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Harry T. Whelan
Medical College of Wisconsin

Ellen V. Buchmann
Medical College of Wisconsin

Noel T. Whelan
Medical College of Wisconsin

Scott G. Turner
Medical College of Wisconsin

Vita Cevenini
NASA - Marshall Space Flight Center

See next page for additional authors

Authors

Harry T. Whelan, Ellen V. Buchmann, Noel T. Whelan, Scott G. Turner, Vita Cevenini, Helen Stinson, Ron Ignatius, Todd Martin, Joan Cwiklinski, Glenn A. Meyer, Brian D. Hodgson, Lisa J. Gould, Mary P. Kane, Gina Chen, and James Caviness

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Harry T. Whelan^{1a,5,7}, Ellen V. Buchmann^{1a},
Noel T. Whelan^{1a,7}, Scott G. Turner^{1a},
Vita Cevenini⁷, Helen Stinson⁷, Ron Ignatius², Todd Martin²,
Joan Cwiklinski^{1a}, Glenn A. Meyer^{1c}, Brian Hodgson^{3,4}, Lisa Gould^{1b},
Mary Kane^{1b}, Gina Chen^{1b}, James Caviness⁶

^{1a}Departments of Neurology, ^{1b}Plastic Surgery, ^{1c}Neurosurgery,
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. This work is supported and managed through the NASA Marshall Space Flight Center - SBIR Program. 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. We present the results of LED-treatment of cells grown in culture and the effects of LEDs on patients' chronic and acute wounds. LED-technology is also biologically optimal for photodynamic therapy of cancer and we discuss our successes using LEDs in conjunction with light-activated chemotherapeutic drugs.

LED-ENHANCEMENT OF CELL GROWTH

Studies on cells exposed to microgravity and hypergravity indicate that human cells need gravity to stimulate 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-term space flight. The application of light therapy with the use of NASA LEDs will significantly improve the medical care that is available to astronauts on long-term space missions. 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 color sensitive chemicals (chromophores, cytochrome systems) inside. Optimal LED wavelengths include 680, 730 and 880 nm and our laboratory has improved the healing of wounds in laboratory animals by using both 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 occurring in space have been reported not to heal until landing on Earth. An LED blanket device may be used for the prevention of bone and muscle atrophy in astronauts. 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. The light is absorbed by mitochondria where it stimulates energy metabolism in muscle and bone, as well as skin and subcutaneous tissue.

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 and the U.S. Food and Drug Administration (FDA) has approved human trials. The use of light therapy with LEDs can help prevent bone and muscle atrophy as well as increase the rate of wound healing in a microgravity environment, thus reducing the risk of treatable injuries becoming mission catastrophes.

Space flight has provided a laboratory for studying wound healing problems due to microgravity, which mimic traumatic wound healing problems here on earth. Improved wound healing may have multiple applications that benefit civilian medical care, military situations and long-term space flight. Laser light and hyperbaric 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 (Conlan, 1996). Lasers provide low energy stimulation of tissues which results in increased cellular activity during wound healing (Beauvoit, 1994, 1995; Eggert, 1993; Karu, 1989; Lubart, 1992, 1997; Salansky, 1998; Whelan, 1999; Yu, 1997) including increased fibroblast proliferation, growth factor synthesis, collagen production and angiogenesis. Lasers, however, have some inherent characteristics that make their use in a clinical setting problematic, such as 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 that may be treated by lasers is limited. Light-emitting diodes (LEDs) 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. 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.

Combat casualty care in Special Operations already have adopted the NASA LED technology for submarines deployed in training with risk of injury. The USS Salt Lake City is currently underway with an LED Array in the Pacific. 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. NASA LEDs 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. The NASA LED arrays are light enough and mobile enough to have already flown on the Space Shuttle numerous times. LED arrays may be used for improved wound healing and treatment of problem wounds as well as speeding the return of deconditioned personnel to full duty performance. Examples include: 1. Promotion of the rate of muscle regeneration after confinement or surgery. 2. Personnel spending long periods of time aboard submarines may use LED arrays to combat muscle atrophy during relative inactivity. 3. LED arrays may be introduced early to speed wound healing in the field. Human trials have begun at the Medical College of Wisconsin, Naval Special Warfare Command, Submarine Squadron ELEVEN and NASA-Marshall Space Flight Center.

