NIR Basic and Clinical Research Review

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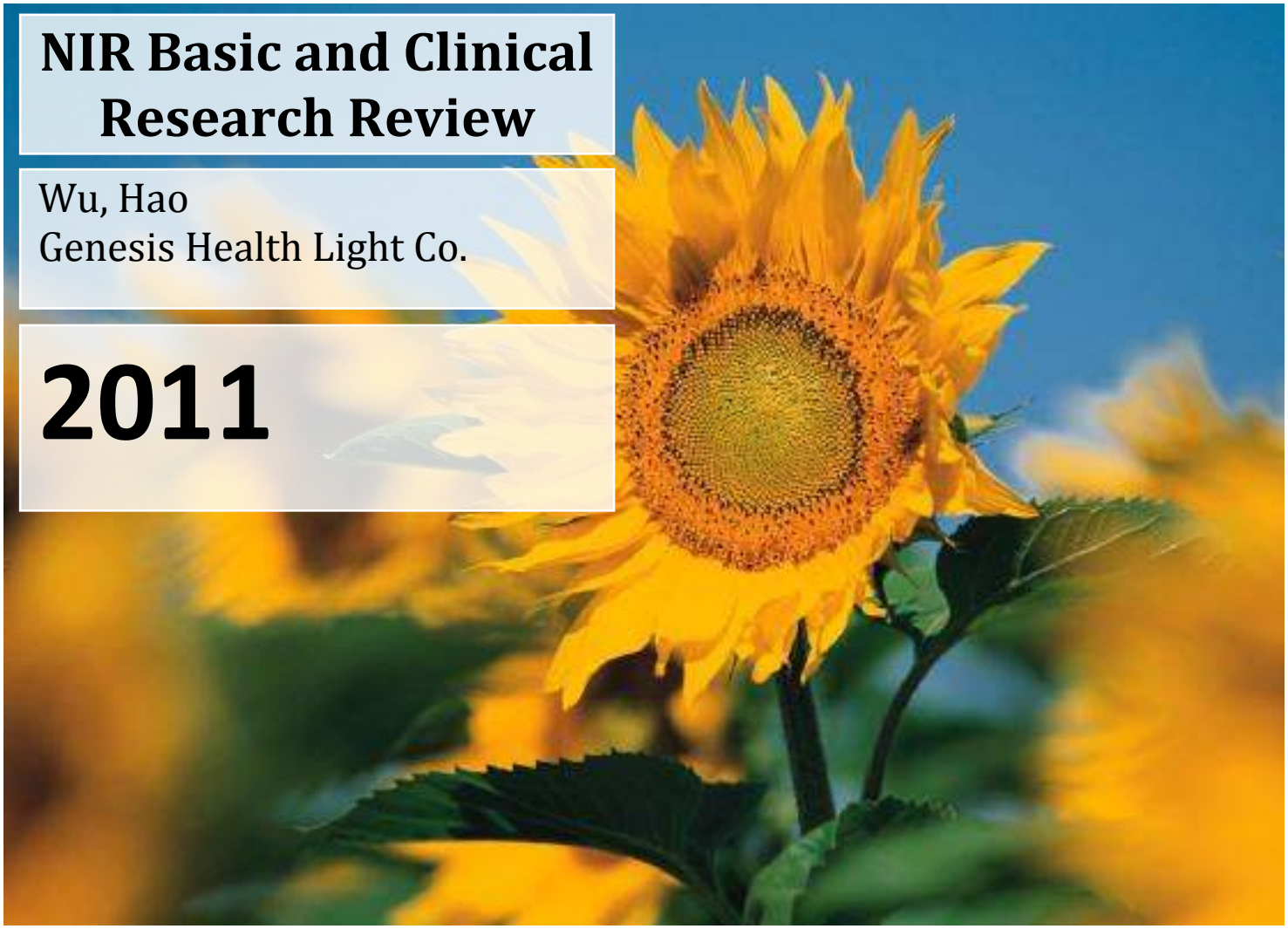

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This is a review of fundamental and clinical researches of near infrared light therapeutic effects. NIR light represents a novel, noninvasive, therapeutic intervention without any side-effects. Researches demonstrate that NIR light treatment stimulates mitochondrial oxidative metabolism in vitro, and accelerates cell and tissue repair in vivo. There are many clinical studies showed NIR can alleviate pain, diminish inflammation and speed up wound healing. NIR has a broad usage in wound healing and aging symptoms like neck pain, back pain, and arthritis. Additionally, NIR has good effects on diabetic foot ulcers.

The review of fundamental and clinical studies on Near Infrared light therapeutic effects

Hao Wu

Genesis Health Light, July, 2011

Abstract

The experience of the pleasant heat of the sun in moderate climatic zones arises from the filtering of the heat radiation of the sun by water vapor in the atmosphere of the earth. The filter effect of water decreases those parts of ultraviolet (UV) and infrared radiation (most parts of infrared-B and -C and the absorption bands of water within infrared-A), which would cause by reacting with water molecules in the skin – only an undesired heat load to the surface of the skin. The left visible light (550-750nm) and near infrared (750-1400nm) (NIR) as a special form of light radiation with a high tissue penetration and a low heat load to the skin surface.

As we know, all plants perform photosynthesis which converts sunlight and water into glucose and oxygen (photo energy-chemical energy). Cellular biologists have determined that our bodies use a similar principle in the final digestive process whereby proteins, fats and sugars are broken down within the mitochondrion membrane into the smallest molecular nutrient elements, called pyruvates and produce ATP energy. Certain light wavelengths such as near infrared, on the low level of the color spectrum stimulate the mitochondrion membrane to produce ATP. ATP is the fuel that all cells utilize to perform cellular activities, including DNA and RNA synthesis, cellular repair and collagen production. One of the primary effects of NIR is on cytochrome C oxidase in the mitochondria. When cells are stressed their mitochondria produce nitric oxide (NO) which displaces oxygen in the electron transport chain. This prevents ATP production and increases oxidative stress leading to inflammation and cell death. NIR photo-dissociates NO from cytochrome C oxidase leading to restoration of ATP production, a reduction of oxidative stress and subsequently a reduction in inflammation. The effects will lead to increase of growth factors secretion and activation of enzymes and other secondary messengers. These will improve tissue repair such as accelerated regeneration of skin, muscle, tendon, ligament, bone and neural tissue, reverse and inhibit nerve conduction in small and medium diameter peripheral nerve fibres that release chronic pain and resolve inflammation. The therapeutic effectiveness of near infrared light (NIR) has been determinate for decades. Genesis health light use NIR as main light resource. NIR is well accepted therapeutic tools in the treatment of infected, ischemic, and hypoxic wounds, along with other soft tissue injuries. Positive effects include acceleration of wound healing, improved recovery from ischemic injury and pain relief. NIR produces a therapeutically usable field of heat in the tissue and increases tissue temperature, oxygen partial pressure and tissue perfusion. These three factors are vital for a sufficient tissue supply with energy and oxygen. As inflammation and wound is the main pathology procedure in the above disorders, the defences of them all depend decisively on a sufficient supply with energy and oxygen.

In this review, we collected published and unpublished studies of NIR effects from fundamental and clinical researches. In the fundamental research, we presents current study on the use of far-red to near-infrared (NIR) light treatment in various in vitro and in vivo models. Low-intensity light therapy, commonly referred to as "photobiomodulation," uses light in the far-red to near-infrared region of the spectrum and modulates numerous cellular functions. Various in vitro and in vivo models of mitochondrial dysfunction were treated with a variety of wavelengths of NIR light. These studies were performed to determine the effect of NIR light treatment on physiologic and pathologic processes. NIR light treatment accelerates wound healing in ischemic rat and murine diabetic wound healing models, attenuates the retinotoxic effects of methanol-derived formic acid in rat models, and attenuates the developmental toxicity of dioxin in chicken embryos. Furthermore, The experimental results demonstrate that NIR light treatment stimulates mitochondrial oxidative metabolism in vitro, and accelerates cell and tissue repair in vivo.

In clinical side, there is a study showed that NIR can considerably alleviate pain (without any exception during 230 irradiations) with substantially less need for analgesics (52–69% less in the groups with NIR compared to the control groups). It also diminishes exudation and inflammation and can show positive immunomodulatory effects. The overall evaluation of the effect of irradiation as well as the wound healing and the cosmetic result (assessed on visual analogue scales) were markedly better in the group with NIR compared to the control group. NIR can advance wound healing (median reduction of wound size of 90% in severely burned children already after 9 days in the group with wIRA compared to 13 days in the control group; on average 18 versus 42 days until complete wound closure in chronic venous stasis ulcers) or improve an impaired wound healing (reaching wound closure and normalization of the thermographic image in otherwise recalcitrant chronic venous stasis ulcers) both in acute and in chronic wounds including infected wounds. After major abdominal surgery there was a trend in favor of the NIR group to a lower rate of total wound infections (7% versus 15%) including late infections following discharge from hospital (0% versus 8%) and a trend towards a shorter postoperative hospital stay (9 versus 11 days). Even the normal wound healing process can be improved. There are studies of NIR treatment on low back pain, neck pain, arthritis and carpal tunnel syndrome. The mentioned effects have been proven in a number of prospective studies.

Experimental results demonstrate that NIR light treatment stimulates mitochondrial oxidative metabolism in vitro, and accelerates cell and tissue repair in vivo. There are many clinical trails showed NIR can alleviate pain, anti-inflammation and accelorate wound healing. NIR has a broad usage in wound healing and degeneration symptoms like neck pain, back pain, and arthritis. Additionally, NIR has good effects on diabetic foot ulcers. NIR light represents a novel, noninvasive, therapeutic intervention without any side-effects.

Contents

Introduction

History:

Interest in the clinical application of light therapy (or phototherapy) has increased in recent years (1-3). The idea that light may be used in medicine has been recognised throughout history. The experience of the pleasant heat of the sun in moderate climatic zones arises from the filtering of the heat radiation of the sun by water vapour in the atmosphere of the earth (4). Ancient Egyptians and Greeks believed the sun could strengthen and heal the body. In the middle ages, sunlight was also considered to be an ally in the battle against virulent diseases such as the plague (5). Since 1960s, people started to research on light power such as laser which was found rapid application in medicine and surgery. The therapeutic properties of low intensity laser were than recognised and referred to as laser therapy (LLLT) (6-8). However, the usage of laser is limited by its speciality of focused light which is monochromatic and coherent. There is only one narrow spectrum for each laser provided for treatment. Analysis of the transmission spectra revealed the optimum range of transmission or penetration for different tissue such as 770-850 nm for blood and 810 nm for spinal cord (Figure 1) (9).

The therapeutic effectiveness of near infrared light (NIR) has been determinate for decades (10-11). It is produced in special radiators whose whole incoherent broad-band radiation of a full spectrum bulb is passed through a cuvette, containing water. The spectrum of filtered light emphasise visible light and infrared-A which is called near infrared light (380-1400 nm) and eliminate ultraviolet and infrared-B and C which make majority heat over the skin. The NIR leads to high penetration properties with a low thermal load to the surface of the skin. Genesis® health light develops the NIR technology and acquires a more effective NIR spectrum (400-1400nm) through a specific filter (Figure 2). Genesis light includes all valuable wavelength in LLLT and also the most penetrated infrared-A.

