Effect of NASA light-emitting diode (LED) irradiation on wound healing

Harry T. Whelan1a,5,7, Robert L. Smits, Jr.1a, Ellen V. Buchmann1a, Noel T. Whelan1a,7, Scott G. Turner1a, David A. Margolis3 Vita Cevenini7, Helen Stinson7, Ron Ignatius2, Todd Martin2, Joan Cwiklinski1a, Alan F. Philippi5, William R. Graf5, Brian Hodgson3,4, Lisa Gould1b, Mary Kane1b, Gina Chen1b, James Caviness6

1aDepartments of Neurology, 1bPlatic Surgery, 1cNeurosurgery,
Medical College of Wisconsin, Milwaukee, WI 53226, (414) 456-4090
2Quantum Devices, Inc Barneveld, WI 53507 (608) 924-3000
3Children`s Hospital of Wisconsin, Milwaukee, WI 53201 (414) 266-2044
44th Dental Battalion, 4th Force Service Support Group, USMCR, Marietta, GA
5Naval Special Warfare Group TWO, Norfolk, VA 23521, (757) 462-7759
6Submarine Squadron ELEVEN, San Diego, CA 92106, (619) 553-8719
7NASA-Marshall Space Flight Center, AL 35812, (256) 544-2121

Abstract - The need to care for a population with chronic wounds is a growing challenge that requires innovative approaches. Two approaches that specifically address the identified pathophysiological processes involved in wound healing are hyperbaric oxygen therapy and light therapy.

Light Emitting Diodes (LED-technology), originally developed for NASA plant growth experiments in space, show promise for delivering light deep into tissues of the body to promote wound healing and human tissue growth. In this paper we review and present our new data of LED treatment on cells grown in culture, on ischemic and diabetic wounds in rat models, and on acute and chronic wounds in humans. Results include: in vitro increases of cell growth of 140-200% in mouse derived fibroblasts, rat derived osteoblasts, and rat derived skeletal muscle. Increases in growth of 155% -171% of normal human epithelial cells in vitro. Wound size decreases of up to 36% in conjunction with HBO in ischemic rat models. Improvement of greater than 40% in musculoskeletal training injuries in Navy SEAL team members. Decreased wound healing time by 50% of crew members aboard a U.S. Naval submarine. And finally, up to a 47% reduction in pain of children suffering from oral mucositis. We believe that the use of NASA Light-Emitting Diodes (LED) for light therapy will greatly enhance the natural wound healing process. This work is supported and managed through the NASA Marshall Space Flight Center – SBIR Program.

Introduction

The need to care for a population with chronic wounds is a growing challenge that requires innovative approaches. Two approaches that specifically address the identified pathophysiological processes involved in wound healing are hyperbaric oxygen therapy and light therapy. Hyperbaric oxygen therapy is currently the standard of care for ischemic, hypoxic, infected, and otherwise slowly healing, problem wounds. We believe that the use of NASA Light-Emitting Diodes (LED) for light therapy alone, and in conjunction with hyperbaric oxygen, will greatly enhance the natural wound healing process. This will save valuable time and resources for both patients and health care facilities. Furthermore, improved wound healing will reduce the risk of infection for the patient, decrease the amount of costly dressings required, and more quickly return the patient to a pre-injury/illness level of activity.

Laser light and hyperbaric oxygen have been widely acclaimed to speed wound healing of ischemic, hypoxic, and infected wounds (Conlan, 1996). Lasers provide low energy stimulation of tissues which

results in increased cellular activity during wound healing (Beauvoit, 1994, 1995). These activities include collagen production and angiogenesis (Abergel, 1987). Hyperbaric oxygen therapy, which is currently standard therapy in the treatment of diabetic ulcers, graft failures, radiation necrosis, and other ischemic wounds, has also been shown to beneficially affect these processes. However, there are a variety of instances in which a patient who may benefit form hyperbaric oxygen is unable or unwilling to be treated in a high-pressure environment. These situations include lack of access to a facility equipped with hyperbaric oxygen, claustrophobia, and certain current or chronic medical conditions which would make hyperbaric oxygen therapy contraindicated. In these instances light therapy provides an option for the patient.

Wound healing has three phases: first a substrate is laid down, then cells proliferate, and finally there is remodeling of tissue. The data published so far suggests that laser biostimulation produces its primary effect during the cell proliferation phase of the wound healing process. It has been demonstrated that mitochondria are receptive to monochromatic near-infrared light and that laser light likely increases respiratory metabolism of certain cells (Beauvoit, 1994, 1995; Tamura, 1993). Processes such as fibroblast proliferation, attachment and synthesis of collagen and procollagen, growth factor production [including keratinocyte growth factor (KGF), transforming growth factor (TGF) and platelet-derived growth factor (PDGF)], macrophage stimulation, lymphocyte stimulation (Mester , 1998) and greater rate of extracellular matrix production have been reported with laser light treatment (Mester, 1973; Lubart, 1992; Miller, 1993; Yu, 1994; Whelan, 1999, 2000, 2001; Sommer, 2001). Animal studies on the enhanced wound healing effect of laser light of low power density have been performed in toads, mice, rats, guinea pigs, and swine (Bibikova 1995, Al-Watban, 1997). Human studies with laser light have demonstrated greater amounts of epithelialization for wound closure and stimulation of skin graft healing (Miller, 1993; Conlan, 1996). An excellent review of recent human experience with near-infrared light therapy for wound healing was published by Conlan, et al in 1996.

Lasers, however, have some inherent characteristics which make there use in a clinical setting problematic, including limitations in wavelength capabilities and beam width. The combined wavelengths of the light for optimal wound healing cannot be efficiently produced, the size of wounds which may be treated is limited (due to laser production of a beam of light; a fact inconsistent with treating large areas), heat production from the laser light itself can actually damage tissue, and the pin-point beam of laser light can damage the eye. NASA developed LEDs offer an effective alternative to lasers. These diodes can be configured to produce multiple wavelengths, can be arranged in large, flat arrays (allowing treatment of large wounds), and produce no heat. It is also of importance to note that LED light therapy has been deemed a nonsignificant risk by the FDA; thus FDA approval for the use of LEDs in humans for light therapy has been obtained.

NASA LEDs stimulate the basic energy processes in the mitochondria (energy compartments) of each cell, particularly when near-infrared light is used to activate the wavelength sensitive constituents inside (chromophores, cytochrome systems). Optimal light wavelengths [proven in prior studies of laser and LED light (Karu, 1989; Lubart, 1992; Beauvoit, 1994, 1995; Whelan, 1999, 2000, 2001; Sommer 2001)] to speed wound healing include 680nm, 730nm, and 880 nm. These wavelengths can be produced accurately be NASA LEDs, which have a bandwidth of 25nm. The depth of near-infrared light penetration into human tissue has been measured spectroscopically (Chance, 1988; Beauviot, 1994, 1995). Spectra taken from the wrist flexor muscles in the forearm and muscles in the calf of the leg demonstrate that most of the photons at wavelengths between 630-800 nm travel approximately 23 cm through the skin surface (light input) and muscle, exiting at the photon detector. Data collection and cataloging to elucidate the absorption coefficients of the various human tissues is currently underway by this principle investigator.

