The NASA Light-Emitting Diode Medical Program - Progress in Space Flight and Terrestrial Applications

Harry T. Whelan, M.D.[1a,2,3], John M. Houle, B.S.[1a],
Noel T. Whelan[1a,3], Deborah L. Donohoe, A.S., L.A.T.G.[1a],
Joan Cwiklinski, M.S.N., C.P.N.P.[1a], Meic H. Schmidt, M.D.[1c],
Lisa Gould, M.D., Ph.D. [1b] , David L. Larson, M.D.[1b],
Glenn A. Meyer, M.D.[1a], Vita Cevenini[3], Helen Stinson, B.S.[3]

[1a]Departments of Neurology, [1b]Plastic Surgery and [1c]Neurosurgery,
Medical College of Wisconsin, Milwaukee, WI 53226, (414) 456-4090
[2]Naval Special Warfare Group TWO, Norfolk, VA 23521, (757) 462-7759
[3]NASA-Marshall Space Flight Center, AL 35812, (256) 544-2121

Abstract. This work is supported and managed through the NASA Marshall Space Flight Center - SBIR Program. Studies on cells exposed to microgravity and hypergravity indicate that human cells need gravity to stimulate cell growth. As the gravitational force increases or decreases, the cell function responds in a linear fashion. This poses significant health risks for astronauts in long termspace flight. LED-technology developed for NASA plant growth experiments in space shows promise for delivering light deep into tissues of the body to promote wound healing and human tissue growth. This LED-technology is also biologically optomal for photodynamic therapy of cancer.

LED-ENHANCEMENT OF CELL GROWTH

The application of light therapy with the use of NASA LED's will significantly improve the medical care that is available to astronauts on long-term space missions. NASA LED's stimulate the basic energy processes in the mitochondria (energy compartments) of each cell, particularly when near-infrared light is used to activate the color sensitive chemicals (chromophores, cytochrome systems) inside. Optimal LED wavelengths include 680, 730 and 880 nm. The depth of near-infrared light penetration into human tissue has been measured spectroscopically (Chance, et al 1988). Spectra taken from the wrist flexor muscles in the forearm and muscles in the calf of the leg demonstrate that most of the light photons at wavelengths between 630-800 nm travel 23 cm through the surface tissue and muscle between input and exit at the photon detector. Our laboratory has improved the healing of wounds in laboratory animals by using NASA LED light and hyperbaric oxygen. Furthermore, DNA synthesis in fibroblasts and muscle cells has been quintupled using NASA LED light alone, in a single application combining 680, 730 and 880 nm each at 4 Joules per centimeter squared.

Muscle and bone atrophy are well documented in astronauts, and various minor injuries occuring in space have been reported not to heal until landing on Earth. Long term space flight, with its many inherent risks, also raises the possibility of astronauts being injured performing their required tasks. The fact that the normal healing process is negatively affected by microgravity requires novel approaches to improve wound healing and tissue growth in space. NASA LED arrays have already flown on Space Shuttle missions for studies of plant growth. The U.S. Food and Drug Administration (FDA) has approved human trials. The use of light therapy with LED's is an approach to help increase the rate of wound healing in the microgravity environment, reducing the risk of treatable injuries becoming mission catastrophes.

Wounds heal less effectively in space than here on Earth. Improved wound healing may have multiple applications which benefit civilian medical care, military situations and long-term space flight. Laser light and hyperbaric