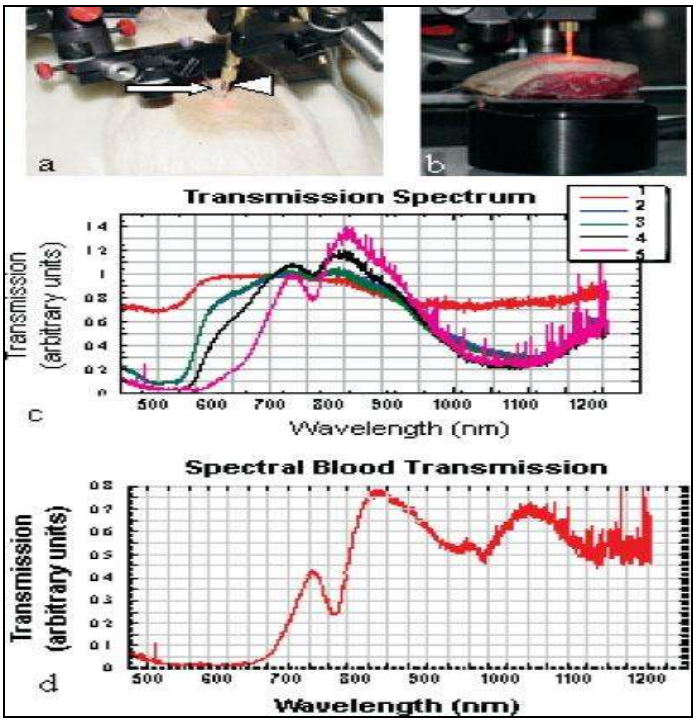


Figure1. Light penetration analysis. a: Photograph of spectrophotometric analysis experimental set-up. The smart fiber (arrow) is inserted below the skin of the rat, the light source (arrowhead) is positioned above the skin for transcutaneous application of light. b: Ex vivo power analysis, a cross section of the rat's dorsal thoracic region was placed between the light source and a power meter. Graphical representation of transmission (in arbitrary units) through each layer of tissue (c) or through blood (d), depending on wavelength (nm). Layer 1, skin; 2, loose connective tissue; 3, dense connective tissue; 4, muscle; 5, vertebral column and spinal cord.

Mechanisms

The light from a 50 w full spectrum bulb go through a filter with the specific fluid and contain the energy-rich wavelengths of NIR which have been described both in vitro and in vivo (12-19). The NIR acts both by thermal effects as well as by non-thermal effects.

1. Thermal effects of NIR are related to a heat energy transfer into tissue. It includes:
   - Reaching capillaries near the surface of the skin by the NIR radiation (primary warming)
   - Heat distribution by the blood (cooling of tissue areas near the surface of the skin, spreading of the heat into the depth)

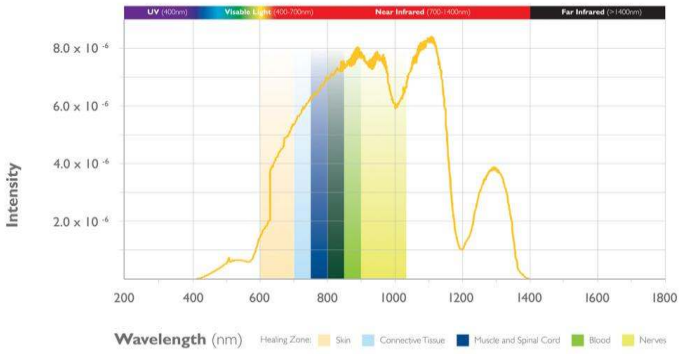


Figure 2 **The spectrum of Genesis pain relief light is from 400 to 1400 nm. The total output power is 4.9 W in high and 2.3 W in low scale. The Power Intensity is 339 mW/cm^2 in high and 161 mW/cm^2 in low scale. The therapeutic windows of skin (600-700nm), connective tissue (700-800nm), muscle and spinal cord (750-850nm), blood (800-900nm) and nerve (900-1100nm) are all included into this spectrum*. The therapeutic window defines the range of wavelengths where light has its maximum depth of penetration in tissue.**

Kimberly R. Byrnes, Ronald W. Waynant, Ilko K. Ilev, Xingjia Wu, Lauren Barna, Kimberly Smith, Reed Heckert, Heather Gerst, and Juanita J. Anders.* ***Light Promotes Regeneration and Functional Recovery and Alters the Immune Response after Spinal Cord Injury.* *Lasers in Surgery and Medicine 36:171-185 (2005)*

Increasing capillary blood flow near the surface of the skin with expansion of the blood flow areas accessible to the radiation and by this augmenting the second mechanism

- Conduction of heat into the depth
- Secondary energy release by stimulation of metabolism caused by the increase of temperature (in accordance with the reaction velocity temperature rule a 3°C higher means approximately 30% more speed of reaction and by this more energy provision and release in the tissue)

Thermal effects of NIR- transfer of heat energy, e.g. bringing an amount of heat into tissue- can lead to a

temperature increase in the tissue and by this to temperature depending effects. Based on its high penetration properties, NIR allows a high energy transfer into tissue combined with a limited temperature increase on the skin. This is especially of importance concerning safety aspects.

2. Non-thermal effects of NIR are based on putting a stimulus on cells and cellular structures as a specific (direct) radiation effect. Reactions of the cells at infrared radiation are target oriented growth of surface extensions (plasmodia) (12), influence on the cytochrome C oxidase (16, 20, 21), target oriented growth of neurons (15), stimulation of wound repair (22, 23), as well as cell protective effects of infrared-A (24-27) including signalling pathway.

As we know, all plants perform photosynthesis which converts sunlight into glucose. Cellular biologists have determined that our body use a similar principle in the final digestive process whereby proteins, fats and sugars are broken down within the mitochondrion membrane into the smallest molecular nutrient elements, called pyruvates and produce ATP energy (28). Certain light wavelength such as near infrared (NIR), on the low level of the color spectrum stimulate the mitochondrion membrane to produce ATP (29). ATP is the fuel that all cells utilize to perform cellular activities, including DNA and RNA synthesis, cellular repair and collagen production (Figure 3). One of the primary effects of NIR is on cytochrome C oxidase in the mitochondria. When cells are stressed or in hypoxic tissues, Nitric Oxide (NO) competitively displaces oxygen in cytochrome C oxidase, reducing ATP and changes in reactive oxygen species (ROS) production that lead to increased oxidative stress and inflammation. NIR photo-dissociates NO from cytochrorme C oxidase leading to restoration of ATP production, a reduction of oxidative stress and subsequently a reduction in inflammation (28). The effects will lead to increase of growth factors secretion and activation of enzymes and other secondary messengers. These will improve tissue repair such as accelerated regeneration of skin, muscle, tendon, ligament, bone and neural tissue, reverse and inhibit nerve conduction in small and medium diameter peripheral nerve fibres that release chronic pain and resolve inflammation (28-32).

NIR has appropriate therapeutic irradiances and no harm for human skin (no induction of matrix metalloproteinase 1) (30, 31), but it has cell protective effects against the damages caused by UV radiation (32, 33). In addition, wavelengths within NIR have been shown to influence adhesive interactions between cells and extracellular matrices (16), playing a regulative role in wound repair processes, and may have a positive effect on cosmetic results (34). Concerning thermal and non-thermal effects of NIR, the mediation by pathways like nitric oxide in vasodilatation or by cytokines or neurotropines should also be taken into account (35).

Figure 3 The water-based filter removes UV and far infrared light only remains Near infrared (NIR); NIR penetrates the skin 3 to 4 cm deep; NIR repairs damaged cells and accelerates the healing process; NIR stimulates the cell's mitochondria to increase energy production.

Wound healing:

Wound healing and infection defence represent processes with an extremely high energy demand (36,37), e.g. function of granulocytes including their antibacterial oxygen radical formation. Hence they depend on a sufficient supply with energy and oxygen quite decisively. On the long run energy must be provided mostly aerobically (with oxygen). Oxygen plays a double role in wound healing: as an agent in the energy production and as a substrate for the oxygen radical formation of the granulocytes (respiratory burst). Wound repair and energy production therefore depend on the integrity of the following three vital factors (36, 37):

- Tissue temperature
- Tissue oxygen partial pressure
- Tissue perfusion

Even one single factor lying clearly in the pathological area can deter energy production and wound healing or makes them both impossible:

- Below 28°C no wound healing is possible because of too slow metabolism in accordance with the reaction velocity temperature rule. The centre of chronic wounds is often relatively hypothermic (38).
- Without a sufficient oxygen partial pressure no aerobic energy production (and no granulocyte function) is possible. The centre of chronic wounds frequently has an oxygen partial pressure near zero which increases markedly the risk of wound infections (39, 40).
- A sufficient tissue blood flow including capillary blood flow is required for the transport of high-energy substrates to the tissue and for the removal of metabolic waste products.

NIR augments the cellular energy provision per time considerably by increasing all three factors, where the effects of NIR on these three factors have been proven by different study groups by means of various methods:

- Tissue temperature, proved in humans by means of a direct measuring of the tissue temperature with stitch probes (41), implanted probes (in 2cm of tissue depth in operation wounds) (34) and thermographically (35, 42, 43), as well as in animal experiments with stitch probes up to 7 cm of tissue depth (44). It shows that NIR can increase the temperature in 2 cm of tissue depth by approximately 2.7°C, the field of heat can reach into a depth of approximately 5-7 cm.
- Oxygen partial pressure in the tissue, proved in humans by means of a direct oxygen partial pressure measurement in the tissue with implanted probes in operation wounds as well as by means of measuring of the oxygen saturation of the haemoglobin with an external white light-measuring probe (45). NIR can increase the oxygen partial pressure in 2cm of tissue depth by approximately 30%.
- Tissue blood flow/capillary blood flow, proved in humans by means of blood flow measurement with laser Doppler perfusion imaging and by means of blood flow measurement at two depths with an external laser Doppler-measuring probe (43-45) as well as in animal experiments by means of color microsphere technique up to 7 cm of tissue depth (44). NIR can increase superficial blood flow to approximately 8 times the amount, the field of increased blood flow can reach into a depth of approximately 5-7 cm.

In contrast to this, hyperbaric oxygenation (HBO) primarily increases only one factor, the oxygen partial pressure in the tissue (46-48). The clinically beneficial effects of NIR on wounds and infections-including the effects to decrease pain, inflammation, and

hypersecretion and to have immunomodulatory effects- can be explained by the improvement in both the energy provision per time (increase of the metabolic rate) and the oxygen supply (e.g. for the function of granulocytes) as well as by non-thermal cellular effects.

Due to its penetration properties, NIR allows a multiple energy transfer into subcutaneous (2-3 cm) without irritating or overheating the skin. As many postoperative wound healing impairments and infections originate primarily in the subcutaneous layer, NIR has advantages for local warming in acute wound healing compared to other sources such as heating bandage systems or hot packs, whose heat is absorbed in the epidermal layers and may cause burning of the skin (35, 49).

Advantages of NIR:

- Decrease of pain, inflammation, and hypersecretion and positive immunomodulatory. All four effects are clinically important. Especially the pain reduction or the pruritus reduction in morphea (50-51) seen in a variety of indications, e.g. in verrucae, herpes, wounds and scleroderma (35, 36). As observed in different study groups, there is less pain, remarkably less need for analgesics, less side-effects of analgesics should be emphasized as an important clinical effect of NIR.
- Contact-free, easily used procedure
- Without expenditure of material
- Usable for a single body region
- No need for a fixing at the body (compared with a warm pack)
- Gentle concerning blood circulation (compared with full bath)
- Usable at all sorts of positioning
- Offers freedom of movement
- Continuously rising temperature without heat shock and overheating of the superficial skin layers
- Subjectively pleasant (even on wounds), therefore unproblematic use also with children
- Good effects in the depth
- Long lasting heat depot
- Low time expenditure for staff
- Easy feasibility
- Limited time expenditure for the patient

NIR is fundamentally better than “red light” (unfiltered infrared), because a considerably higher irradiance is possible with more warming in the depth and less heating of the surface and also a better alternative to “wet warm packs” and other heating methods.

Beside the possibility that NIR radiators are used in hospitals or in offices of physicians or surgeons, they can be used directly at home or similarly in a nursing home: especially when for a longer period of time a wound shall be irradiated once or twice daily and the patient itself or his family or an ambulatory nursing care service takes care to use the radiator appropriately.