LED-PHOTODYNAMIC THERAPY FOR CANCER

Astronauts in deep space are subjected to increased-levels of radiation, compared to low-earth orbit environments. Cancer protection strategies are therefore a subject of our NASA-LED photodynamic therapy program. Photodynamic therapy (PDT) is a cancer treatment modality that recently has been improved using LED space technology. 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. 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.

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 improve 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. In deep space, the LED-PDT technology may be capable of early cancer surveillance and treatment before radiation-induced tumors can develop in astronauts.

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 than 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.

Photodynamic Therapy with NASA LED Human Subjects

Preclinical and clinical studies of LED-photodynamic therapy were reported previously (Whelan, et al., 1993, 1999, 2000; Schmidt, 1996, 1999), and continue under our FDA approval.

Improvements Realized from LED Arrays

Red laser light is frequently produced using an argon ion or KTP/YAG laser beam that is converted by a dye module, usually to 630 nm. For longer wavelengths of light improved technology is required. Laser conversion to near-infrared wavelengths is inherently costly and inefficient, but allows for light to be delivered by fiberoptics. For non-fiber optic delivery of light, LEDs offer an alternative. LEDs have been frequently used to emit low power, broad spectrum light of 25-30 nm bandwidths in an array of semiconductor chips. In recent years, improvements in semiconductor technology have substantially increased the light output of LED chips. Dr. Whelan's laboratory has already used LED array light successfully in cancer treatment (Whelan, et al., 2000), with Photofrin, and has published animal data with Photofrin, Benzoporphyrin Derivative (BPD) and lutetium texaphyrin (Schmidt, et al., 1996, 1999; Whelan, et al., 2000). Resulting human skin cancer treatment trials with LED-based PDT using BPD have occurred, and FDA approval for use of LED-based PDT in children and adults with brain tumors has prompted further human studies. BPD has a spectral photoactivation/absorption peak of 680 nm and lutetium texaphyrin has an absorption peak at 730 nm, both involving high, long-lived quantum yields for triplet states that produce singlet oxygen cytotoxic to cancer cells.

Currently, PDT with Photofrin has become standard therapy for lung cancer (superficial microinfiltrating, palliative endobronchial) and advanced esophageal cancer (adenocarcinoma, squamous cell carcinoma). Furthermore, balloon adapters developed by Quantum Devices and tested in Dr. Whelan's laboratory and in his brain tumor patients show promise for adaptation to esophageal use to flatten out the folds of tissue lining, in which cancer cells could otherwise hide from light. Barrett's esophagus investigators at University of Tennessee (Knoxville) have already displayed an interest in this LED balloon adaptor application. LED arrays, and LED probes for skin cancer, psoriasis and rheumatoid arthritis treatment using BPD, and advances of the other cancer PDT regimens to use of the newer photosensitizers all promise to launch the NASA LED Medical Program to full PDT commercialization rapidly, with the test-site strategy described.

WOUND HEALING WITH NASA LED

Experiments using an ischemia animal model system provide pre-clinical data relevant to human healing problems, chronic non-healing wounds.

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, we still have very little understanding of what constitutes a chronic wound, particularly at the molecular level, and have 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 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 1 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 showed to speed 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 1 at day 14 when the points are converging due to the fact that the wounds are healing.

Analysis of the biochemical makeup of the wounds at days 4, 7, and 14 is currently underway. 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 2. 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.

The synergistic effects of HBO and LED (880 nm) can be seen easily in Figure 3. 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 (880) 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.