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oxygen have been widely acclaimed to speed wound healing in ischemic, hypoxic wounds. An excellent review of recent human experience with near-infrared light therapy for wound healing was published by Conlan, et al in 1996. Lasers provide low energy stimulation of tissues which results in increased cellular activity during wound healing (Beauvoit, 1989, 1995; Eggert, 1993; Karu, 1989; Lubart, 1992, 1997; Salansky, 1998; Whelan, 1999; Yu, 1997). Some of these activities include increased fibroblast proliferation, growth factor synthesis, collagen production and angiogenesis. Lasers, however, have some inherent characteristics which make their use in a clinical setting problematic, including limitations in wavelength capabilities and beam width. The combined wavelengths of light optimal for wound healing cannot be efficiently produced, and the size of wounds which may be treated by lasers is limited. Light-emitting diodes (LED's) offer an effective alternative to lasers. These diodes can be made to produce multiple wavelengths, and can be arranged in large, flat arrays allowing treatment of large wounds. Our experiments suggest potential for using LED light therapy at 680, 730 and 880 nm simultaneously, alone and in combination with hyperbaric oxygen therapy, both alone and in combination, to accelerate the healing process in Space Station missions, where prolonged exposure to microgravity may otherwise retard healing. NASA LED's have proven to stimulate wound healing at near-infrared wavelengths of 680, 730 and 880 nm in laboratory animals, and have been approved by the U.S. Food and Drug Administration (FDA) for human trials. Furthermore, near-infrared LED light has quintupled the growth of fibroblasts and muscle cells in tissue culture. The NASA LED arrays are light enough and mobile enough to have already flown on the Space Shuttle numerous times. LED arrays may prove to be useful for improving wound healing and treating problem wounds, as well as speeding the return of deconditioned personnel to full duty performance. Potential benefits to NASA, military, and civilian populations include treatment of serious burns, crush injuries, non-healing fractures, muscle and bone atrophy, traumatic ischemic wounds, radiation tissue damage, compromised skin grafts, and tissue regeneration.

LED-PHOTODYNAMIC THERAPY FOR CANCER

Photodynamic therapy (PDT) is a cancer treatment modality that recently has been applied as adjuvant therapy for brain tumors. PDT consists of intravenously injecting a photosensitizer, which preferentially accumulates in tumor cells, into a patient and then activating the photosensitizer with a light source. This results in free radical generation followed by cell death. The development of more effective light sources for PDT of brain tumors has been facilitated by applications of space light-emitting diode array technology; thus permitting deeper tumor penetration of light and use of better photosensitizers. Lutetium Texaphyrin (Lutex) and Benzoporphyrin Derivative (BPD) are new, second generation photosensitizers that can potentially imrove PDT for brain tumors. Lutex and BPD have major absorption peaks at 730 nm and 680 nm respectively, which gives them two distinct advantages. First, longer wavelengths of light penetrate brain tissue easily so that larger tumors could be treated; and second, the major absorption peaks mean that more of the drug is activated upon exposure to light. Tumoricidal effects of Lutex and BPD have been studied *in vitro* using canine glioma and human glioblastoma cell cultures. Using light-emitting diodes (LED) with peak emissions of 728 nm and 680 nm as a light source, a greater then 50 percent cell kill was measured in both cell lines by tumor DNA synthesis reduction. The effectiveness of Lutex and BPD against tumor cells in vitro thus established, we have taken the first step toward determining their *in vivo* efficacy by performing experiments to determine the largest doses of both Lutex, or BPD, and light that can be administered to dogs before toxicity is seen, i.e. the maximum tolerated dose (MTD). Using this dose allows us to effect maximum tumor cell destruction during in vivo studies.

For longer wavelengths of light, the improved NASA LED-technology is required. LED's are an effective alternative to lasers for PDT. Laser conversion to near-infrared wavelengths is inherently costly and inefficient, using an argon ion or KTP/YAG laser beam that is converted by a dye module, usually to 630 nm. LED's have been frequently used to emit longer wavelength broad spectrum near-infrared light of 25-30 nm bandwidths. LED lamps traditionally consist of an array of semiconducting LED chips. In recent years, improvements in semiconductor technology have substantially increased the light output of LED chips. A novel type of LED chip is based on the semiconductor Aluminum Gallium Arsenide (AlGaAs). These LED chips have been manufactured to emit light with peak wavelengths of 680 and 730 nm, which are optimal wavelengths for the absorption spectrum of the new photosensitizers used for cancer PDT.

Human trials have begun at the Medical College of Wisconsin, Naval Special Warfare Command and NASA-Marshall Space Flight Center.

Photodynamic Therapy with NASA LED Human Subjects

Preclinical studies of LED-photodynamic therapy were reported previously (Whelan, 1993, 1999; Schmidt, 1996, 1999).