Recommendations for the use of NIR:

The following fundamental recommendations can be given for clinical use of NIR:

- Typically NIR acts only on bare skin, i.e. NIR does not penetrate clothes or most kinds of bandages or wound dressings.
- If possible, irradiation should be vertical to the skin; irradiation distance should be 1 inch (2.5 cm) which put light window on the skin area. It allows touch of light window on the skin since a washable silicon ring covered the window (Figure 4). Irradiation time should be refer to indications, usually from 10 to 20 mins
- Special caution, i.e. a shorter irradiation should be taken in patients with an impaired sensation or a deteriorated ability to express themselves, and when irradiating cold tissue or tissue badly supplied with blood or an area with low subcutaneous tissue such as tibial border.

Figure 4. Example of an irradiation of a wound with a NIR radiator (Genesis® Health Light CS1000)

Acute wounds and especially chronic wounds, intractable wounds or infected problem wounds should be irradiated with NIR ideally once or twice per day or at least three times per week for 20-30 mins each since longer irradiation times per day are often helpful. NIR does not replace other sensible/necessary therapeutic procedures such as the important compression garment therapy of chronic venous stasis ulcers of the lower legs but complements them. Correspondingly the therapy with NIR has to be embedded in an overall therapeutic concept. NIR can be used independently from therapy preferences concerning wound management such as moist wound management. Typically for NIR irradiation the wound has to be uncovered as most bandages or wound dressings (with the exception of e.g. some tested transparent foils) are not adequately permeable for NIR. According to modern concepts for the assessment of wound healing also other end-points and variables of interest aside from a complete wound closure have to be used like reduction of pain, improvement of quality of scars, clinically relevant shortening of the time of wound healing and improved quality of healing (52). Nowadays great importance is placed on the reduction or avoidance of pain in order to improve the wound healing and to avoid the formation of a pain memory with chronification of the pain (53), associated with the application of management strategies of common acute and chronic wounds.

Safety of NIR

NIR in clinical use at appropriate irradiances has been described since more than 15 years as helpful and safe (54-60). In accordance with previous studies (24-26), NIR is unlike ultraviolet especially not implicated in photoaging of the skin, mediated by the collagenase matrix metalloproteinase 1 (MMP-1). Investigations of human skin fibroblasts after single exposures between 15 minutes and 8 hours to NIR or 15-45 minutes to ultraviolet-A (UV-A) radiation at two physiologic temperatures as well as after repeated exposures with NIR are presented in: single exposure of cultured human dermal fibroblasts to UV-A radiation yielded a very high increase in MMP-1 mRNA expression (11-fold expression for conventional Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) and 76-fold expression for quantitative real-time RT-PCR) and a dose-dependent decrease in cell survival. In contrast, even at an investigated disproportionally high irradiance NIR did not produce cell death and did not induce an increase in MMP-1 mRNA expression. Additionally, repeated exposure to NIR did not induce MMP-1 mRNA expression.

Clinical indications for NIR

Clinical indications for NIR are in physiotherapy, sports medicine and orthopedics: the clinical application of NIR can be in preventive, therapeutic, regenerative, or rehabilitative intention. Muscular hardenings, myoeloses, lumbago, diseases of the rheumatic disorders circle, arthritis, contusions, fibromyalgia, regeneration after sports, postoperative rehabilitation.

Fundamental Studies

Low-intensity light therapy, commonly referred to as “photobiomodulation”, by light in the near-infrared (NIR) modulates numerous cellular functions. Clinical and experimental applications of photobiomodulation have

expanded over the past 30 years (61). NIR is well-accepted therapeutic tools in the treatment of infected, ischemic, and hypoxic wounds, along with other soft tissue injuries (62-65). Positive effects include acceleration of wound healing, improved recovery from ischemic injury in heart, and attenuated degeneration in the injured optic nerve (64, 66, 67).

At the cellular level, NIR can modulate fibroblast proliferation, attachment and synthesis of collagen and procollagen, promote angiogenesis, and stimulate macrophages and lymphocytes by improving energy metabolism within the mitochondria. In addition, NIR has demonstrated the ability to promote the production of growth factors, such as keratinocyte growth factor (KGF), transforming growth factor (TGF), and platelet-derived growth factor (PDGF) (65, 68-70). Optimal wavelengths within 630-1000 nm along with a minimal energy density of 4 J/cm2 have been proven effective at stimulating biological processes (64, 70-73). Lasers are limited in their ability to deliver monochromatic NIR light. Combined wavelengths cannot easily be reproduced with lasers, and the beam width makes it difficult to treat large areas. Moreover, lasers emit a fair amount of heat, which has the potential to produce tissue damage.

Photoacceptor

Within mammalian tissues, there are three major photoacceptor molecules: hemoglobin, myoglobin, and cytochrome c oxidase (74). Of these three, cytochrome c oxidase is the only one that is involved in energy metabolism and production, as it comprises complex IV of the electron transport chain located within the mitochondria. Thus, cytochrome c oxidase has been postulated as the photoacceptor molecule for the biological effects of photobiomodulation. The evidence to support cytochrome c oxidase as the primary photoacceptor has been steadily growing. Cellular proliferation studies comparing the action spectrum following laser irradiation compared to the absorption spectra of possible photoacceptor molecules have suggested cytochrome c oxidase as the primary photoacceptor (20). In addition, it has been demonstrated that up to 50% of NIR light is absorbed by mitochondrial chromophores, including cytochrome c oxidase (75, 76). In studies using primary cultured neurons and tetrodotoxin
(TTX)—a voltage-dependent sodium channel blocker that impedes neuronal impulses, decreases ATP demand, and downregulates cytochrome c oxidase—NIR light treatment has been shown to reverse the toxic effects of TTX. This is accomplished by reverting levels of cytochrome c oxidase back to control levels in TTX-exposed NIR light treated neurons and up regulating the enzyme's activity in NIR light treated control neurons (77). Furthermore, the action and absorption spectra in the NIR wavelengths, compared to the action and absorption spectra of cytochrome c oxidase activity and ATP content in neurons exposed to TTX that received NIR light treatment, parallel each other (Figure.5). NIR light treatment has also partially restored cytochrome c oxidase activity in primary cultured neurons exposed to 10–100 μM potassium cyanide (KCN), significantly reduced cell death in neurons exposed to 300 μM KCN, significantly restored ATP levels in neurons treated with 10 μM KCN, and enhanced the effect of photobiomodulation by pretreating neurons with NIR light prior to exposure to 10–100 μM KCN in vitro.

Wound healing in vitro and in vivo

There is a growing need for safe and efficacious therapeutic intervention for the treatment of chronic wounds. The process of wound healing occurs in three phases: first, a substrate is laid down; second, cell proliferation occurs; and third, remodeling of the tissue takes place. Photobiomodulation exerts its biological effect during the proliferative phase of wound healing. In vitro experimentation utilizing NIR light treatments at various wavelengths has shown significantly increase cell growth in a variety of cell lines, including murine fibroblasts, rat osteoblasts, rat skeletal muscle cells, and normal human epithelial cells (78). Accelerated wound

healing following photobiomodulation has also been demonstrated in a number of in vivo models, including toads, mice, rats, guinea pigs, and swine (79, 80). In an in vivo rat model of ischemic wounds, a decrease in wound size and acceleration of wound closure has been demonstrated in rats treated with 880-nm NIR light [Ref 4, Review of Photo].

To determine if NIR light treatment can improve impaired healing, we used a murine model of diabetic healing, which is characterized by a delayed re-epithelialization (81). Polyvinyl acetal (PVA) sponges were implanted subcutaneously in the dorsum of genetically diabetic mice (BKS.Cg-m +/+ Leprdb). The mice were subsequently treated with 670-nm NIR light, and wounds were harvested for RNA analysis.

Microarray analysis revealed that basement membrane and tissue regenerating genes were significantly up-regulated in mice that received NIR light treatments as compared to controls. Integrins, nidogens, laminin, actin, and kinesin motor proteins were up-regulated. All of these proteins are necessary at specific time points for wound-induced epithelial cell migration and differentiation (81). Up-regulation of these genes is one possible mechanism by which NIR photobiomodulation can accelerate wound closure. Semaphorins/collapsins are another group of genes that were significantly up-regulated in mice receiving NIR light treatments. Specifically, murine semaphorin H is involved in the inhibition of sensory peripheral nerve ingrowth. Murine semaphorin H, along with other semaphorin/collapsin proteins, is involved in pain management. Pain has been shown to slow the healing process by the recruitment of inflammatory cells to the site of injury (81). Decreasing pain via NIR light could aid in the acceleration of wound closure.

Genes that were down-regulated in NIR light treated mice include cytokine receptors, interleukin-1, interleukin-10, and macrophage inflammatory protein–2. A decrease in these genes encoding for proteins associated with the inflammatory response results in a decrease in pain, which in turn increases the ability of tissue-regenerating proteins to facilitate wound closure. Another group of genes that were down-regulated in response to NIR light treatment were those encoding proapototic proteins. Activator of apoptosis harakiri (HRK), programmed cell death 1 protein precursor (PDCD-1; PD-1), and receptor-interacting protein (RIP) were all down-regulated.

Treatment for Retinal Toxicity in vivo

Mitochondrial dysfunction plays a central role in the pathogenesis of numerous retinal and neurodegenerative diseases, including age-related macular degeneration, Leber's hereditary optic neuropathy, and Parkinson's and Alzheimer's disease (82).

Furthermore, mitochondrial dysfunction has been shown to play an integral role in the development of retinal toxicity resulting from methanol intoxication (83, 84). The neurotoxic agent in methanol intoxication is the metabolite formic acid. Formic acid is a mitochondrial toxin that specifically inhibits cytochrome c oxidase in the retina and optic nerve, resulting in blindness (85, 86). To determine if exposure to monochromatic NIR light protects the retina against the toxic actions of methanol-derived formic acid, Eells J.T. and his colleagues employed a rat model of methanol toxicity (87).

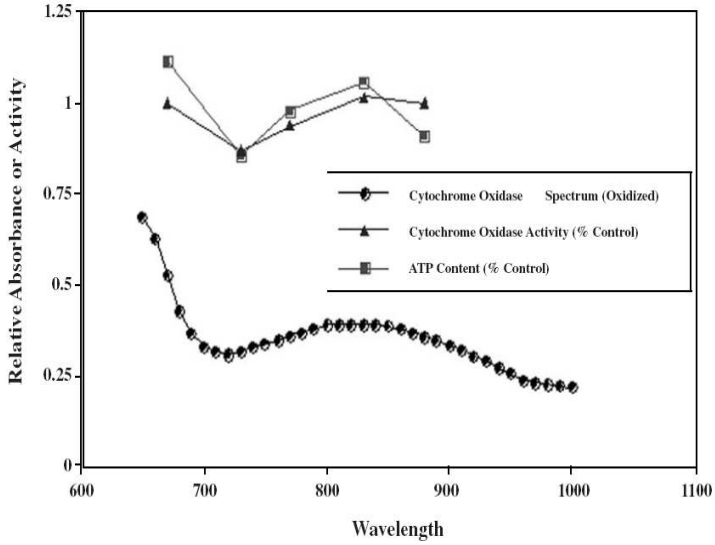
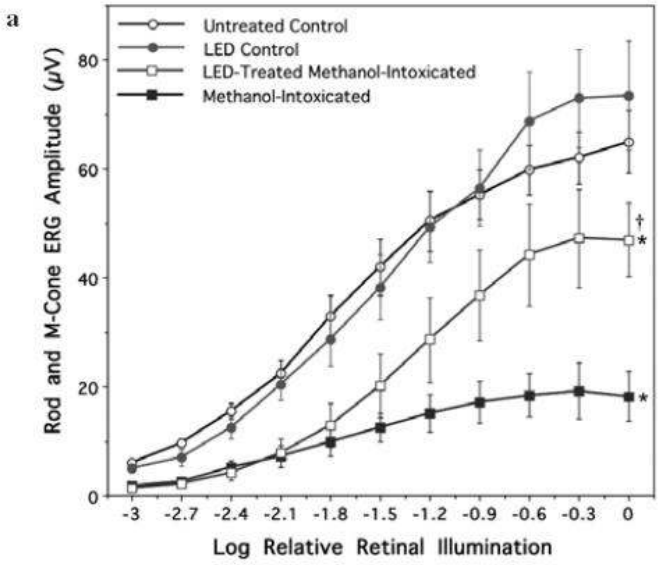


Figure 5. Action and absorption spectra in the near-infrared (NIR) region of the spectrum for cytochrome C oxidase as compared to the relative cytochrome c oxidase activity and ATP content in

tetrodotoxin (TTX)- exposed neurons treated with NIR light at varying wavelengths expressed as percent of controls.