LED-In Vitro Studies

In order to better understand the effects of LEDs on cell growth and proliferation, we have measured radiolabeled thymidine incorporation in vitro in several cell lines treated with LED light at various wavelengths and energy levels. As previously reported, 3T3 fibroblasts (mouse derived skin cells) responded extremely well to laser and LED light exposure (Lubart, 1992, 1993; Yu, 1994, 1997; Yamada, 1991; Whelan, 1999, 2000, 2001). Cell growth increased 150-200% over untreated controls. Additionally, osteoblasts (rat derived) have been reported to have increased DNA synthesis and increased cellular growth

rate when exposed to laser light (Yamada, 1991). With LED treatment, we have found that these cells demonstrated a growth-phase specificity, responding only when cells are in the growth phase. In these experiments, fibroblasts and osteoblasts, at a concentration of 1x104 cells/well, were seeded in 24 well plates with a well diameter of 2 square centimeters. DNA synthesis was determined on the second, third, and fourth days in culture for both fibroblasts (Figure 1) and osteoblasts (Figure 2). Exposure to LED irradiation accelerated the growth rate of fibroblasts and osteoblasts in culture for 2 to 3 days (growing phase), but showed no significant change in growth rate for cells in culture at 4 days (stationary phase). These data are important demonstrations of cell-to-cell contact inhibition, which occurs in vitro once cell cultures approach confluence. This is analogous, in vivo, to a healthy organism, which will regenerate healing tissue, but stop further growth when healing is complete. It is important to note that LED treatment accelerates normal healing and tissue regeneration without producing overgrowth or neoplastic transformation.

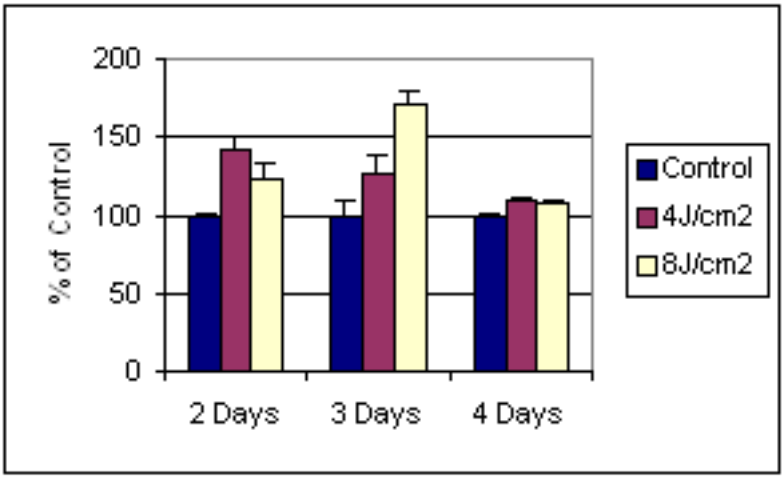


Figure 1. Growth phase specificity of 3T3 fibroblasts; combined wavelengths; 4J/cm2 vs. 8J/cm2; 50mW/cm2.

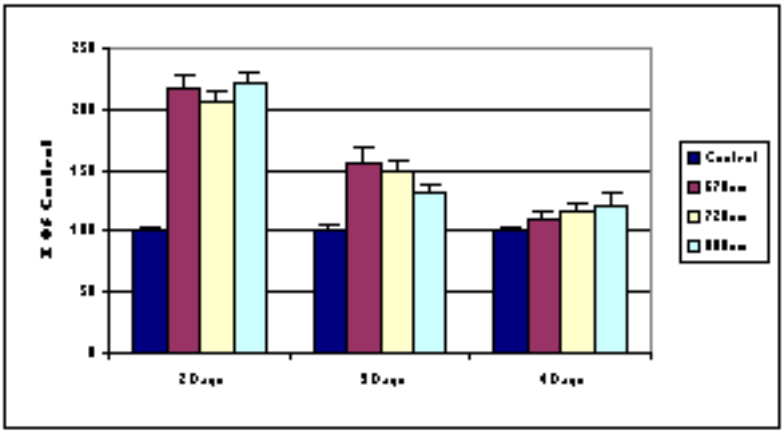


Figure 2. Growth phase spHarry T. Whelan1a,5,7, Robert L. Smits, Jr.1a, Ellen V. Buchmann1a,

Noel T. Whelan1a,7, Scott G. Turner1a, David A. Margolis3
Vita Cevenini7, Helen Stinson7, Ron Ignatius2, Todd Martin2,
Joan Cwiklinski1a, Alan F. Philippi5, William R. Graf5, Brian Hodgson3,4,
Lisa Gould1b, Mary Kane1b, Gina Chen1b, James Caviness6

1aDepartments of Neurology, 1bPlastic Surgery, 1cNeurosurgery, Medical College of Wisconsin, Milwaukee, WI 53226, (414) 456-4090 2Quantum Devices, Inc Barneveld, WI 53507 (608) 924-3000 3Children's Hospital of Wisconsin, Milwaukee, WI 53201 (414) 266-2044 44th Dental Battalion, 4th Force Service Support Group, USMCR, Marietta, GA
5Naval Special Warfare Group TWO, Norfolk, VA 23521, (757) 462-7759 6Submarine Squadron ELEVEN, San Diego, CA 92106, (619)553-8719 7NASA-Marshall Space Flight Center, AL 35812, (256) 544-2121ecificity of osteoblasts; individual wavelengths; 8J/cm2; 50mW/cm2.

A series of experiments have recently been completed using an L-6 musculoskeletal cell line (rat derived). These cells were exposed to the LED light at both combined wavelengths and individual wavelengths (670nm, 728nm, and 880nm), energy densities of 4 and 8J/cm2, and an intensity of 50mW/cm2. Results demonstrated a cell growth increase of about 140% over untreated controls, particularly at 8J/sq cm energy as shown in Figure 3.

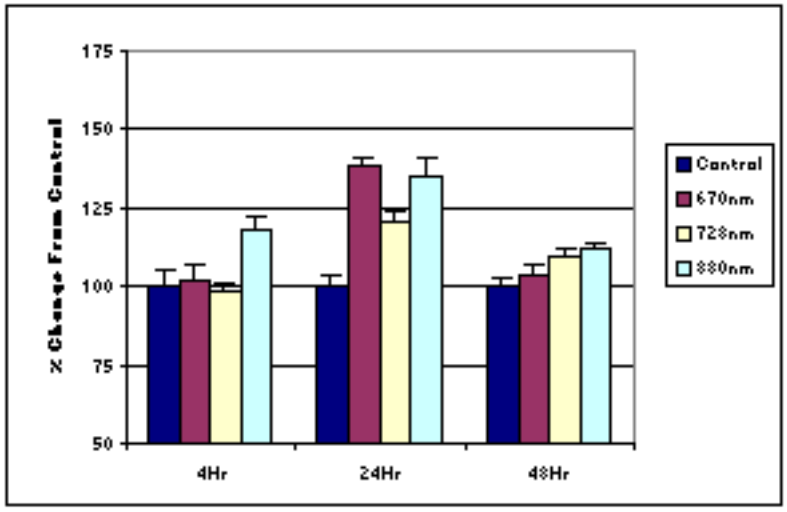


Figure 3. Growth phase specificity of L-6 skeletal muscle
Cells treated with individual wavelengths at 8J/cm2; 50mW/cm2.

In addition, experiments are now complete using a normal human epithelial cell line in order to possibly explain the continued, dramatic results of LED light therapy in preventing oral mucositis in cancer patients (presented later in paper). Cell growth increased 155% over untreated controls at 670nm and 4J/cm2 energy density (50mW/cm2 power density), as shown in Figure 4. An increase of 171% over untreated controls was obtained with a wavelength of 880nm and 8J/cm2 energy density (53mW/cm2 power density), as shown in Figure 5.