The first patient treated with the NASA LED probe in our Photodynamic Therapy Phase II study, is a 20 year old female who underwent PDT on May 4, 1999 for a brain tumor (anaplastic ependynoma). She had had two previous PDT treatments with **laser** light in another area of the brain. The most recent tumor recurrence formed in a different location of the brain than the previous two sites and is thought to be the result of the laser light not being able to penetrate the tissue deep enough to prevent recurrence. As is evidenced by the graph below (Figure 1), LED and photofrin together allow for deeper penetration of tissue, thereby exposing surrounding tissue which may contain stray cancer cells to the LED light.

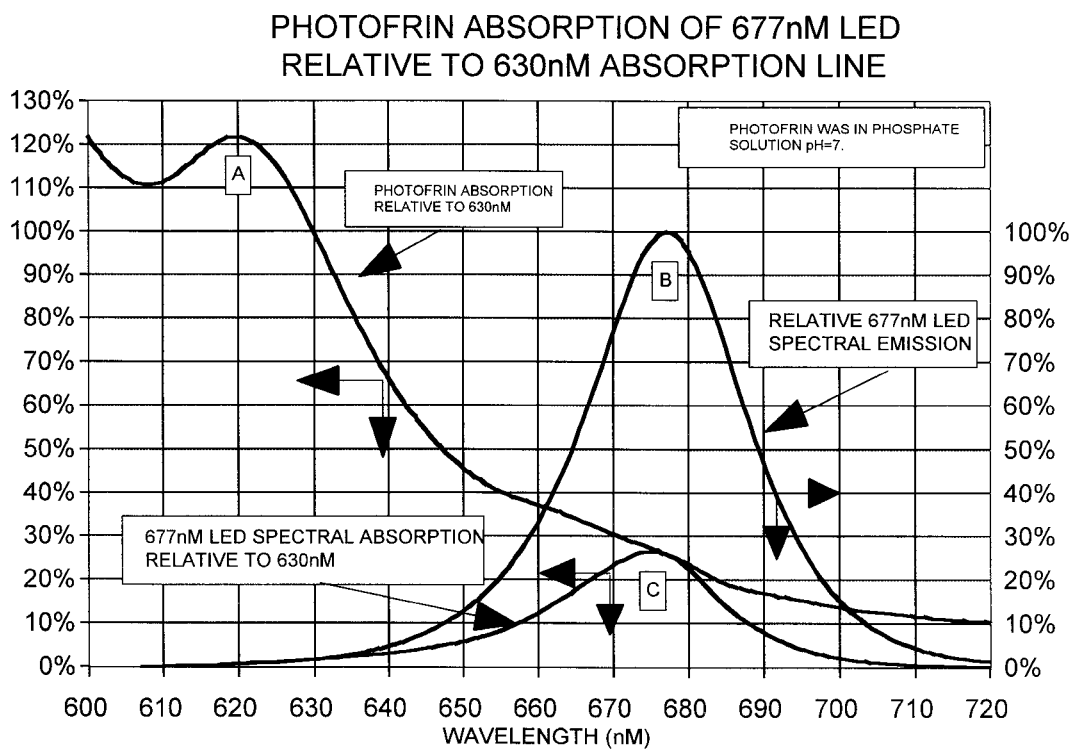


FIGURE 1. Spectral comparison of LED and Photofrin. Photofrin absorption of 677-nm LED relative to 630-nm absorption line. *A*, Photofrin absorption curve relative to 630 nm. *B*, spectral emission of the LED. *C*, integration of the LED spectral emission and the Photofrin absorption.

Post surgically, she experienced some edema as is expected from brain surgery, and was treated successfully, and discharged on May 14, 1999. Follow-up MRI scans do not demonstrate tumor growth. A subsequent 21 year-old male with brain tumor (glioblastoma) was treated August 25, 1999, with LED-photodynamic therapy, he tolerated the procedure well and shows no evidence residual tumor on subsequent neuro imaging.