Results from these studies demonstrate that three brief 670-nm NIR light treatments of 2 min and 24 sec delivered at 5, 25, and 50 h of methanol intoxication significantly attenuated the retinotoxic effects of methanol-derived formate during intoxication (Figure 6). In addition, NIR light treatment protected the retina from the histopathologic changes induced by methanol-derived formate. These findings provide a link between the actions of monochromatic far-red to NIR light on mitochondrial oxidative metabolism in vitro and retinoprotection in vivo. Moreover, they have provided the impetus for ongoing investigations of thetherapeutic efficacy of NIR light therapy in other models of retinal disease.



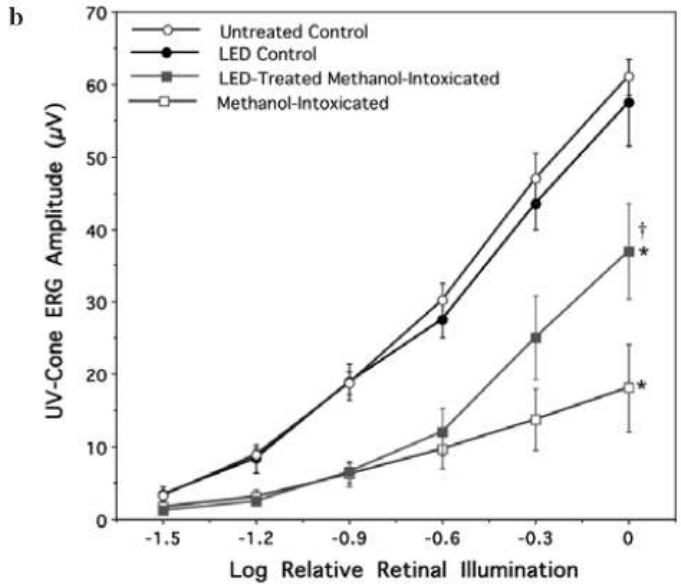


Figure 6 (a) 670 nm NIR light treatment increases rod and M-cone ERG amplitude in LED-treated methanol-intoxicated rats as compared to methanol-intoxicated rats. (b) 670 nm NIR light treatment improves retinal function by increasing ultraviolet (UV)-cone ERG amplitude in LED-treated methanol-intoxicated rats as compared to methanol-intoxicated rats as compared to methanol-intoxicated rats.

Clinical Studies

Post-operation Application

A prospective, randomized, controlled, double-blind study with 111 patients who had undergone major abdominal surgery at the university hospital Heidelberg, Germany, and thereafter underwent 20 minutes irradiation 2 times per day (starting on the second postoperative day) showed a significant and relevant pain reduction combined with a markedly decreased dose of required analgesics in the group with NIR and visible light VIS (NIR[+VIS], approximately 75% NIR, 25% VIS) compared to a control group with only VIS: during 230 single irradiations with NIR (+VIS) pain decreased without any exception (median of decrease of pain on postoperative days 2-6 was 13.4 on a 100 mm visual analogue scale VAS 0-100), while pain remained unchanged in the control group ($p<0.001$, see Figure 7) (41). The median of decrease of pain on the third

postoperative day was 18.5 versus 0.0, the median difference between the groups was 18.4 (99% confidence interval 12.3/21.0), p<0.001.

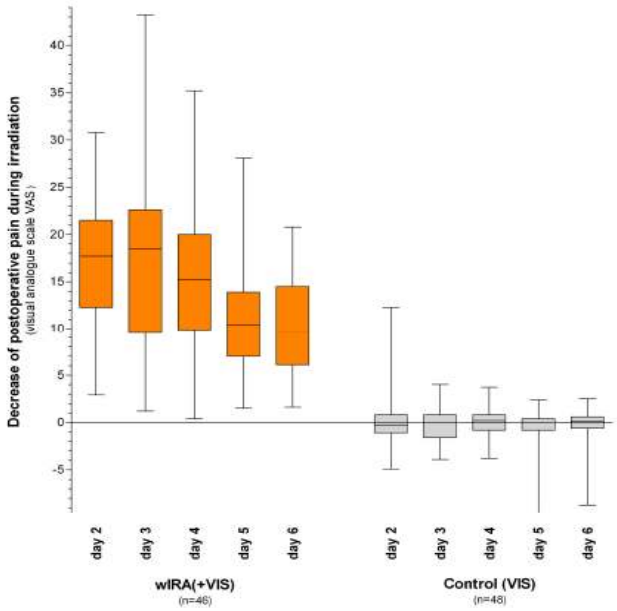


Figure 7: Decrease of postoperative pain during irradiation in the group with NIR and VIS and in the control group with only VIS assessed with a visual analogue scale; given as minimum, percentiles of 25, median, percentiles of 75, and maximum (box and whiskers graph with the box representing the interquartile range). During 230 single irradiations with NIR(+VIS) the pain decreased without any exceptions, while pain remained unchanged in the control group (p<0.001) [Ref19, NIR in acute and chronic pain].

The required dose of analgesics was 52-69% lower (median differences) in the subgroups with NIR(+VIS) compared to the control subgroups with only VIS (median 598 versus 1398 mL Ropivacaine, p<0.001, for peridural catheter analgesia; 31 versus 102 mg Piritramide, p<0.001, for patient-controlled analgesia; 3.4 versus 10.2 g Metamizole, p<0.001, for intravenous and oral analgesia, see Figure 7).

During irradiation with NIR (+VIS) the subcutaneous oxygen partial pressure rose markedly by 32% and the subcutaneous temperature by 2.7°C (both measured at a tissue depth of 2 cm), whereas both remained unchanged in the control group. After irradiation, the median of the subcutaneous oxygen partial pressure was 41.6 (with NIR) versus 30.2 mm Hg in the control group (median difference between the groups 11.9 mm Hg (+39%), 99% confidence interval 8.4/15.4 mm Hg (+28%/+51%),

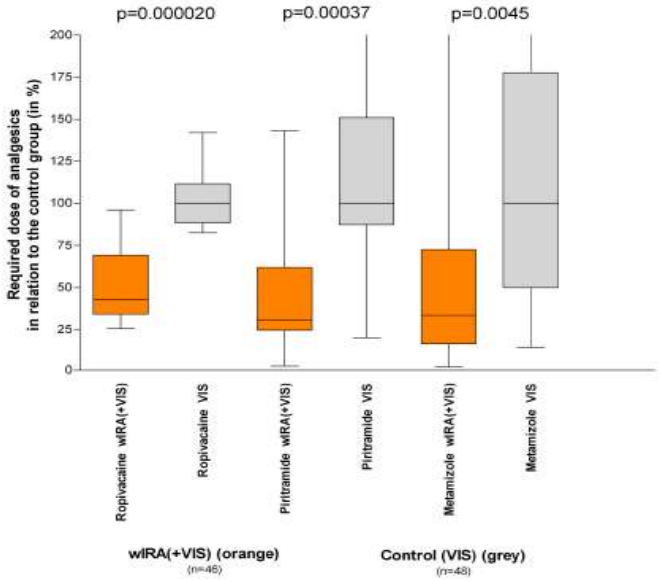


Figure 5: Required dose of analgesics of the subgroups with NIR and VIS in relation to the control subgroups with only visible light (medians of control subgroups=100); given as minimum, percentiles of 25, median, percentiles of 75, and maximum (box and whiskers graph with the box representing the interquartile range. The required dose of analgesics was 52-69% lower (median differences) in the subgroups with NIR(+VIS) compared to the control subgroups with only VIS [Ref19, NIR in acute and chronic pain].

p<0.001 (Figure 6); the median of the subcutaneous temperature was 38.9 versus 36.4°C (median difference between the groups 2.6°C, 99% confidence interval 2.1/2.9°C, p<0.001) (Figure 7). The baseline values (before irradiation) of the subcutaneous oxygen partial pressure rose from the second to the tenth postoperative day by 3.4 versus 0.3 mm Hg (median difference between the groups 3.1 mm Hg (+10%), 99% confidence interval 1.9/3.7 mm Hg, p<0.001). The baseline values for the subcutaneous temperature rose by 0.4 versus -0.3°C (median difference 0.6°C, 95% confidence interval 0.2/0.8°C, p<0.001).

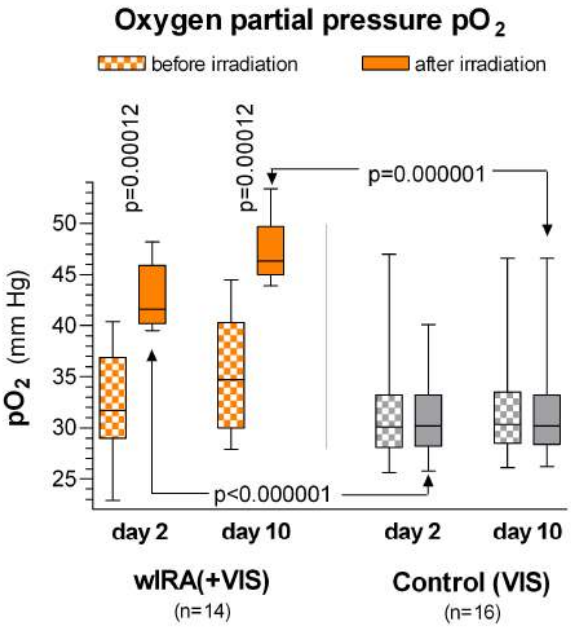


Figure 6. Subcutaneous oxygen partial pressure at a tissue depth of 2 cm on the postoperative days 2 and 10 in the group with NIR and visible light (VIS) and in the control group with only VIS. given as minimum, percentiles of 25, median, percentiles of 75, and maximum (box and whiskers graph with the box representing the interquartile range. During irradiation with NIR(+VIS), the subcutaneous oxygen partial pressure rose markedly by more than 30%, whereas it remained unchanged in the control group.

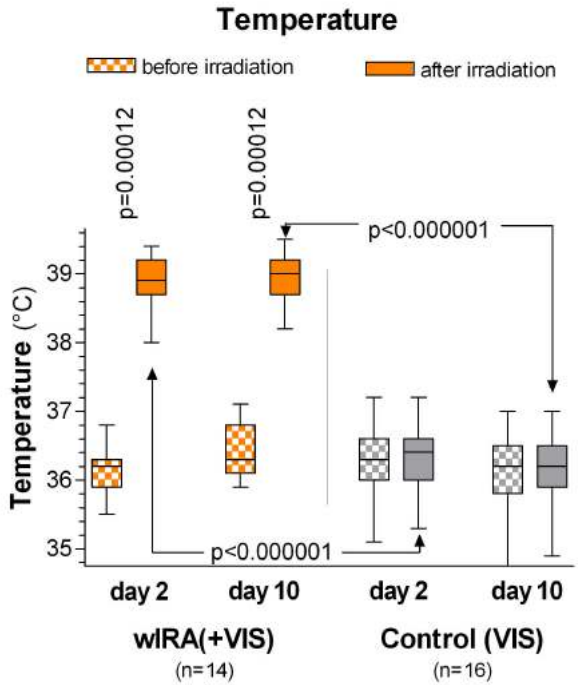


Figure 7 subcutaneous temperature at a tissue depth of 2 cm on the postoperavtive days 2 and 10 in the group with NIR and VIS and in control group with only VIS, given as minimum, percentiles of 25, median, percentiles of 75, and maximum (box and whiskers graph with the box representing the interquartile range. During irradiation with NIR(+VIS) the subcutaneous temperature rose markedly by approximately 2.7°C, whereas it remained unchanged in the control group.