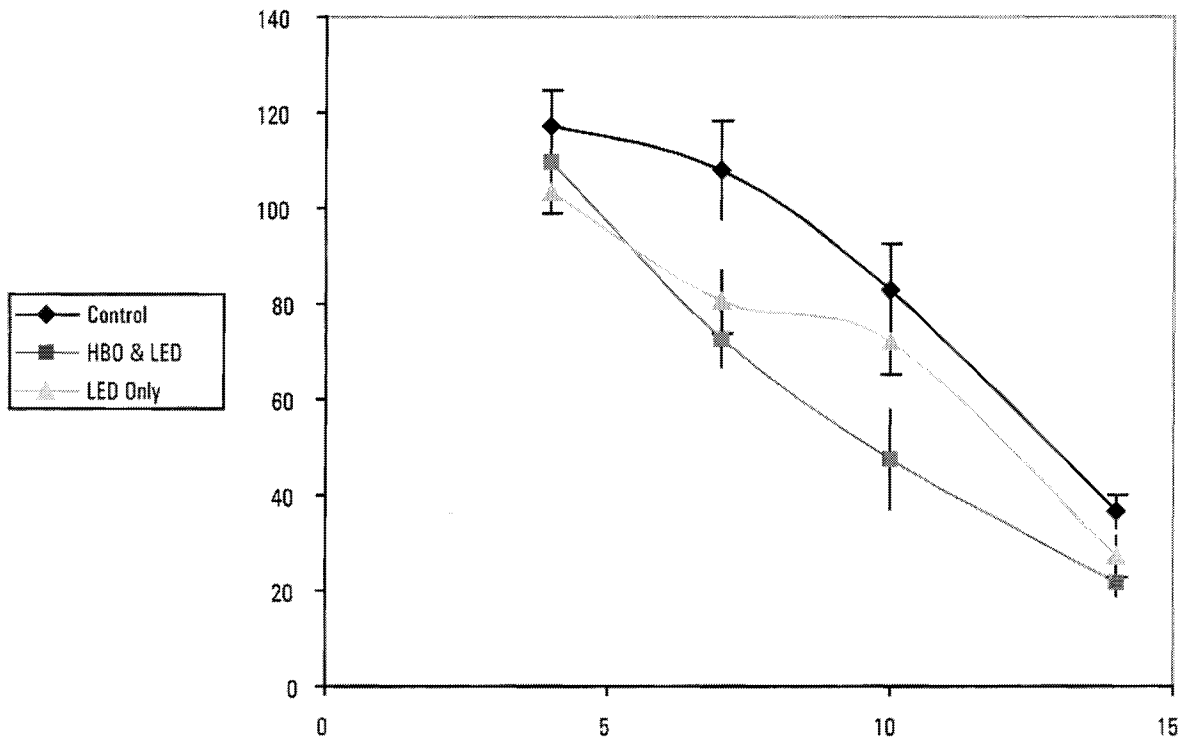


FIGURE 1. Change in wound size (%) in rat ischemic wound model Time (Days).

LED-Wound Healing in Human Subjects

Preclinical and clinical LED-Wound Healing studies were reported previously (Whelan et al., 1999, 2000); and additional human trials are summarized below:

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. An LED array with 3 wavelengths combined in a single unit (670, 720, 880 nm) was delivered to Naval Special Warfare Group-2 in Norfolk and a data collection system has been implemented for musculoskeletal training injuries treated with NASA 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). Data have also been received from Naval Special Warfare Command (Norfolk & San Diego) where 18-20 patients per day are being treated with NASA-LEDs and results indicate >40% improvement in musculoskeletal training injuries. Data has also been received from the USS Salt Lake City (submarine SSN 716 on Pacific deployment) reporting 50% faster (7 day) healing of lacerations in crew members compared to untreated control healing (approximately 14 days).