WOUND HEALING WITH NASA LED

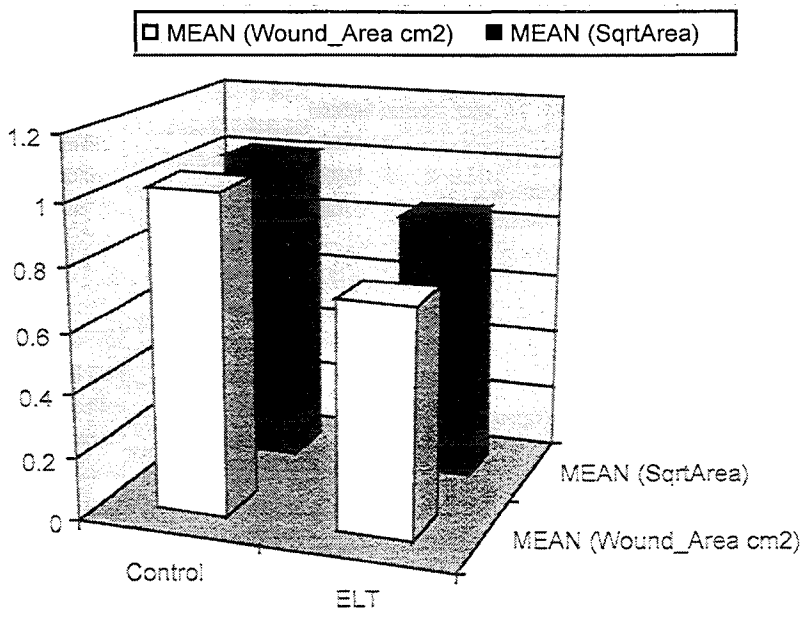
Preclinical LED-Wound Healing studies were reported previously (Whelan, 1999).

LED-Diabetic Mice

Type II, Diabetic Mice with excisional skin wounds were treated with LED's at 680, 730, and 880 nm, 4J/cm^2. LED treatment produced increased healing rates, compared to surgical controls. Refer to table of diabetic mouse data using 4 J/cm^2 of energy (Figure 2).

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 2). This analysis was carried out using the SAS statistical software package, published by The SAS Institute, Inc.

The group means are as follows:



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

FIGURE 2. LED-Treatment Wound Area-vs-Control.

Variables

Wound_Area	The size of the wound, in cm^2.
SqrtArea	The square root of Wound_Area, this 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.
ELT	LED External/Light Therapy

LED-Human Subjects

Pt. #1 is a 31 year-old white female insulin dependent diabetic (type 1) with end-stage renal disease status post renal transplant failure and bilateral below knee amputations. Her left amputation site has poor healing compared to the right. She was receiving Hyperbaric Oxygen therapy because of bilateral arterial insufficiency ulcers on her hands. In January of 1999 she had a hangnail on her right third finger and sores developed at the tip. Multiple fissures and sores on both hands further occurred. Sores covered with black eschar developed and her hands are dry with very limited range of motion. She has similar processes on both hands and had similar processes developing on her feet prior to undergoing bilateral below the knee amputations. At that time she complained of cold-induced cyanosis of the fingers and has been developing fissuring of the skin on her hands and some small spots on her fingers. She is a nonsmoker and has been on dialysis for her renal failure. The renal failure and the arterial insufficiency ulcers of her extremities have been felt to be due to vasculitis. She does not use alcohol and has no drug allergies. She had renal transplant for renal failure in 1996 with removal of the transplanted kidney in 1998. She has received Prednisone and Cytoxan for her vasculitis and she also takes Synthroid for hypothyroidism. She has had problems in the past with gastroparesis and had a temporary percutaneous endoscopic gastrostomy tube in place, but has never had a diagnosis of gastroesophageal dysmotility. She has no history of calcinosis and she is anuric because of renal failure. A renal biopsy performed December 5, 1997 of her transplanted kidney showed "focal necrotizing glomerular nephritis, no evidence of acute rejection, no eveidence of recurrent diabetic nephopathy". She received 20 treatments of 8 J/cm^2 NASA LED light therapy to all surfaces of both hands. Prior to starting treatment her hands were cold and dusky, lacking sensation. They had many eschar covered ulcerations. After light treatment we noted an improvement in color and hands were warm to the touch. Her complaints of pain and itching indicated a return of sensation.