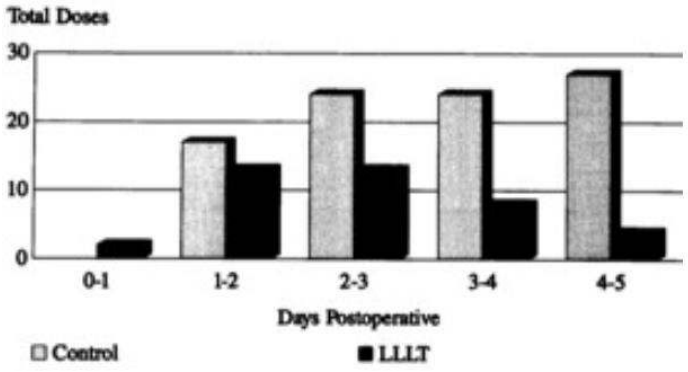
The overall evaluation of the effect of irradiation, including wound healing, pain and cosmesis, assessed on a VAS (0-100 with 50 as the indifferent point of no effect) by the surgeon (median 79.0 versus 46.8, median difference 27.9, 99% confidence interval 17.2/37.3, p<0.001) was considerably better in the group with NIR compared to the control group. This was also true for single aspects: wound healing assessed on a VAS by the surgeon (median 88.6 versus 78.5, p<0.001) or the patient (median 85.5 versus 81.0, p=0.04, trend) and cosmetic result assessed on a VAS by the surgeon (median 84.5 versus 76.5, p<0.001) or the patient (median 86.7 versus 73.6, p<0.001). The principal finding of this study was that postoperative irradiation with NIR can improve even the normal wound healing process.

Kevin C. Moore and his colleagues investigate NIR treatment on reducing extent and duration of postoperative pain (88). Twenty consecutive patients for elective cholecystectomy were randomly allocated for either NIR or as controls. The trial was double blind. Patients for NIR received 6-8 minute treatment (830 nm, 60 mW/cm^2) to the wound area immediately following skin closure prior to emergence from GA. All patients were prescribed on demand postoperative analgesia (IM or oral according to pain severity). Recordings of pain scores (0-10) and analgesic requirements were noted by an independent assessor. There was a significant difference in the number of doses of narocotic analgesic (IM) required between the two groups. Controls n=5.5: NIR n=2.5. No patient in the NIR group required IM analgesia after 24 h. Similarly the requirement for oral

analgesia was reduced in the NIR group. Controls n=9: NIR n=4. Control patients assessed their overall pain as moderate to severe compared with mild to moderate in the NIR group (Figure 8).

Oral Mucositis

Chemotherapy and/or radiation therapy is administered prior to bone marrow transplant (BMT). Mucositis, especially oral mucositis (OM), is a common debilitating side effect of this treatment. The development of these ulcerations causes severe pain, compromises the ability of the patient to eat and drink independently, and can lead to infection and to increased morbidity (89-91). Since NIR photobiomodulation accelerates wound healing and increases cell proliferation, this treatment was used in an attempt to treat pediatric BMT patients prophylactically to prevent the development of oral mucositis (92). The first clinical trial of NIR light treatment as a preventative treatment for the development of OM was performed at the Children's Hospital of Wisconsin in Milwaukee, Wisconsin.



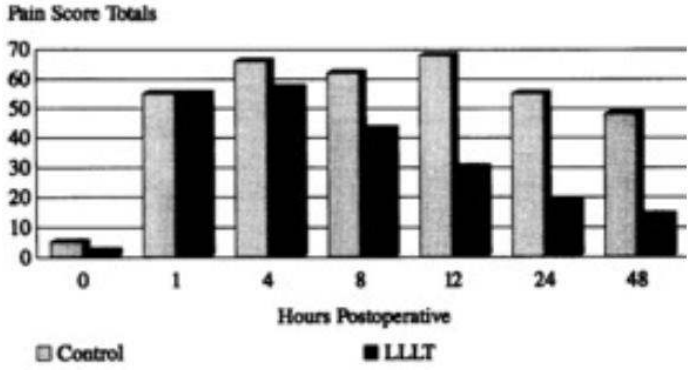


Figure 8. Deduction of analgesic and pain score between light treatment and control group are significant (p<0.001).

Thirty-two pediatric patients receiving myeloablative therapy were treated with 670-nm NIR light once a day for 14 days post-BMT at an energy density of 4 J/cm2. Patients received NIR light treatment on the left extraoral epithelium and sham treatment on the right. Subsequent to the light treatment, patients were asked to rate left and right buccal pain as compared to throat pain, which served as an untreated control. NIR light treatment produced a significant reduction in left and right buccal pain (48% and 39%, respectively) when compared to throat pain. In addition, the incidence of OM in this patient population was decreased, with only 53% of patients developing OM, when compared to historical epidemiological data, which suggests that 70–90% of the patient population receiving BMT should have developed OM (Figure 9). The results of this clinical trial demonstrate that NIR light treatment may be an effective preventive countermeasure to the development of OM in cancer patients. This study served as the foundation for the current multi-centered, double-blinded trial underway.

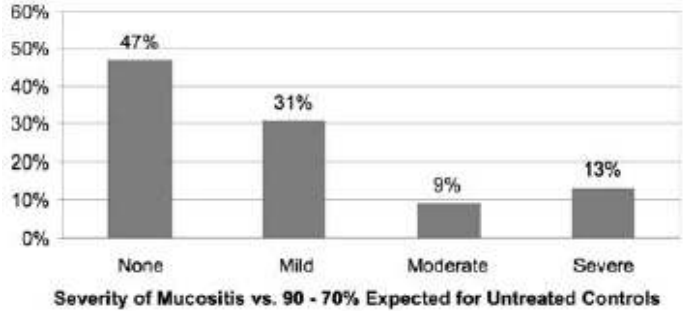


Figure 9. Reduced incidence of oral mucositis in patients that received 670-nm NIR light treatments once daily for 14 days post-bone marrow transplant (BMT) as compared to historical epidemiological data.

A group of doctor in Brazil investigated the clinical effects of NIR on prevention and reduction of severity of conditioning-induced oral mucositis (OM) for hematopoietic stem cell transplantation (HSCT) (92). They randomized 38 patients who underwent autologous (AT) or allogeneic (AL) HSCT. A NIR was used, emitting light at 660 nm, 50 mW, and 4 J/cm^2, measured at the fiberoptic end with 0.196 cm^2 of section area. The evaluation of OM was done using the Oral Mucositis Assessment Scale (OMAS) and the World Health Organization (WHO) scale. In the NIR group, 94.7% of patients had an OM grade (WHO) lower than or equal to grade 2, including 63.2% with grade 0 and 1, whereas in the controls group, 31.5% of patients had an OM grade lower than or equal to grade 2 ($P < 0.001$). Remarkably, the hazard ratio (HR) for grades 2, 3, and 4 OM was 0.41 (range, 0.22-0.75; P<0 .002) and for grades 3 and 4 it was 0.07 (range, 0.11-0.53; P <0.001). Using OMAS by the calculation of ulcerous area, 5.3% of the NIR group presented with ulcers of 9.1 cm2 to 18 cm2, whereas 73.6% of the control group presented with ulcers from 9.1 cm2 to 18 cm2 (P <0.003). Our results indicate that the use of upfront NIR in patients who have undergone HSCT is a powerful instrument in reducing the incidence of OM and is now standard in our center (Figure 10).

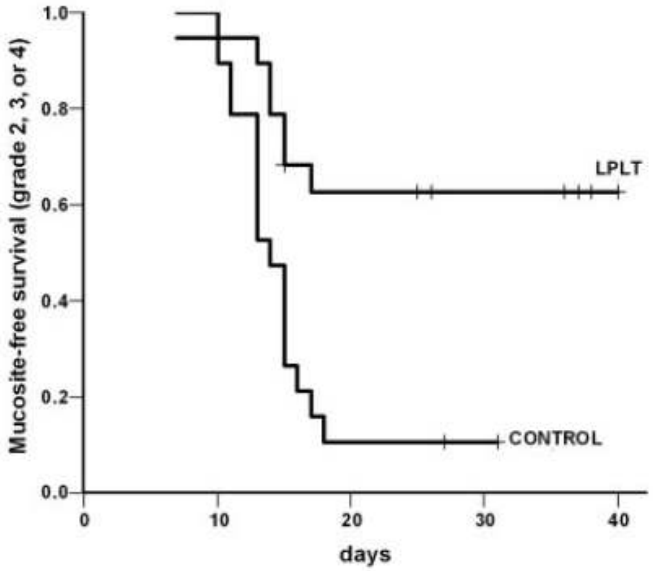


Figure 10 Kaplan-Meier mucositis-free survival. LPLT is the NIR group which has a significantly higher survival rate than the control group.

Pain Relief

Bjordal JM reviewed published papers on possible mechanisms of NIR therapy in acute pain (93). Literature search of (i) controlled laboratory trials investigating potential biological mechanisms for pain relief and (ii) randomized placebo-controlled clinical trials which measure outcomes within the first 7 days after acute soft tissue injury. There is strong evidence from 19 out of 22 controlled laboratory studies that NIR can modulate inflammatory pain by reducing levels of biochemical markers (PGE(2), mRNA Cox 2, IL-1beta, TNFalpha), neutrophil cell influx, oxidative stress, and formation of edema and hemorrhage in a dose-dependent manner (median dose 7.5 J/cm^2, range 0.3-19 J/cm^2). Four comparisons with non-steroidal anti-inflammatory drugs (NSAIDs) in animal studies found optimal doses of NIR and NSAIDs to be equally effective. Seven randomized placebo-controlled trials found no significant results after irradiating only a single point on the skin overlying the site of injury, or after using a total energy dose below 5 Joules. Nine randomized placebo-controlled trials (n = 609) were of acceptable methodological quality, and irradiated three or more points and/or more than 2.5 cm^2 at site of

injury or surgical incision, with a total energy of 5.0-19.5 Joules.

Results in these nine trials were significantly in favor of NIR groups over placebo groups in 15 out of 18 outcome comparisons. Poor and heterogeneous data presentation hampered statistical pooling of continuous data. Categorical data of subjective improvement were homogeneous (Q-value = 7.1) and could be calculated from four trials (n = 379) giving a significant relative risk for improvement of 2.7 (95% confidence interval [CI], 1.8-3.9) in a fixed effects model. NIR can modulate inflammatory processes in a dose-dependent manner and can be titrated to significantly reduce acute inflammatory pain in clinical settings.

Chow RT et al report the formation of 830 nm NIR-induced, reversible axonal varicosities, using immunostaining with beta-tubulin, in small and medium diameter, TRPV-1 positive, cultured rat DRG neurons. Light also induced a progressive and statistically significant decrease ($p<0.005$) in mitochondrial membrane potential (MMP) in mitochondria in and between static axonal varicosities (94). In cell bodies of the neuron, the decrease in MMP was also statistically significant ($p<0.05$), but the decrease occurred more slowly. Importantly they also report that 830 nm NIR blocked fast axonal flow, imaged in real time using confocal laser microscopy and JC-1 as mitotracker. Control neurons in parallel cultures remained unaffected with no varicosity formation and no change in MMP. Mitochondrial movement was continuous and measured along the axons at a rate of 0.8 microm/s (range 0.5-2 microm/s), consistent with fast axonal flow. Photoacceptors in the mitochondrial membrane absorb light and mediate the transduction of laser energy into electrochemical changes, initiating a secondary cascade of intracellular events. In neurons, this result in a decrease in MMP with a concurrent decrease in available ATP required for nerve function, including maintenance of microtubules and molecular motors, dyneins and kinesins, responsible for fast axonal flow. Light induced neural blockade is a consequence of such changes and provide a mechanism for a neural basis of light-induced pain relief. The repeated application of light in a clinical setting modulates nociception and reduces pain.