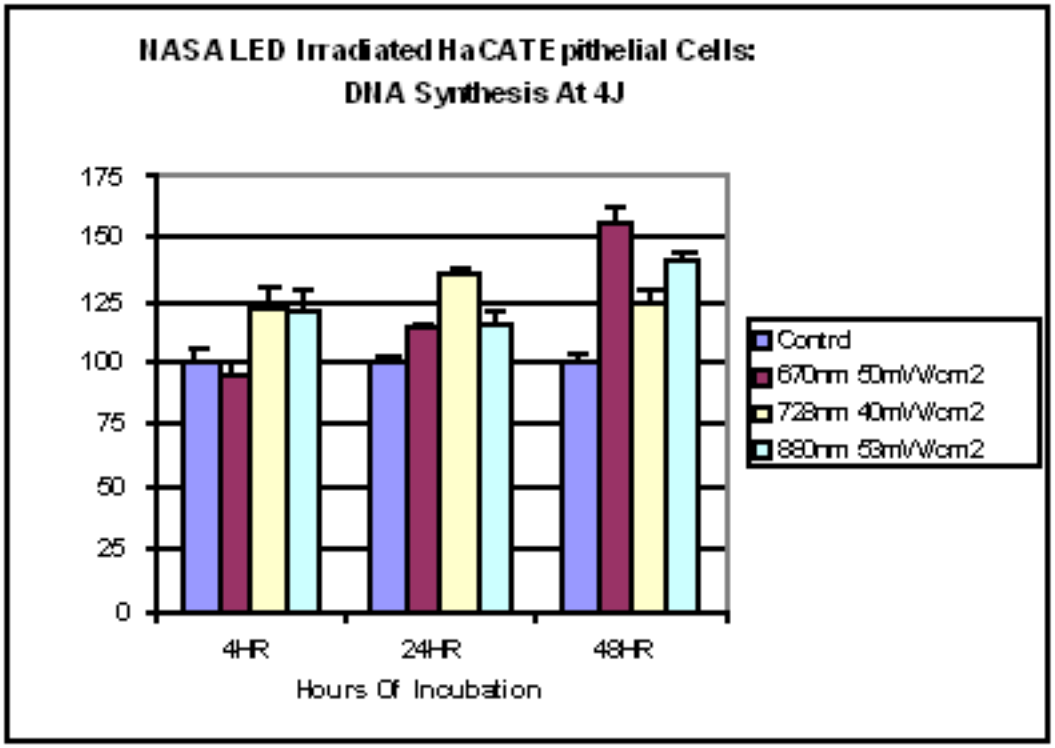


Figure 4. LED response at 4J/cm2, 50mW/cm2 using individual wavelengths of 670nm, 728nm and 880nm. (% change from control vs # of hours after LED treatment).

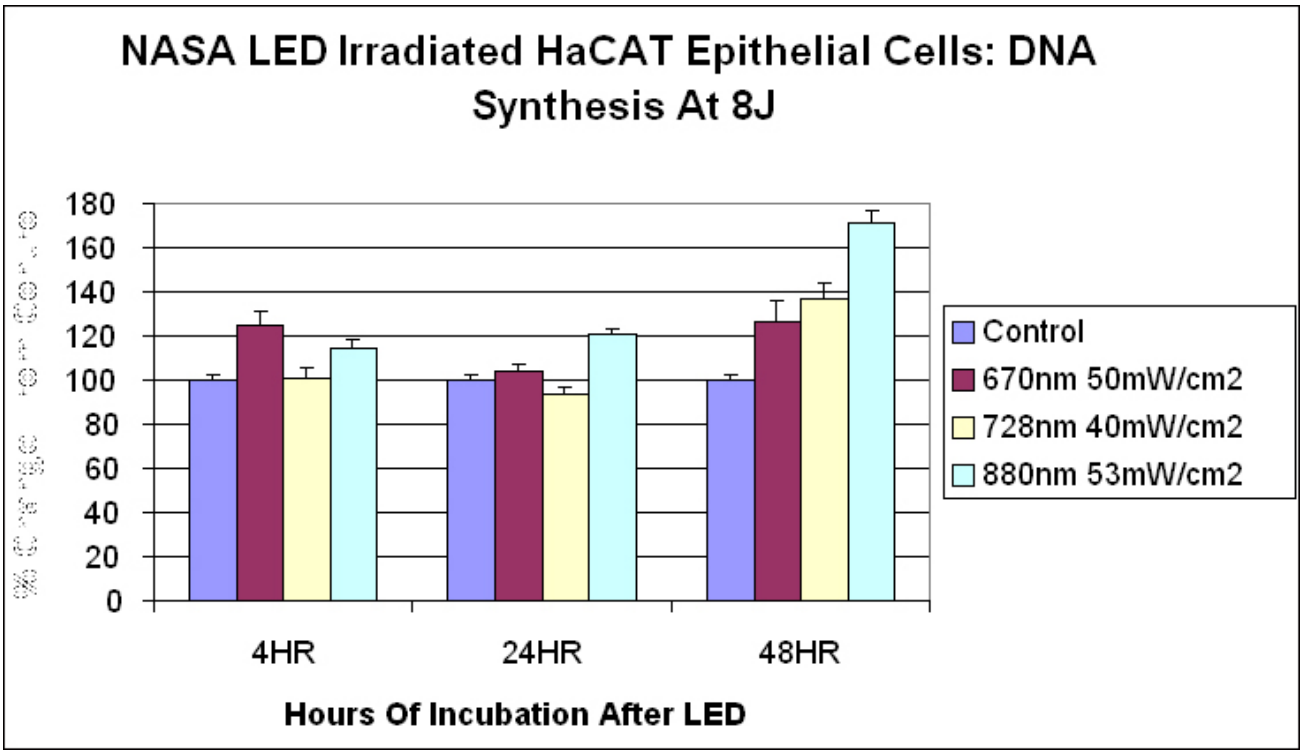


Figure 5. LED response at 8J/cm2, 50mW/cm2 using individual wavelengths of 670nm, 728nm and 880nm. (% change from control vs # of hours after LED treatment).

Collagen synthesis of the HaCAT epithelial cells was determined by measuring tritiated proline incorporation using a modified method described by Peterkofsky and Diegelmann (1971). HaCAT epithelial cells were seeded in two 12-well tissue culture plates with 600?l DMEM containing 10% FBS, 1% penicillin/streptomycin, and 6?l L-proline [2,3-3H]. The media was free of non-essential amino acids. One plate was used as the control and the other was treated with the 670nm NASA-LED at 8J/cm2. After 24h incubation in 5% CO2 at 37?C, two 200?l aliquots of media were removed from each well. One aliquot was used to quantitate total protein by trichloroacetic acid (TCA) precipitation. The precipitated proteins were collected by suction onto a glass fiber filter and allowed to dry overnight at room temperature. The second aliquot was incubated for 90 minutes at 37?C with highly purified bacterial collagenase that degraded the collagen in the sample. The remaining noncollagen protein also underwent TCA precipitation. The following day, scintillation fluid was added and the samples were counted in a scintillation counter. Collagen content was determined by subtracting the noncollagen protein from the total protein. Figure 6 shows that the HaCAT epithelial cells that were NASA LED treated synthesized more than twice the amount of collagen than that of the control cells.