PT #2 is a 76 year-old white male, borderline diabetic with two open sores on his right medial malleolus. These wounds were traumatic, caused when he was wounded by mortar fire during World War II in the Battle of the Bulge. He has had open sores in that area since that time. These wounds have required two split thickness grafts. One was performed in 1945 and another in 1965. Both grafts have been of questionable success. Mr. Marek has been treated with 52 Hyperbaric Oxygen Treatments to date and has received 19 treatments of 8J/cm^2 of NASA LED therapy to his ankle wound. At the start of NASA LED treatment the area had opened into one large 2cm X 3cm wound draining sanguinous fluid. Since treatment we have seen an increase in tissue granulation from the inside towards the outer edges of the wound. Area has decreased to 1.5cm X 2.8cm and there is a significant decrease in drainage. Transcutaneous Oxygen Measurement (T.C.pO_2) readings have shown an improvement in vasculature to the area. (Figure 3)

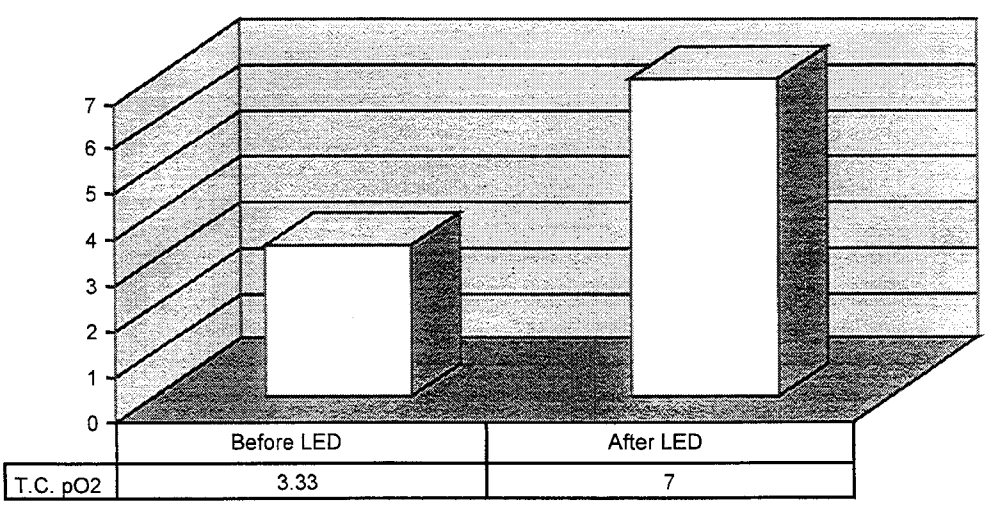


FIGURE 3. Effect of NASA LED Treatment of Ankle Wound Sustained in Combat During WW11 Battle of the Bulge at 8 Joules/cm^2 demonstrating improvement in T.C.pO_2 (y-axis).

Further In Vitro LED Cell Growth Studies

In-vitro studies continue with the 3T3 Fibroblasts, and L6 Rat Skeletal Muscle cell line. Stimulation with LED's clearly shows proliferation of both cell lines with the DNA synthesis assay. The first graph below shows the 3T3 Fibroblasts with stimulation of 4 J/cm^2, 8 J/cm^2 and 12 J/cm^2 of energy and proliferation of cells within 3 hours after exposure to LED (Figure 4). The second graph below depicts growth of the L6 Skeletal Muscle over a period of 48 hours after exposure to 4 J/cm^2 of energy (Figure 5). These data demonstrate *immediate* benefit of LED exposure in-vitro, as in the 3T3 Fibroblasts, but also *over a period of time*, over 48 hours with the L6 Muscle Cells.