Neck Pain

A systematic review and meta-analysis of randomised placebo or active-treatment controlled trials on efficacy of low intensity light therapy (LLLT) in neck pain written by Roberta T. Chow show that LLLT reduces pain immediately after treatment in acute neck pain and up to 22 weeks after completion of treatment in patients with chronic neck pain. They identified 16 randomised controlled trials and that included 820 patients. Two trials (95,96) provided data for light therapy of acute neck pain, one treating acute whiplash associated disorders and one treating acute neck pain of no defined cause. The other 14 trials reported response of chronic non-specific neck pain without radiculopathy to laser therapy (97-110). Of the studies included, 648 (79%) of the sample of patients with chronic neck pain were women, and patients had a mean age of 43 years (SD 9.8), mean symptom duration of 90 months (SD 36.9), and mean baseline pain of 56.9 mm (SD 7.5) on a 100 mm visual analogue scale in any trial. Analysis of categorical data for immediate before and after LLLT effects showed that LLLT groups in the two trials (95, 96) of acute neck pain had a significant RR of 1.69 (95% CI 1.22 – 2.33) for improvement immediately after treatment versus placebo. Methodological quality varied between these two studies. Five trials of chronic neck pain reported categorical data, and all were high-quality trials with methodological scores of 3 or more. RR of pain improvement with LLLT was 4.05 (2.74 – 5.98) compared with placebo at the end of treatment. Analysis of data from visual analogue scale showed that in patients in 13 groups in 11 trials, pain intensity was reduced by a mean value of 19.86 mm (10.04 – 29.68) compared with placebo groups. Seven trials with eight LLLT groups provided follow-up data for 1 – 22 weeks after end of treatment. The pain-relieving effect in the

short term (<1 month) persisted into the medium term (up to 6 months). Five studies provided evidence for improvement in disability at end the of LLLT treatment. Several questionnaire-based outcome measures were used—specifically, the neck pain and disability scale (111), Northwick Park neck pain questionnaire (112), short form 36 (113), Nottingham health profile (114), and neck disability index (115).

The light parameters and application techniques, including treatment protocols, were heterogeneous. Light irradiation was applied to an average of 11 points (range 3-25) in the neck. Energy delivered per point ranged from 0.06 to 54 J, with irradiation durations of 1-600 s. Patterns of treatment ranged from a one-off treatment to a course of 15 treatments, which were administered daily to twice a week. On average, participants received a course of ten treatments. Visible (632.8 and 670.0 nm) and near infrared (820-830, 780, and 904 nm) wavelengths were used at average power outputs ranging from 4 to 450 mW, in pulsed and continuous wave mode.

A randomized, double-blind, placebo-controlled study of low-level light therapy (LLLT) in 90 subjects with chronic neck pain was conducted with the aim of determining the efficacy of 300 mW, 830 nm NIR in the management of chronic neck pain (116).Subjects were randomized to receive a course of 14 treatments over 7 weeks with either active or sham laser to tender areas in the neck. The primary outcome measure was change in a 10 cm Visual Analogue Scale (VAS) for pain. Secondary outcome measures included Short-Form 36 Quality-of-Life questionnaire (SF-36), Northwick Park Neck Pain Questionnaire (NPNQ), Neck Pain and Disability Scale (NPAD), the McGill Pain Questionnaire (MPQ) and Self-Assessed Improvement (SAI) in pain measured by VAS. Measurements were taken at baseline, at the end of 7 weeks' treatment and 12 weeks from baseline. The mean VAS pain scores improved by 2.7 in the treated group and worsened by 0.3 in the control group (difference 3.0, 95% CI 3.8-2.1). Significant improvements were seen in the active group compared to placebo for SF-36-Physical Score (SF36 PCS), NPNQ, NPAD, MPQVAS and SAI. The results of the SF-36 - Mental Score (SF36 MCS) and other MPQ component scores (afferent and sensory) did not differ significantly between the two groups. Low-level light therapy (LLLT), at the parameters used in this study, was efficacious in providing pain relief for patients with chronic neck pain over a period of 3 months (Figure 11).

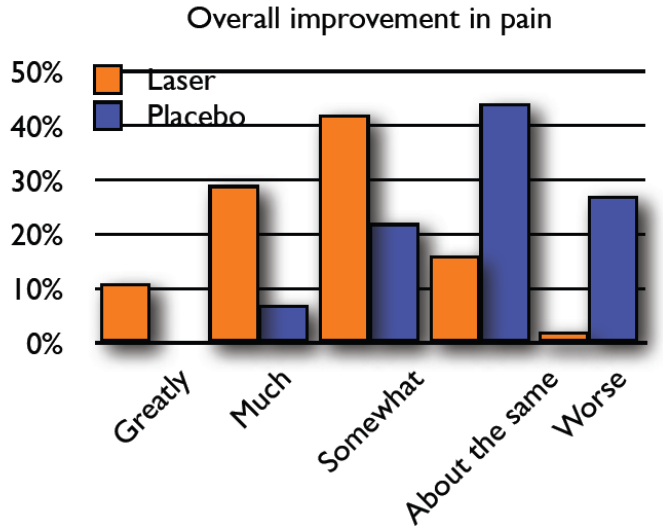


Figure 11. Overall improvement in pain between light and placebo group.

Low Back Pain

Gur A, Karakoc investigated NIR on chronic low back pain (LBP) in 2003 (117). This study included 75 patients divided into three groups: NIR + exercise 25, NIR alone 25 and exercise alone 25. Visual analogue scale (VAS), Schober test, flexion and lateral flexion measures, Roland disability questionnaire (RDQ) and modified Oswestry Disability Questionnaire (MODQ) were used in the clinical and functional evaluations pre and post therapeutically. A physician, who was not aware of the therapy undertaken, evaluated the patients. Significant improvements were noted in all groups with respect to all outcome parameters, except lateral flexion ($p<0.05$). NIR therapy seemed to be an effective method in reducing pain and functional disability in the therapy of low back pain.

Konstantinovic et al studied the clinical effects of NIR therapy in patients with acute low back pain with

radiculopathy (118). Acute LBP with radiculopathy is associated with pain and disability and the important pathogenic role of inflammation. NIR has shown significant anti-inflammatory effects in many studies. A randomized, double-blind, placebo-controlled trial was performed on 546 patients. Group A (182 patients) was treated with nimesulide 200 mg/day and additionally with active NIR, group B (182 patients) was treated only with nimesulide; and group C (182 patients) was treated with nimesulide and placebo light. NIR was applied behind the involved spine segment using a stationary skin-contact method. Patients were treated 5 times weekly, for a total of 15 treatments, with the following parameters: wavelength 904nm, frequency 5000 Hz, 100 mW average power, power density of 20 mW/cm^2 and dose of 3 J/cm^2, treatment time 150 sec at whole doses of 12 J/cm^2. The outcomes were pain intensity measured with a visual analogue scale (VAS); lumbar movement, with a modified Schober test; pain disability, with Oswestry disability score; and quality of life, with a 12-item short-form health survey questionnaire (SF-12). Subjects were evaluated before and after treatment (Figure 12). Statistically significant differences were found in all outcomes measured ($p<0.001$), but were larger in group A than in B ($p<0.0005$) and C ($p<0.0005$). The results in group C were better than in group B ($p<0.0005$). The results of this study show better improvement in acute low back pain treated with NIR used as additional therapy.

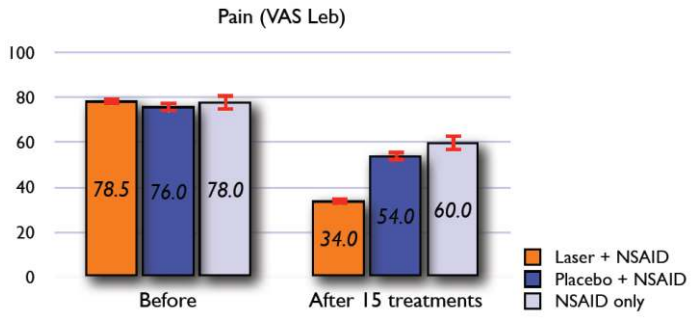


Figure 12. Before and after light treatment, show pain VAS improvement in three groups of light+NSAID, Placebo+NSAID and NSAID only. Light+NSAID is significantly better than Placebo and drug only groups.

Wound Healing

NIR has been proven to improve wound healing based on clinical studies (10, 35, 36, 41, 42, 46) and protect cells (31, 32). Acute wounds and especially chronic wounds, non-healing wounds or infected problem wounds should be irradiated with NIR at least three times per week and ideally once or twice per day for 20-30 minutes each. Longer irradiation times are possible and often helpful. NIR does not replace other sensible/necessary therapeutic procedures such as compression garment therapy of chronic venous stasis ulcers of the lower legs, but complements them. Correspondingly the therapy with NIR has to be embedded in an overall therapeutic concept. We reviewed ___ published and unpublished clinical studies of NIR treating chronic and acute wound. Clinical outcome has been evaluated by alleviation of pain, less comsuption of analgesics, reduction of exudation and inflammation, positive immunomodulatory effects and better cosmetic result.

Acute Wound Healing

A prospective, randomized, controlled, double-blind study with 45 severely burned children was carried out at the Children's Hospital Park Schonfeld, Kassel, Germany (119). A 30 minutes irradiation was applied once a day (starting on the first day, with the day of burn being day 1). In the group with NIR and VIS, a markedly faster reduction of wound size was seen in comparison to a control group with only VIS. On the fifth day (after 4 days with irradiation), the descision was taken as to whether surgical debridement of necrotic tissue was necessary because of deeper (second degree, type b) burns (11 of 21 in the group with NIR, 14 of 24 in the control group) or whether non-surgical treatment was possible (second degree, type a). The patients treated conservatively were kept within the study and irradiated until reepithelialisation was complete.

The patients in the group with NIR showed a markedly faster reduction of wound area: a median reduction of wound size of 50% was reached already after 7 days compared to 9 days in the control group, a median

reduction of wound size of 90% was already achieved after 9 days compared to 13 days in the control group (Figure 13).

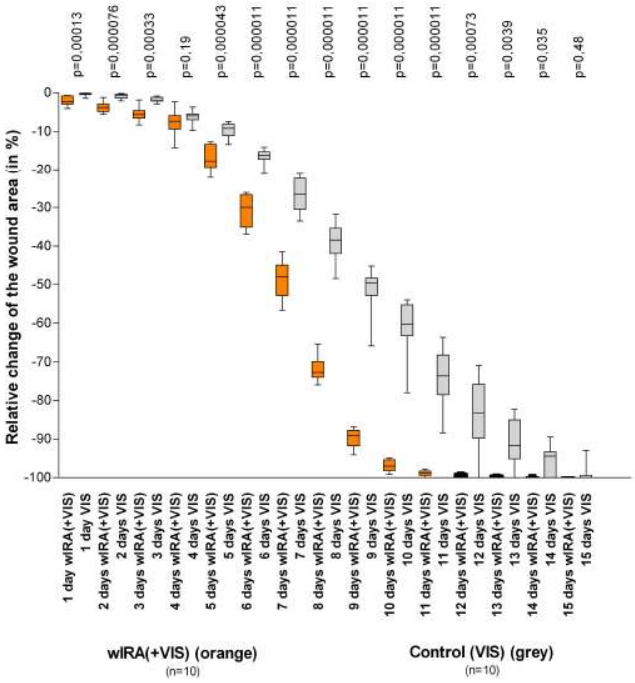


Figure 13. Relative change of wound area in severely burned children as a function of duration of treatment (in days) in the group with NIR and VIS and in the control group with only VIS, given as minimum, percentiles of 25, median, percentiles of 75, and maximum.
The figure presents the data from those 10+10 = 20 children (out of 21+24 = 45 children), who had second degree, type a burns (not second degree, type b burns) and were consequently treated non-surgically until complete cutaneous regeneration occurred including irradiation (starting on the day of the burn, until complete reepithelialization) with NIR(+VIS) or with only VIS (control group). Patients in the group with NIR showed a markedly faster reduction of wound area compared to the control group: a median reduction of wound size of 50% was reached in the group with NIR already after 7 days compared to 9 days in the control group, a median reduction of wound size of 90% was achieved in the group with NIR already after 9 days compared to 13 days in the control group.