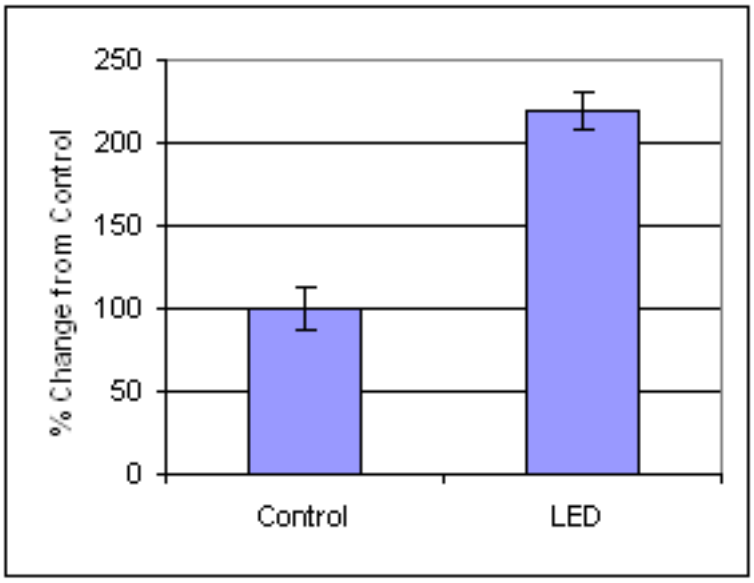


Figure 6. HaCAT Epithelial Cell Collagen Synthesis at 8J/cm2, 50mW/cm2, 670nm. 24 hour 3H proline incorporation.

LED-Wound Healing in Rats

An ischemic wound is a wound in which there is a lack of oxygen to the wound bed due to an obstruction of arterial blood flow. Tissue ischemia is a significant cause of impaired wound healing which renders the wound more susceptible to infection, leading to chronic, non-healing wounds. Despite progress in wound healing research, there is still very little understanding of what constitutes a chronic wound, particularly at the molecular level. Consequently, there is minimal scientific rationale for treatment.

In order to study the effects of NASA LED technology and hyperbaric oxygen therapy (HBO), we developed a model of an ischemic wound in normal Sprague Dawley rats. Two parallel, 11-cm incisions were made 2.5 cm apart on the dorsum of the rats, leaving the cranial and caudal ends intact. The skin was elevated along the length of the flap and two punch biopsies created the wounds in the center of the flap. A

sheet of silicone was placed between the skin and the underlying muscle to act as a barrier to vascular growth, thus increasing the ischemic insult to the wounds. The four groups, each consisting of 15 rats, in this study include: the control (no LED or HBO), HBO only, LED (880 nm) only, and LED and HBO in combination. The HBO was supplied at 2.4 atm for 90 minutes, and the LED was delivered at a fluence of 4J/cm2 and 50mW/cm2 for fourteen consecutive days. A future study will incorporate the combination of three wavelengths (670nm, 728nm, and 880nm) in the treatment groups.

The wounds were traced manually on days 4, 7, 10, and 14. These tracings were subsequently scanned into a computer and the size of the wounds was tracked using SigmaScan Pro software. Figure 7 depicts the change in wound size over the course of the 14-day experiment. The combination of HBO and LED (880 nm) proves to have the greatest effect in wound healing in terms of this qualitative assessment of wound area. At day 7, wounds of the HBO and LED (880nm) group are 36% smaller than those of the control group. That size discrepancy remains even by day 10. The LED (880nm) alone also speeds wound closure. On day 7, the LED (880 nm) treated wounds are 20% smaller than the control wounds. By day 10, the difference between these two groups has dropped to 12%. This is due to the fact that there is a point when the wounds from all of the groups will be closed. Hence, the early differences are the most important in terms of determining the optimal effects of a given treatment. This can be seen in Figure 7 at day 14 when the points are converging due to the fact that the wounds are healing.

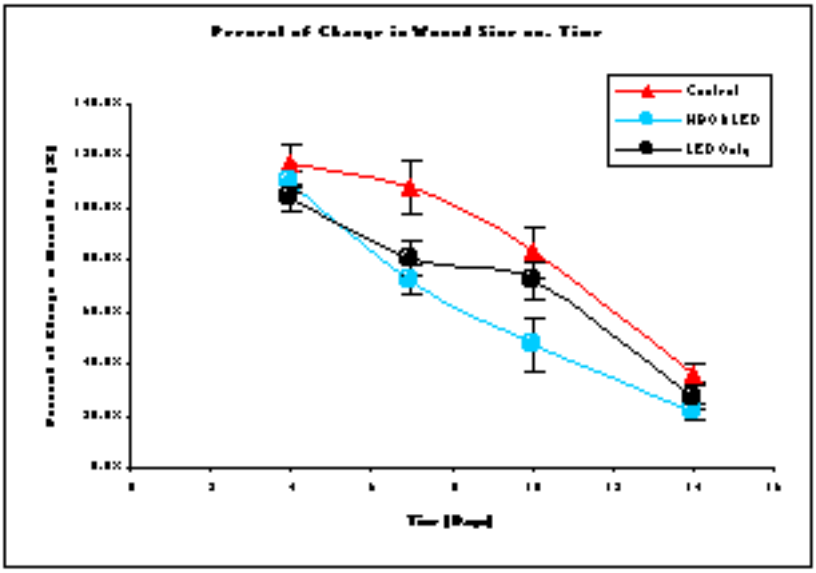


Figure 7. Change in wound size in rat ischemic wound model vs. time (days).

Analysis of the biochemical makeup of the wounds at days 0, 4, 7, and 14 was performed. The day 0 time point was determined by evaluating the punch biopsy samples from the original surgery. The levels of basic fibroblast growth factor (FGF-2) and vascular endothelial growth factor (VEGF) were determined using ELISA (enzyme linked immunosorbent assay). The changes in the VEGF concentration throughout the 14-day experiment can be seen in Figure 8. The LED (880 nm) group experiences a VEGF peak at day 4 much like the control group. In contrast, the hyperoxic effect of the HBO suppresses the day 4 peak, and instead, the HBO groups peak at day 7. The synergistic effect of the HBO and LED (880 nm) can be seen at day 4. The VEGF level for the group receiving both treatments is markedly higher at day 4 than the HBO only group. The HBO and LED (880 nm) treated group also experiences the day 7 peak characterized by the HBO treatment. Hence, there is a more uniform rise and fall to the VEGF level in the combined treatment group as opposed to the sudden increases seen in the control, LED only, and HBO only groups. By day 14,

the HBO treated groups have dropped closer to the normal level than the LED (880 nm) only or control groups.

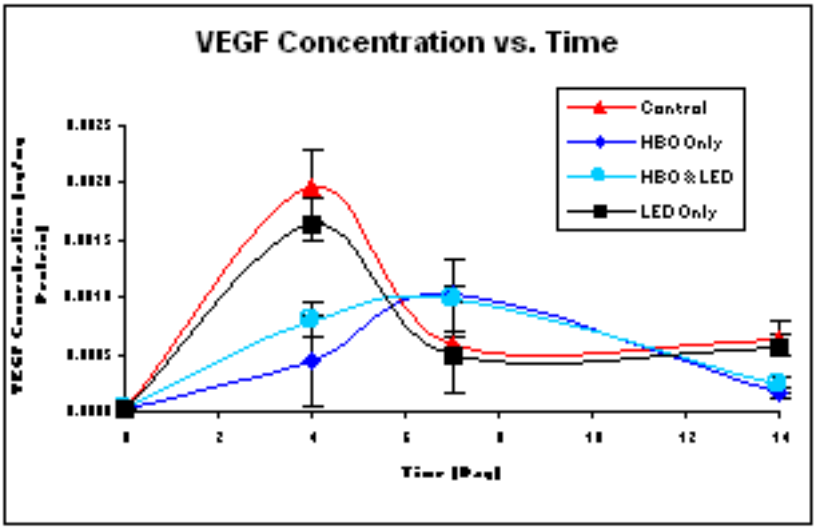


Figure 8. Change in vascular endothelial growth factor (VEGF) concentration (?g/mg protein) vs. time (days).