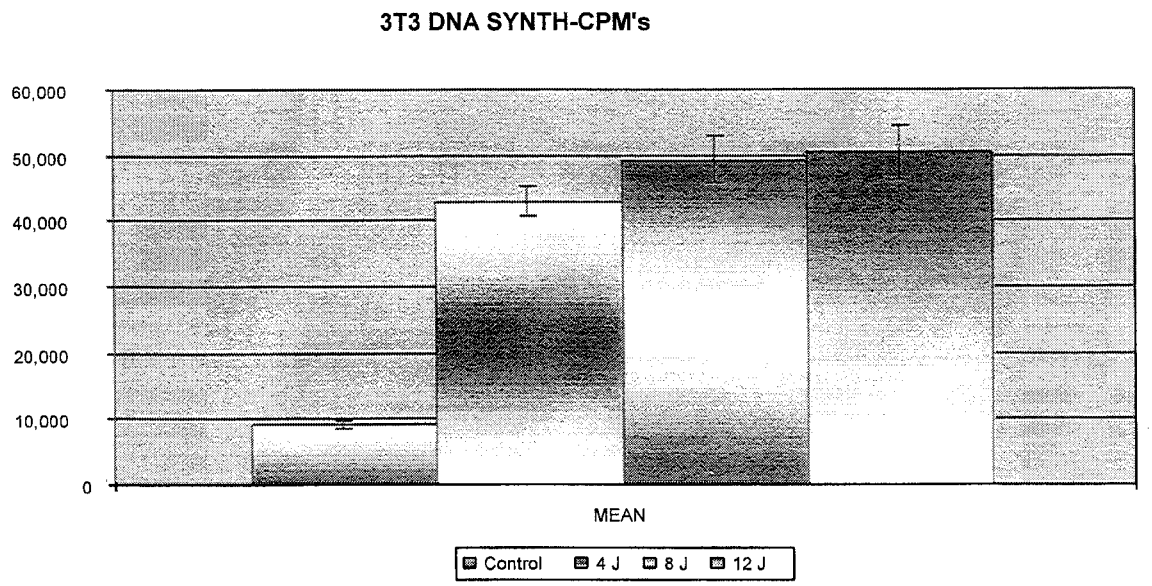


FIGURE 4. 3T3 Fibroblast DNA Synthesis 3 Hour Incubation - LED dose-response 4,8 & 12 J/cm^2.

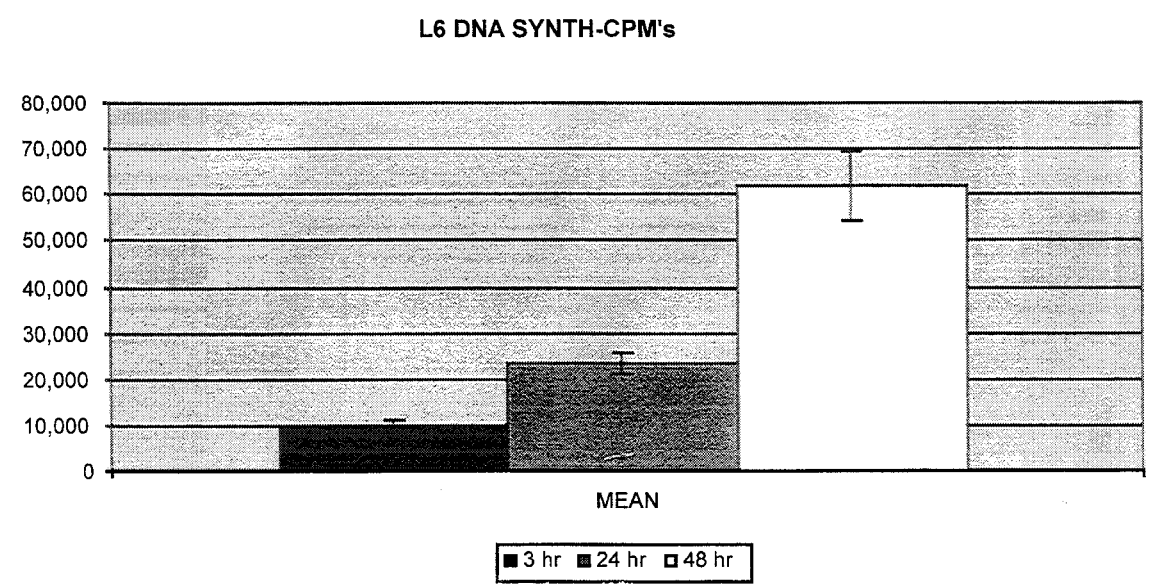


FIGURE 5. L6 Rat Skeletal Muscle - LED-treated at 680, 730 & 880 nm with 4 J/cm^2 each.

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