, two, three, four, five, six, eight, ten, twelve, and sixteen weeks after the fracture by each site investigator. The time to union was significantly shorter for the fractures that were treated with ultrasound than it was for those that were treated with the placebo. 61 +/- 3 days compared with 98 +/- 5 days; $p < 0.0001$.[26]

3) A recent systematic review and meta-analysis (Busse et al 2002) has carefully considered the evidence in respect to the effect of low intensity pulsed ultrasound on the time to fracture healing. They conclude that the evidence from randomised trials where the data could be pooled (3 studies, 158 fractures) that the time to fracture healing was significantly reduced in the ultrasound treated groups than in the control groups and the mean difference in healing time was 64 days.[27]

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