The synergistic effects of HBO and LED (880 nm) can also be seen easily in Figure 9. The pattern of the changes in basic fibroblast growth factor (FGF-2) concentration is similar to that of the VEGF data. It is clear that the LED (880 nm) day 4 peak is higher than the day 4 peak of the control group. These peaks can be attributed to the hypoxic effect of the tissue ischemia created in the surgery. The hyperoxia of the HBO therapy has a greater effect on suppressing the FGF-2 concentration at day 4 than the VEGF concentration at the same time point. The synergy of the two treatments is evident when looking at the HBO and LED (880 nm) treated group. The concentration of FGF-2 at day 4 is significantly enhanced by the LED (880 nm) treatment. Whereas, the level would normally drop off by day 7 for a LED-only treated wound, the HBO effect seizes control causing the concentration of FGF-2 to plateau. Hence, an elevated FGF-2 concentration is achieved throughout the greater part of the 14 day treatment with both HBO and LED (880nm) therapies. Further analysis of the excised wounds will include matrix metalloproteinase 2 and 9 (MMP-2 and MMP-9) determination by ELISA, histological examination, and RNA extraction

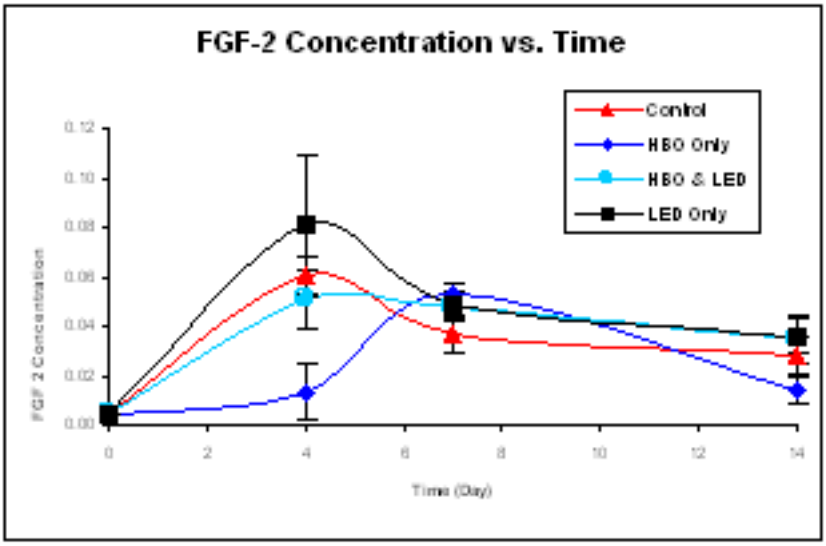
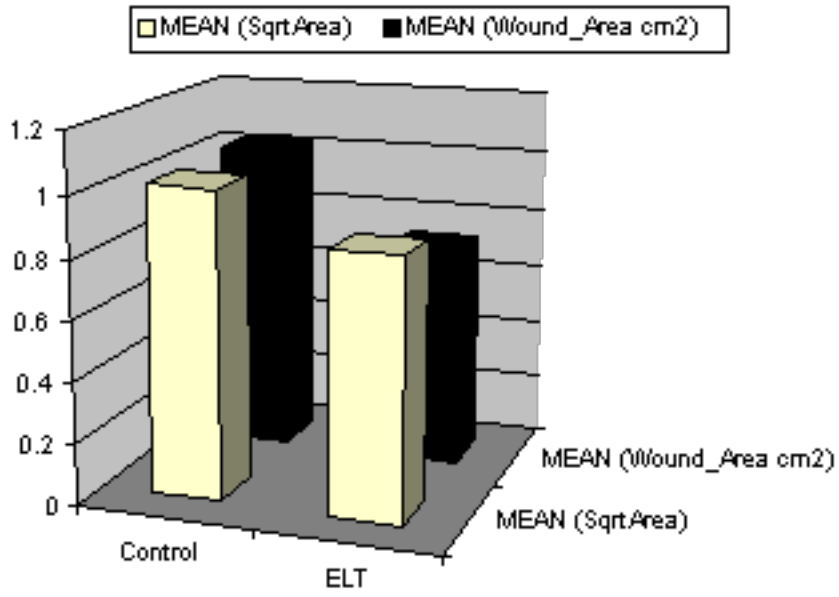


Figure 9. Change in basic fibroblast growth factor (FGF-2) concentration (?g/mg protein) vs. time (days).

A wound healing impaired type 2 diabetic mouse model has also been studied. As previously reported, genetically diabetic mice treated with low level laser irradiaiton demonstrated significantly enhanced wound closure grossly, and improved wound epithelialization, cellular content, granulation tissue formation, collagen deposition, and extensive neovascularization on histological evaluation (Yu, 1997). In our study, type 2 diabetic mice with excisional skin wounds were treated with LEDs at individual wavelengths of 680nm, 730nm, and 880 nm at 4J/cm2 and 50mW/cm2. LED treatment produced increased healing rates, compared to surgical controls as seen in Figure 10.

A repeated measures analysis was conducted using a General Linear Model with SqrtArea as the dependent variable and Treat as the independent variable. The interaction effect Day*Treat is significant (p-value = 0.0095), indicating that there is a significant difference between treatments on some days. This test is of primary interest in this situation, because it shows that the treatments are effective for some part of the treatment period (Figure 10). This analysis was carried out using the SAS statistical software package, published by The SAS Institute, Inc



	Control	ELT
MEAN (SqrtArea)	1.0121	0.8548
MEAN (Wound_Area cm2)	1.0244	0.7307

Figure 10. Type 2 Diabetic Mice with excisional skin wounds treated with combined LED wavelengths, 4J/cm2, 50mW/cm2.

The square root of wound area is used in the dependent variable in the analysis. This transformation was needed to correct for non-constant error in the General Linear Model. SqrtArea could be interpreted as being proportional to the radius of a circular wound.

LED-Wound Healing in Human Subjects

Clinical LED wound healing studies have been reported previously (Whelan, 1999, 2000, 2001); additional data on human trials are summarized below.

Military Special Operations are characterized by lightly equipped, highly mobile troops entering situations requiring optimal physical conditioning at all times. Wounds are an obvious physical risk during combat operations. Any simple and lightweight equipment that promotes wound healing and musculoskeletal rehabilitation and conditioning has potential merit. An LED array with 3 wavelengths combined in a single unit (670nm, 720nm, and 880 nm) was delivered to Naval Special Warfare Group-2 (SEALS) in Norfolk, VA. Treatment was with 4J/cm2. A data collection system has been implemented for musculoskeletal training injuries treated with LEDs. Data collection instruments now include injury diagnosis, day from injury, range of motion measured with goniometer, pain intensity scales reported on scale 1-10, girth-circumferential measurements in cm, percent changes over time in all of the aforementioned parameters, and number of LED-treatments required for the subject to be fit-for-full-duty (FFD). These injuries were sustained 1 month to 1 year prior to LED treatment and had been chronic and unimproving in nature. See summary of data in Figure 11.