After 9 days, the median reduction in wound area was 89.2% versus 49.5%, the median difference between the groups was a 39.5% reduction of the wound area (99% confidence interval 34.4%/43.0%, p<0.001). The median difference between the groups existed already after one day with p<0.001 and after 2,5,6,7,8,9,10 and 11 days with p<0.001. In addition, the group with NIR showed superior results in terms of the overall surgical assessment of the wound and the assessment of effects irradiation (the latter as a trend up to 3 months after the burn) compared to the control group.

In a prospective, randomized, controlled study with 12 volunteers at the University Medical Centre Charite, Berlin, Germany, volunteers were inflicted with 4 experimental superficial wounds (5 mm diameter). In this acute wound model, wounds were generated by a suction cup technique, with the roof of the blister being removed with a scalpel and sterile forceps (day 1). There were 4 different treatments used and investigated over 10 days: no therapy, NIR(+VIS) only (approximately 75% NIR, 25% VIS; 30 minutes irradiation once a day), only dexpanthenol (=D-panthenol) cream once a day, NIR (+VIS) and dexpanthenol cream once a day. Healing of the small experimental wounds was, from a clinical point of view, excellent with all 4 treatments. Therefore there were only small differences between the treatments with slight advantages seen with the combination NIR (+VIS) and dexpanthenol cream and with dexpanthenol cream alone as far as relative change of wound size and assessment of feeling of the wound area were concerned.

Laser scanning microscopy, however, together with a scoring system revealed differences between the 4 treatments concerning the formation of the stratum corneum (from first layer of corneocytes to full formation) especially on days 5-7: the fastest formation of the stratum corneum was seen in wounds treated with NIR (+VIS) and dexpanthenol cream, second was NIR (+VIS) alone, third dexpanthenol cream alone and lastly, untreated wounds. Bacterial counts of the wounds (taken every two days) showed that NIR (+VIS) and the combination of NIR (+VIS) with dexpanthenol cream were able to inhibit the colonisation with physiological skin flora up to day 5 when compared with the two other groups (untreated group and group with dexpanthenol cream alone). At any investigated time, the amount of colonisation under therapy with NIR (+VIS) alone was

lower (interpreted as being more suppressed) compared with the group with NIR (+VIS) and dexpanthenol cream.

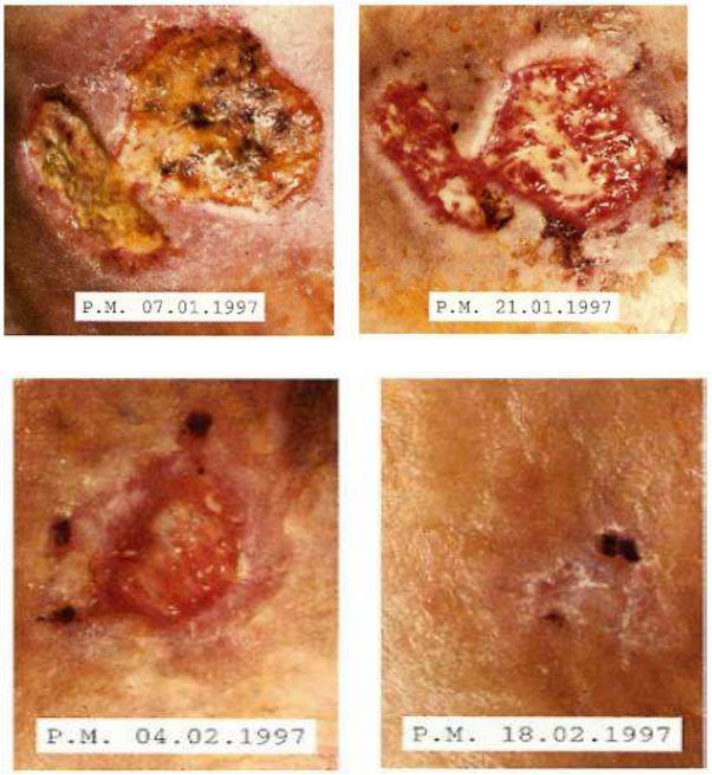


Figure 14. Example of a healing process of a chronic venous stasis ulcer of the lower leg under therapy with NIR, three times a week 30 minutes irradiation with NIR and VIS (Study Basel, Switzerland): initial findings, result after 2 weeks, after 4 weeks and after 6 weeks (healed).

Chronic Ulcers

In a prospective, randomized, controlled study of 40 patients with chronic venous stasis ulcers of the lower legs, irradiation with NIR and visible light VIS 30 minutes three times per week over 6 weeks accelerated the wound healing process (on average 18 versus 42 days until complete wound closure, residual ulcer area after 42 days 0.4 cm^2 versus 2.8 cm^2) as well as in a statistically significant ($p<0.001$) and led to a reduction of the required dose of pain medication in comparison to the control group of patients treated with the same standard care (wound cleansing, wound dressing with antibacterial gauze, and compression therapy) without concomitant irradiation (120). Figure 14 depicts a successful course of treatment.

Another prospective, primarily designed randomized, controlled study of 10 patients with non-healing chronic venous stasis ulcers of the lower legs (ulcer size up to 5 cm in diameter) including extensive thermographic investigations resulted under therapy with NIR (+VIS) (maximum total irradiance 185 mW/cm^2, approximately 140 mW/cm^2-75% NIR and 45 mW/cm^2-25% VIS) in an accelerated wound healing process with complete or almost complete wound healing (96-100% reduction of wound area) in 7 of 10 patients and a reduction of the ulcer size in another 2 of 10 patients (35).

An example of a successful course of therapy with NIR (+VIS) irradiation is demonstrated in Figure 15 with normal view, thermographic image and temperature profile across the ulcer, in each case before therapy and after completion of therapy (35).

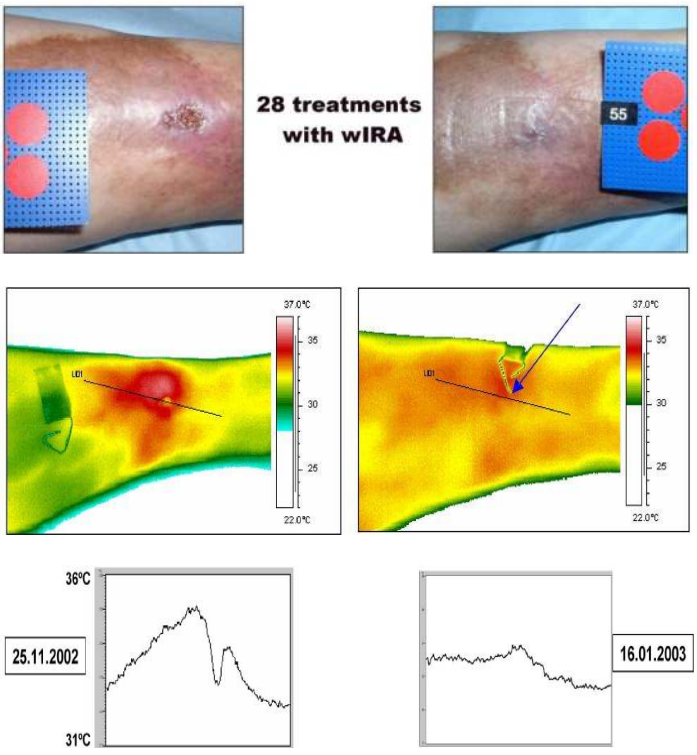


Figure 15: Example of a healing process of a chronic venous stasis ulcer of the lower leg under therapy with NIR (28 times 30 minutes irradiation with NIR and VIS within 52 days=approximately 7 weeks)

with normal view, thermographic image, and temperature profile across the ulcer, in each case to the left before therapy and the the right after completion of the course of therapy. The arrow and the long arm of the piece of wire in the thermography image point to the place where the wound has been. Diameter of the red circles: 16mm.

Among the 6 patients without concomitant problems (peripheral occlusive arterial disease, smoking or lacking compression garment therapy) a complete or almost complete wound healing (96-100% reduction of wound area) was achieved without any exception. In contrast only in 1 of 5 ulcers of the 4 patients with peripheral occlusive arterial disease, smoking or lacking compression garment therapy a complete or almost complete wound closure was accomplished. However, even in these 4 patients with 5 ulcers clear reductions of wound area were reached in 4 of the 5 ulcers. In addition the study showed a clear reduction of pain and required pain medication (e.g. from 15 to 0 pain tablets per day) and a normalization of the thermographic image. Prior to the start of therapy typically the ulcer border was hyperthermic, accompanied with a relative hypothermic ulcer base, partly associated with several degrees of temperature gradiance. At the end of the course of therapy the temperature differences were mostly balanced. All assessments using visual analog scales which include pain sensation of the patient in the wound area, overall rating of the effect of the irradiation by the patient and by the clinical investigator, overall assessment of the feeling of the patient of the wound area, overall evaluation of the wound healing process by the clinical investigator, overall assessment of the cosmetic appearance by the patient and by the clinical investigator were improved remarkably during the period of irradiation therapy and commensurate with the improvement of the quality of life (35).

A prospective, randomized, controlled, blinded study with a planned cohort of approximately 50 patients with non-healing chronic venous stasis ulcers of the lower legs is performed at the university medical centre Freiburg, Germany, department of dermatology. A descriptive interim evaluation of the first 23 patients with compression garment therapy, wound cleansing and non-adhesive wound dressings (standardized regarding wound conditions) and 30 minutes irradiation five times per week over 9 weeks (and additional 4 weeks without irradiation) demonstrated an advanced wound healing and better granulation in the group with NIR (+VIS) (maximum total irradiance 185 mW/cm^2, approximately 140 mW/cm^2 NIR 75% and 45 mW/cm^2 VIS 25%) compared to a control group with VIS. Concerning wound bacteria the group with NIR (+VIS) showed – compared to the control group with only VIS – after a temporary increase from the beginning of therapy until week 5, a decrease of the bacterial load in the wounds from week 7 over week 9 until the end of the study at week 13. Bacteriological differentiation revealed in the time period with decreasing bacterial level of the wounds both a decrease of physiologic flora of the skin, called contamination flora (coagulase negative staphylococci, micrococcus sppp., corynebacterium spp., streptococcus spp.) and a decrease of pathogenic flora (S. aureus, P. aeruginosa, haemolytic streptococci, K. pneumonia, K. oxytoca, E. coli). The early phase with an increase in the size of the bacterial inoculums (until week 3) is mainly due to non-pathogenic contaminants. In summary, after a phase with increased bacterial growth for the first 3 weeks, the group with NIR showed a decrease of bacterial growth. The better healing of the wounds might explain the impeded growth of bacteria, which vice versa results in an accelerated healing of these lesions even in the presence of significant and severe comorbidities and challenges.

Examples of using NIR to treat chronic venous stasis ulcers of the lower legs:

- 88 years old woman with an infected (lightly malodorous) crustaceous ulcer (of the right distal medial lower leg), which had existed for 13 months and had increased despite of conservative dermatological therapy including local antisepsis, systemic antibiotic, and non-adhesive wound dressing up to 10 cm in

diameter. Chronic venous insufficiency with marked stasis related edemas of the lower legs and extensive stasis dermatitis, diabetes mellitus type II (orally treated), slight overweight, and decreased amount of daily motion. Under irradiation with NIR (+VIS) 30 minutes once daily, compression garment therapy, local antisepsis, non-adhesive wound dressing and possibility to end the systemic antibiotic therapy a complete wound closure was reached within approximately five months. The course of the treatment is depicted in Figure 16 (121).