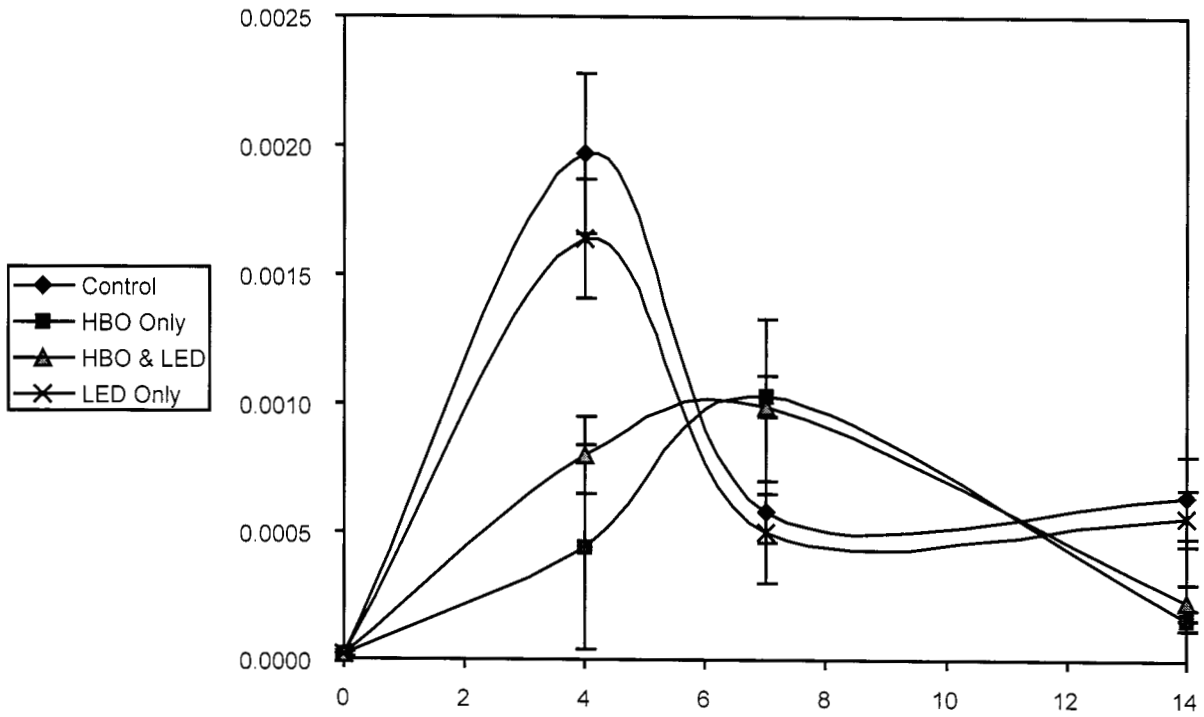


FIGURE 2. Change in vascular endothelial growth factor (VEGF) concentration ($\mu\text{g}/\text{mg}$ Protein) vs. Time (Day) in rat ischemic wound model.