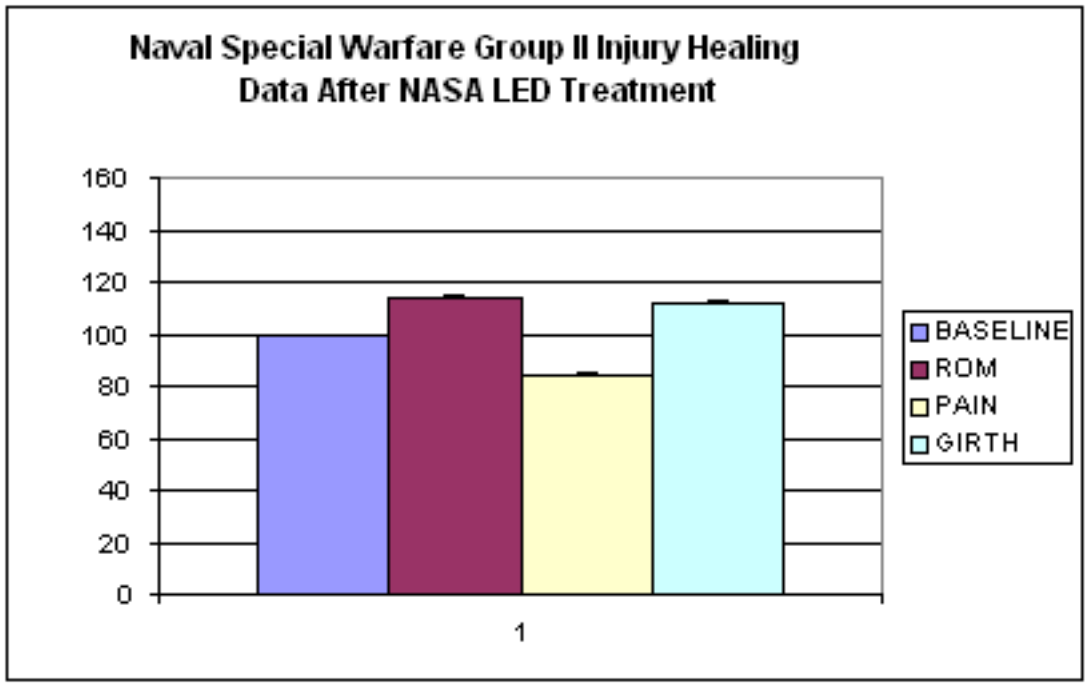


FIGURE 11. Cumulative results of data from 11 patients (SEALS) showing improvement in range of motion, pain, and girth reported as % change from chronic, unimproving injured baseline after LED treatment at 4J/cm2, 10mW/cm2.

In collaboration with U.S. Navy Submarine Squadron ELEVEN, data has also been received from the USS Salt Lake City (submarine SSN 716 of the U.S. Naval Pacific Fleet). Submarine atmospheres are low in oxygen and high in carbon dioxide, which compounds the absence of crew exposure to sunlight, making wound healing slower than on the surface. Reports indicate a 50% faster healing of lacerations in crew members treated with a LED array with 3 wavelengths combined in a single unit (670nm, 720nm, 880 nm) compared to untreated control healing (7 days compared to approximately 14 days respectively). Complete analysis of data is still underway. Receipt of control data from submarines without LED arrays on board continues, and another submarine recently deployed from U.S. Naval Station - San Diego on a six-month mission is equipped with a LED Snap-Light array on board.

In addition, we have recently begun using NASA LEDs to promote healing of acute oral lesions in pediatric leukemia patients. As a final life-saving effort, leukemia patients are given healthy bone marrow from an HLA-matched donor. Prior to the transplant, the patient is given a lethal dose of chemotherapy in order to destroy his/her own, cancerous bone marrow. Because many chemotherapeutic drugs, as well as radiation therapy, kill all rapidly dividing cells indiscriminately, the mucosal linings of the gastrointestinal tract are often damaged during the treatment. As a result of these GI effects, greater than 1/3 of patients treated with cytotoxic drugs develop ulcers in their mouths (oral mucositis) and/or suffer from nausea and diarrhea. Oral mucositis, which causes severe pain, bleeding, an increased risk for infection, and compromised ability to chew and swallow, is a significant risk for this population. Current treatment for mucositis addresses pain management and infection prevention. The use of oral agents to promote cleansing, debridement, and comfort are recomended, and prophylactic oral antiviral and antifungal agents have been used to minimize infections. Because lasers have been shown to speed healing of oral mucositis (Barasch, 1995; Cowen, 1997), we have recently expanded the wound-healing abilities of LED light therapy to include these oral lesions.

A 4J/cm2, 50mW/cm2 dose of 670nm light from LEDs was applied daily to the outside of each patient's left cheek beginning on the day of bone marrow transplantation. The status of their oral mucosa, mouth, and throat pain were assessed three times a week by two calibrated dental clinicians. Each side of the mouth was scored using the Schubert Oral Mucositis Index (OMI), the mucosa was photographed, and mouth and throat pain were assessed using a 1-10 Visual Analog scale (Schubert, 1992). We have now completed treatment to half of our intended patient population, and have noticed some very encouraging trends, but statistical significance will require more patients, as intended in our current study design. We have assessed left cheek, right cheek, and throat pain in each patient, and have noted that there is no statistical difference in perceived pain on either side of the mouth, consistent with the expected tissue penetration (23 cm) of LED light. Throat pain, however, was consistently higher than mouth pain, and because our light does not extend into this region, we have used this pain as our control. Although mouth and throat pain were initially similar, mouth pain peaked at 86% of throat pain on day 5 after transplant and subsequently fell to only 53% of reported throat pain by day seven (Table 1 & Figures 11, 12). The greatest difference between throat and mouth pain was reported on day seven, when, surprisingly, oral mucosal ulceration is believed to be worst in untreated patients.

Table1

	Day 1	Day 2	Day 3	Day 4	Day 5	Day6	Day 7
Control (Throat)	100	100	100	100	100	100	100
Right Cheek	98.56	67.81	84.78	79.78	86.16	63.79	52.73
Left Cheek	104.1	71.21	81.48	82.32	86.82	60.93	53.27
Cont. SEM	33.2	22.9	20.7	18.1	17.8	18.9	21.7
R SEM	34.2	25.2	20.4	23.5	24.5	24.1	17.9
L SEM	33.8	24	20.6	24.1	24.3	25.9	17.7