- 77 year old woman with a pruriginous, erosive stasis dermatitis for 8 years. Contact allergy to scent-mixture. Intermittent topical therapies with hydrocortisone and tacrolimus. In November 2005 development of an ulcer of the lower leg. Local treatments with povidon iodine, ammoniumbituminosulfonat and compression garment therapy of the lower leg resulted in a reduction of ulcer size, but no healing of the ulcer was achieved. In September 2006 irradiations with NIR (+VIS) for 30 minutes each were started, see initial situation of Figure 12. The first 7 irradiations were performed within the first 3 weeks and additional 6 irradiations within the next 5 weeks. After 13 irradiations the ulcer and the stasis dermatitis in the treated area were healed, see course of the treatment in Figure 17. Up to now (approximately 1 year follow-up) the patient is free of complaints.

Approximately 80% of ulcers of the lower legs are venous stasis ulcers (or ulcers at least dominated by venous problems) and systematic studies about the use of NIR concerning ulcers of the lower legs have been performed up to now in venous stasis ulcers (or ulcers at least dominated by venous problems). From a theoretical point of view undesired effects like an arterial steal effect might be considered when thinking about the use of NIR for mixed arterial-venous ulcers or even for arterial ulcers. The following examples show that NIR can be used even in these indications, if irradiance is chosen appropriately low and if irradiation is monitored carefully.

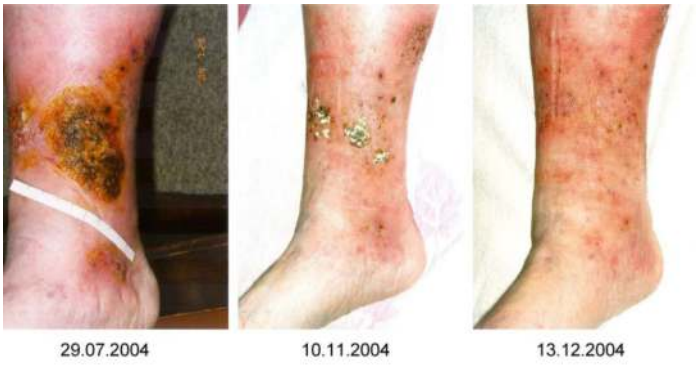


Figure 16. Example of a healing process of a chronic venous stasis ulcer of the lower leg under therapy with NIR, once daily 30 mins with NIR(+VIS): initial findings, $3_{1/2}$ month, $4_{1/2}$ month healed.

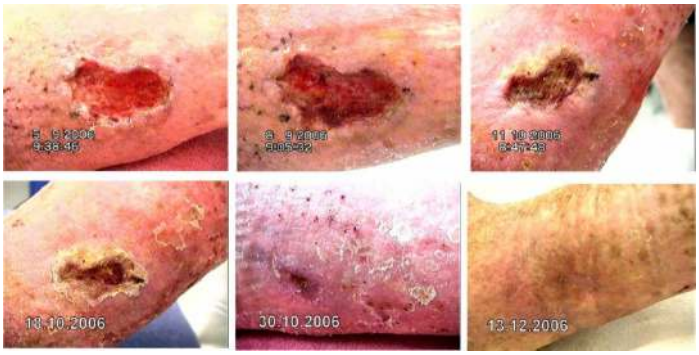


Figure 17. Example of a healing process of a chronic venous stasis ulcer of the lower leg under therapy with NIR, 13 irradiations of 30 mins with NIR and VIS within 8 weeks, the first 7 irradiations within the first 3 weeks, additional 6 irradiations within the next 5 weeks. After 13 irradiations the ulcer and the stasis dermatitis in the treated area were healed: initial findings, 3 days, 5 weeks, 6 weeks, 8 weeks and 14 weeks.

Example 1: the size of an already for years existing mixed arterial-venous ulcer of the lower leg of a smoker in a nursing home decreased within 16 days with 14 NIR (+VIS) treatments of 15 minutes from 4.2 x 2.5 cm to 1.5 x 0.5 cm, the overall evaluation of the wound healing process (visual analog scale VAS 0-100) by the nurse improved within these 16 days from 6 to 75, the pain in the wound area (VAS 0-100) decreased from 48 to 33, the

assessment of the feeling of the patient of the irradiation (VAS -50/+50, 0 as indifferent point) improved from +4 to +16, and the overall rating of the effect of the irradiation (VAS -50/+50, 0 as indifferent point) by the nurse improved from 0 to +26 (121).

Example 2: chronic venous insufficiency (third degree to Widmer) combined with peripheral occlusive arterial disease (second degree to Fontaine) and poorly controlled diabetes mellitus (type II). Already within approximately 10 days with NIR (+VIS) therapy a clear trend to heal with nearly complete healing of three smaller ulcers of the lower leg was achieved (121).

Example 3: chronic venous insufficiency combined with peripheral occlusive arterial disease and diabetes mellitus with microangiopathy; approximately 7 cm large ulcer of the lower leg in the area of the tibia (with small second ulcer): under NIR (+VIS) therapy within 15 days clear reduction of scabs, begin of granulation, nearly complete reduction of inflammatory redness of the surrounding skin. A marked increase of oxygen saturation of haemoglobin between before and after a single NIR treatment, especially in the depth of tissue, could be measured with an external probe (121).

Osteoarthritis

We did an observational study on knee osteoarthritis (OA) in 2010 (122). There were 30 patients with knee OA collected through family doctors in Hamilton, Ontario, Canada. All the patients had pain in at least one knee for the last 48 hours and lasted for more than two months. The age of the patients is from 40 to 65. There were 14 men and 16 women with mean age at 55.3 and 56.2. The symptomatic leg was positioned under NIR light (400-1400nm, 5W, 340 mW/cm^2) for 30 minutes. Thereinto, 10 minutes for each side knee eye and 10 minutes for a pain point indicated by patient self. Patients were asked to do 1 time/day and total 4 weeks treatment. They were asked to mark their pain on a 10 cm line with left end means no pain and right end extreme pain (VAS) at before and after treatment. A quality of life measurement was performed by using a questionnaire called West Ontario and McMaster arthritis symptom evaluation form (WOMAC) to fill in before and after the treatment. Score changes for pain, stiffness and functional difficulty of effected knee were collected.

After treatment, there was 56% patient had a significant decrease of WOMAC pain score (>5) and VAS length (>2cm). There 48% and 32% patients had a big improvement of stiffness and functional difficulty (>2 and >10), respectively. The T-test of comparing means of before and after treatment of VAS and WOMAC was statistically significant for the above changes ($p<0.001$). Therefore, the NIR light can improve knee OA symptoms after 4 weeks treatment.

Pain is a major symptom in cervical osteoarthritis (COA). Near infrared (NIR) therapy has been claimed to reduce pain in musculoskeletal pathologies. Ozdemir F and his colleagues evaluated the analgesic efficacy of NIR therapy and related functional changes in COA (123). Sixty patients between 20 and 65 years of age with clinically and radiologically diagnosed COA were included in the study. They were randomised into two equal groups according to the therapies applied, either with LPL or placebo laser. Patients in each group were investigated blindly in terms of pain and pain-related physical findings, such as increased paravertebral muscle spasm, loss of lordosis and range of neck motion restriction before and after therapy. Functional improvements were also evaluated. Pain, paravertebral muscle spasm, lordosis angle, the range of neck motion and function were observed to improve significantly in the NIR group, but no improvement was found in the placebo group. NIR seems to be successful in relieving pain and improving function in osteoarthritic diseases (Figure 18).

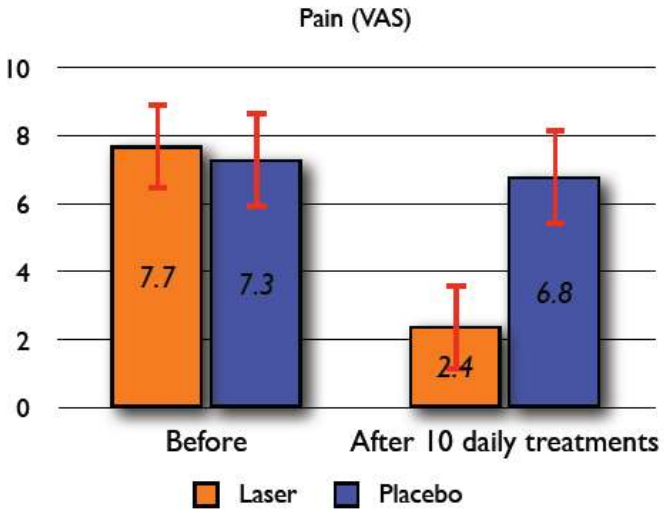


Figure 18. Show Pain VAS before and after treatment in light treatment and placebo groups. Before the treatment there is no difference between light and placebo group, while after treatment light group is significantly lower than placebo group in pain score.

Carpal Tunnel Syndrome

From 2010, a pilot study of NIR improves carpal tunnel syndrome (CTS) was performing on 35 subjects from a chiropractor centre in Hamilton, Ontario, Canada (124). The age of the patients was from 45 to 65, mean at 56.3. A confirmed diagnosis of CTS was given to all the participants within last 10 years. Patient's palm, wrist and elbow were lighted by NIR light (400-1400nm, 5W, 340 mW/cm^2) for 30 minutes (10 minute/position). The treatment interval is one week for 1 time/day. Before and after the treatment, patients were assessed with quality of life by Levine questionnaire which focus on CTS symptom and functional scale. Any scale improvement more than 20% will be recognized as a significant change.

After treatments, there were 26 patients got a significant improvement for symptom scale (74%) and 21 good results for functional scale (60%). The percentage of total improvement is 84% for symptom and function. Comparing the mean scale of symptom and function between before and after treatment, the p value were both less than 0.001 and means a significant improvement was made in this group of patients (Figure 19).

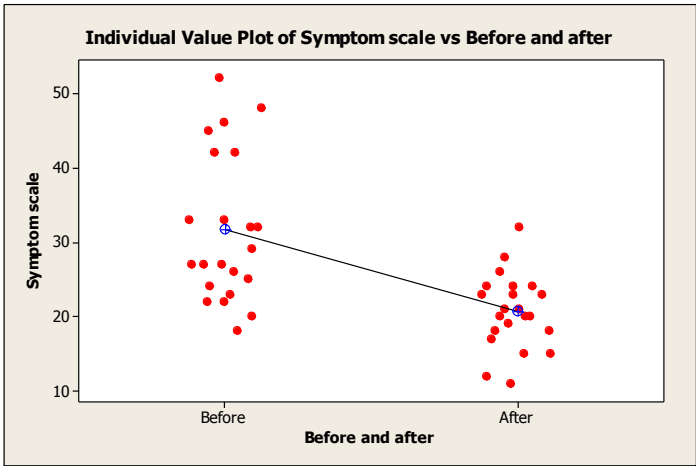


Figure 19. After treatment, the mean value of symptom scale of Carpal tunnel syndrome is significantly lower than before the treatment (p<0.001).

Conclusion

Experimental results demonstrate that NIR light treatment stimulates mitochondrial oxidative metabolism in vitro, and accelerates cell and tissue repair in vivo. There are many clinical trails showed NIR can alleviate pain (reduce need for analgesics), diminish inflammation and speed up wound healing. NIR has a broad usage in wound healing (post-operation, carpal tunnel syndrome, et al) and aging symptoms like neck pain, back pain, and arthritis. Additionally, NIR has good effects on diabetic foot ulcers. NIR light represents a novel, noninvasive, therapeutic intervention without any side-effects.

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