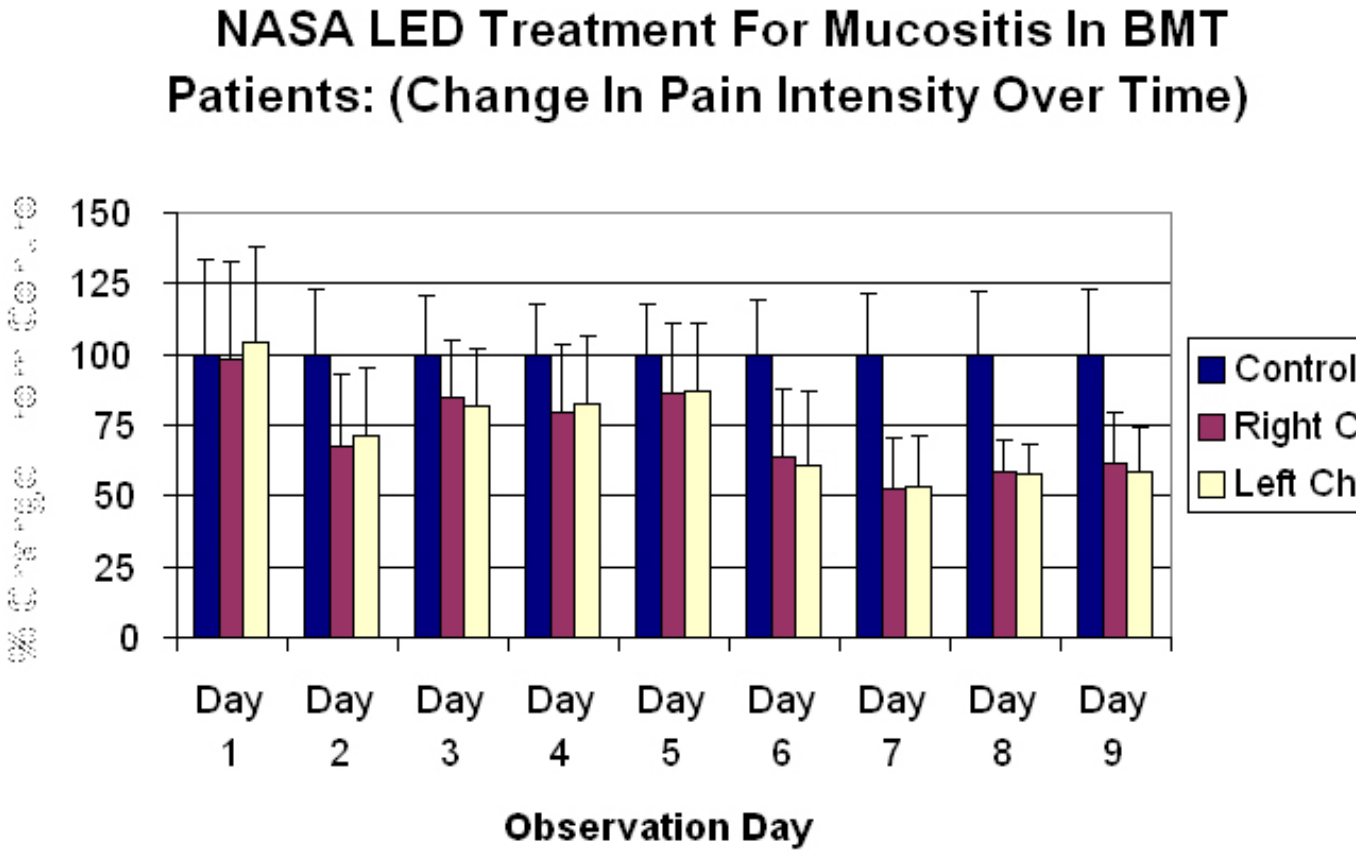


Figure 12. Decrease in pain intensity over time after daily treatment with NASA LED light at 670nm, 4J/cm2, 50mW/cm2.

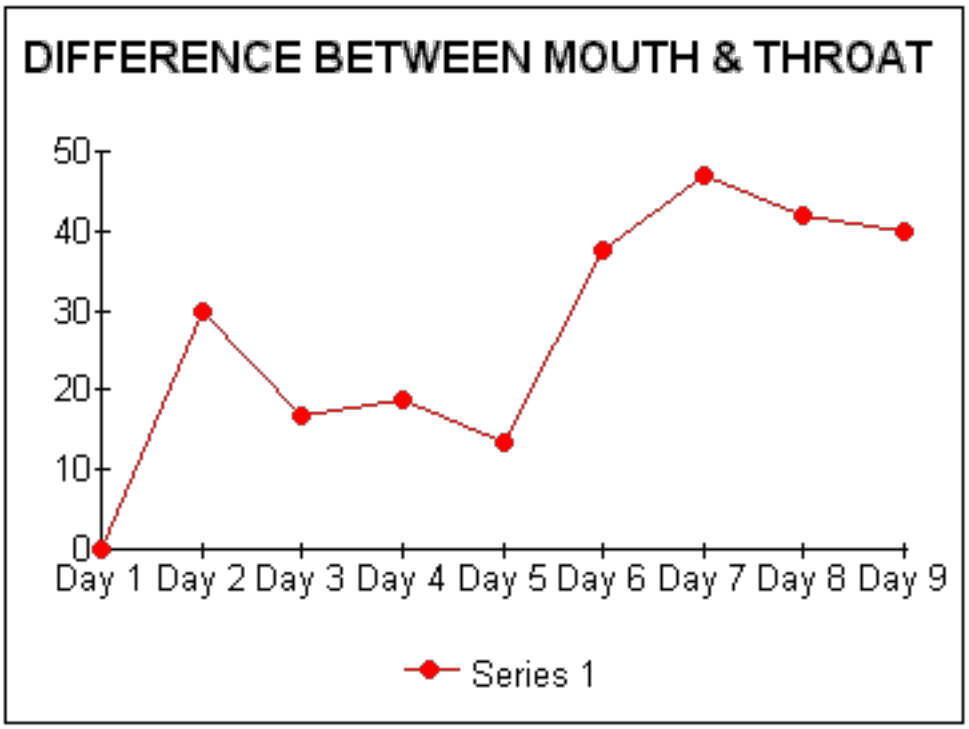


Figure 13. The difference between LED treated (mouth) and untreated control (throat) becomes more dramatic over time, with daily treatment using NASA LED light at 670nm, 4J/cm2, 50mW/cm2.

Additionally, we are determining extent of ulceration, healing rate in mm2/day, and healing time in days for these patients, and we will compare these values with epidemiological control data. A chart review is also in progress to assess morphine pump use and requirement for intravenous feedings in LED treated patients compared to controls. Contact with the FDA`s Richard Felten of the General Surgery Devices Branch has produced an avenue for guidance to final data collection and FDA-approval of this technology as the standard-of-therapy for treatment of mucositis. FDA review of our current data and protocol design is ongoing, and has already led to an FDA recommendation for expanding our study to include at least 3-more academic medical centers, in addition to our own, to be supplied with NASA LED arrays by Quantum Devices, Inc. A multi-site trial is being planned through the International Bone Marrow Transplant Registry.

Research Collaboration

We are now investigating new collaborations with the Defense Advanced Research Projects Agency (DARPA) for further military applications of NASA LED wound healing technology in military medicine. Several uniquely military situations and indications could be addressed in the new collaboration. These include burns, injuries from chemical agents, radiation, highly infected wounds (which are typical for the hygienic conditions occurring in battlefields), infectious diseases, and external wounds occurring in environments with no solar irradiation, low oxygen and high carbon dioxide (submarines and space environments). The dramatic results with use of near-infrared NASA LED light to prevent digestive mucosal lesions (mucositis) and pain in cancer patients, after high-dose chemotherapy and radiation, suggest the potential for military use of near-infrared light to treat U.S. troops exposed to chemical and radioactive warfare agents in the field (Karu, 1994). These life-saving applications require especially accelerated wound healing, rapid reduction of infections, and pain modulation.

ACKNOWLEDGMENTS
We wish to thank Karen Zeqiri for assistance in manuscript preparation. The LED arrays were provided by Quantum Devices, Inc., Barneveld, WI. We also gratefully acknowledge the Department of Defense, Air Force Material Command, Armstrong Laboratories, Davis Hyperbaric Laboratory, Brooks Air Force Base, TX for providing the hyperbaric chamber used in this research. The hyperbaric oxygen treatments of our human subjects were performed by Estelle Woodard, C.R.T., C.H.T. This work was supported by the National Aeronautics and Space Administration, Marshall Space Flight Center SBIR grants: NAS8-99015 and NAS8-97277, Children's Hospital Foundation, the MACC Fund, and Quantum Devices, Inc.

REFERENCES
Abergel R.P., Lyons R.F., Castel J.C., Dwyer R.M., Uitto J. "Biostimulation of wound healing by lasers: Experimental approaches in animal models and in fibroblast cultures," J. Dermatol. Surg. Oncol. 13(2), 127-133 (1987).
Al-Watban F.A. "Laser Acceleration of Open Skin Wound Closure in Rats and its Dosimetric Dependence," Lasers in the Life Sciences. 7(4), 237-47 (1997).
Al-Watban F.A., Zhang X.Y. "Comparison of Wound Healing Process Using Argon and Krypton Lasers," Biochemica et Biophysica Acta. 1091(2), 140-4 (1991).
Barasch A., Peterson D.E., Tanzer J.M., D`Ambrosio J.A., Nuki K., Schubert M.M., Franquin J.C., Clive J., Tutschka P. "Helium-Neon Laser Effects on Conditioning-Induced Oral Mucositis in Bone Marrow Transplantation Patients," Cancer. 76, 2550-2556 (1995).
Beauvoit B., Evans S.M., Jenkins T.W., Miller E.E., Chance B. "Contribution of the Mitochondrial Compartment to the Optical Properties of the Rat Liver: A Theoretical and Practical Approach," Analytical Biochemistry. 226, 167-174 (1995).
Beauvoit B., Kitai T., Chance B. "Correlation Between the Light Scattering and the Mitochondrial Content of Normal Tissues and Transplantable Rodent Tumors," Biophysical Journal. 67, 2501-25 10 (1994).
Bibikova A., Oron, U. "Regeneration in Denervated Toad (Bufo viridis) Gastrocnemius Muscle and the Promotion of the Process by Low Energy Laser Irradiation," The Anatomical Record. 241, 123-128 (1995).
Chance B., Nioka S., Kent J., McCully K., Fountain M., Greenfield R., Holtom G., "Time-Resolved Spectroscopy of Hemoglobin and Myoglobin in Resting and Ischemic Muscle," Analytical Biochemistry. 174, 698-707 (1988).
Conlan M.J., Rapley J.W., Cobb C.M. "Biostimulation of Wound Healing by Low-Energy Laser Irradiation," J. Clin. Periodont. 23, 492-496 (1996).
Cowen D., Tardieu C., Schubert M., Peterson D., Resbeut M., Faucher, C., Franquin J.C. "Low Energy Helium-Neon Laser in the Prevention of Oral Mucositis in Patients Undergoing Bone Marrow Transplant: Results of a Double Blind Random Trail," Int. J. Radiation Oncology Biol. Phys. 38(4), 697-703 (1997).
Eggert H.R., Blazek V. "Optical Properties of Normal Human Brain Tissues In The Spectral Range of 400 to 2500 nm," Advances in Experimental Medicine & Biology. 333, 47-55 (1993).
Hartmann KM., Hoppe W., Lohmann W., Marke H., Ziegler H. "Action spectroscopy," Biophysics. 115-131
(1983).
Karu T. "Photobiology of low-power laser effects," Health Physics. 56(5), 691-704 (1989).
Karu T. "Photochemical Effects Upon the Cornea, Skin and Other Tissues (Photobiology Of Low-Power Laser Effects)," Health Physics. 56, 69 l-704 (1989).
Karu T.I., Pyatibrat L., Kalendo G. "Irradiation with HeNe Laser can Influence the Cytotoxic Response of HeLa cells to Ionizing Radiation," International Journal of Radiation Biology. 65(6), 691-7 (1994).
Lubart R., Friedman H., Sinyakov M., Cohen N., Breitbart H. "Changes in Calcium Transport in Mammalian Sperm Mitochondria and Plasma Membranes Caused by 780 nm Irradiation," Lasers in Surg & Med. 21, 493-499 (1997).
Lubart R., Wollman Y., Friedman H., Rochkind S., Laulicht L. " Effects of Visible and Near-Infrared Lasers on Cell Cultures," Journal of Photochemistry & Photobiology. 12(3), 305-310 (1992).
Mester A.F., Nagylucskay S., Mako E., Hoffmann G., Serenyi M. "Experimental Imunological Study with Radiological Application of Low Power Laser," Laser in Medicine. 509-512 (1998).
Mester E.., Jaszsagi-Nagy E. "The Effects of Laser Radiation on Wound Healing and Collagen Synthesis," Studia Biophysica. Band 35, Heft 3, 227-230 (1973).
Mester E., Nagylucskay s., Triza S., Mester A. "Stimulation of Wound Healing by Means of Laser Rays,"

Acta Chirurgica Academiae Scientiarum Hungaricae. 19(2), 163-70 (1978).
Mester E., Spivy T., Szende B., Tota J.G. "Effect of laser rays on wound healing," Am. J. Surg. 122, 532-535
(1971).
Miller M., Truhe T. "Lasers in dentistry: An overview," Journal of the ADA. 124, 32-35 (1993).
Peterkofsky, Diegelmann. Biochemistry. 10, 988-94 (1971).
Salansky N. "Low Energy Photon Therapy for Wound Healing," Intnl Med Instr, Canadian Defense Ministry,
Personal Communication. (1998).
Schmidt M.H., Bajic D.M., Reichert K.W. II, Martin T.S., Meyer G.A., Whelan H.T. "Light-Emitting Diodes as a Light Source for Intra-Operative Photodynamic Therapy," Neurosurgery. 38(3), 552-556 (1996).
Schmidt M.H., Reichert K.W. II, Ozker K., Meyer G.A., Donohoe D.L., Bajic D.M., Whelan N.T., Whelan H.T., "Preclinical Evaluation of Benzoporphyrin Derivative Combined with a Light-Emitting Diode Array for Photodynamic Therapy of Brain Tumors," Pediatr Neurosurg. 30, 225-231 (1999).
Schubert M.M., Williams B.E., Lloid M.E., Donaldson G., Chapko M.K., "Clinical Assessment Scale for the Rating of Oral Mucosal Changes Associated With Bone Marrow Transplantation; Development of an Oral Mucositis Index," Cancer. 69, 2469-2477 (1992).
Sommer A.P., Pinheiro A.L.B., Mester A.R., Franke R.P., Whelan H.T. "BiostimulatoryWindows in Low Intensity
Laser Activation: Lasers, Scanners and NASA`s LightEmitting Diode Array System," Journal of Clinical Laser
Medicine and Surgery. 19:29-34 (2001).
Whelan H.T., Schmidt M.H., Segura A.D., McAuliffe T.L., Bajic D.M., Murray K.J., Moulder J.E., Strother D.R.,
Thomas J.P., Meyer G.A. "The Role of Photodynamic Therapy in Posterior Fossa Brain Tumors: A Pre-Clinical
Study in a Canine Glioma Model," Journal of Neurosurgery. 79(4), 562-8 (1993).
Whelan H.T., Houle J.M., Donohoe D.L., Bajic D.M., Schmidt M.H., Reichert K.W., Weyenberg G.T., Larson D.L., Meyer G.A., Caviness J.A. "Medical Applications of Space Light-Emitting Diode Technology-Space Station and Beyond," Space Tech. & App. Int'l. Forum. 458, 3-15 (1999).
Whelan H.T., Houle J.M., Whelan N.T., Donahue, D.L., Cwiklinski J., Schmidt M.H., Gould L., Larson D.L., Meyer G.A., Cevenini V., Stinso H. "The NASA Light-Emitting Diode Medical Program- Progress in Space Flight and Terrestrial Applications," Space Tech. & App. Int'l. Forum. 504, 37-43 (2000).
Whelan H.T., Buchmann E.V., Whelan N.T., Turner S.G., Cevenini V., Stinson H., Ignatius R., Martin T., Cwiklinski J., Meyer G.A., Hodgson B., Gould L., Kane M., Chen G., Caviness J. "NASA Light-Emitting Diode Medical Applications From Deep Space to Deep Sea," Space Tech. & App. Int'l. Forum. 552, 35-45 (2001).
Yamada, Kunio. "Biological Effects of Low Power Laser Irradiation on Clonal Osteoblastic Cells (MC3T3-E1)," J.
Jpn. Orthop. Assoc. 65, 787-799 (1991).
Yu W., Naim J.O., Lanzafame R.J. "The Effect Of Laser Irradiation On The Release Of bFGF From 3T3 Fibroblasts," Photochemistry & Photobiology 59, 167-70 (1994).
Yu W., Naim J.O., Lanzafame R.J. "Effects of Photostimulation on Wound Healing in Diabetic Mice," Lasers Surg Med. 20, 56-63 (1997).
Yamada, Kunio. "Biological Effects of Low Power Laser Irradiation on Clonal Osteoblastic Cells (MC3T3-E1)," J. Jpn. Orthop. Assoc. 65, 787-799 (1991).