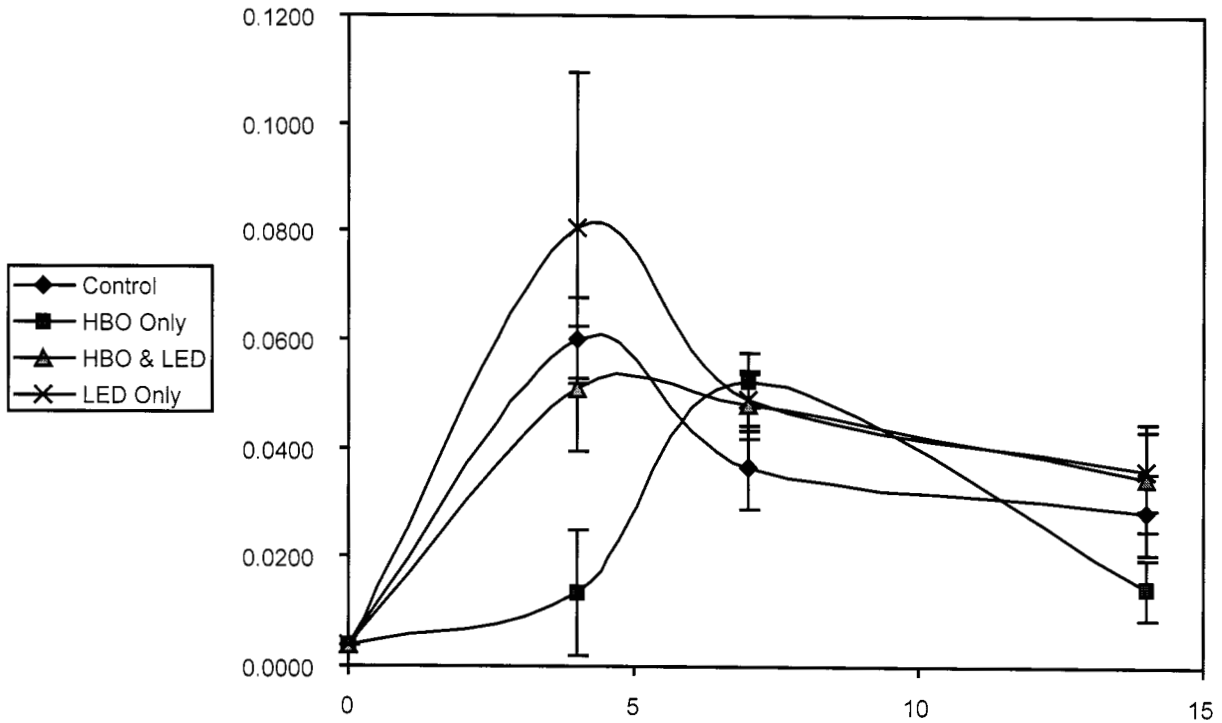


FIGURE 3. Change in basic fibroblast growth factor (FGF-2) concentration ($\mu\text{g}/\text{mg}$ Protein) vs. Time (Day) in rat ischemic wound model.

In addition to ischemic and chronic wound healing, 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 chemo and radiation therapy in order to destroy their own, cancerous, bone marrow. Because many chemotherapeutic drugs as well as radiation therapy kill all rapidly dividing cells indiscriminately, the mucosal linings of the mouth and gastrointestinal tract are often damaged during the treatment. As a result of these GI effects, patients often develop ulcers in their mouths (oral mucositis), and suffer from nausea and diarrhea. Oral mucositis is a significant risk for this population as it can impair the ability to eat and drink, and poses a risk for infection in this immunocompromized patient. Because lasers have been shown to speed healing of oral mucositis (Barasch, et al., 1995), we have recently expanded the wound-healing abilities of NASA LEDs to include these oral lesions. Beginning on the day after the last dose of chemotherapy, we treat one side of the mouth with a 688nm LED at 4J/cm² daily until the lesions are healed. Dental clinicians monitor the rate of healing by using an Oral Mucositis Index (Schubert, et al., 1992) and a Visual Analog Scale to assess mouth pain. Although many BMT patients must receive intravenous feeding due to their oral mucositis, all of the patients we have treated with LEDs have been able to eat, drink, and talk. All have had nausea, diarrhea, and sore throats, indicating mucositis elsewhere in their GI tract, but their oral cavities have been markedly less affected by mucosal ulcers. This study has only included 10% of our target subject number (3/30), and the data so far is preliminary (figure1), but reports by the attending oncologists reveal that these patients have developed significantly less oral mucositis than was expected, especially Patient 2 who received Melphalan, which is notorious for causing severe mucositis. All patients have had Patient Controlled Analgesia (PCA) with morphine sulfate, but all have reported that it was not their mouths that caused them to activate it.

Patient #1

16 year old male with recurrent Hodgkin's Disease
Received radiation therapy and Cytosin, ARA-C prior to Bone Marrow Transplant
Began LED treatment for Mucositis on June 22, 2000
LED treatments ended on July 10, 2000 when patient was discharged from the hospital.
Tolerated oral feeds and reported infrequent use of analgesics for mouth pain.

Patient #2

16 year old male with AML (recurrent)
Received radiation therapy and Melphalan, Methotrexate, and Cyclosporin prior to BMT
Began LED treatment for Mucositis on June 22, 2000
Completed LED treatments on July 21, 2000
Tolerated oral feeds and reported infrequent use of analgesics for mouth pain.

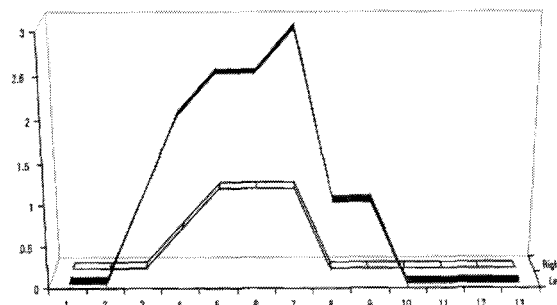
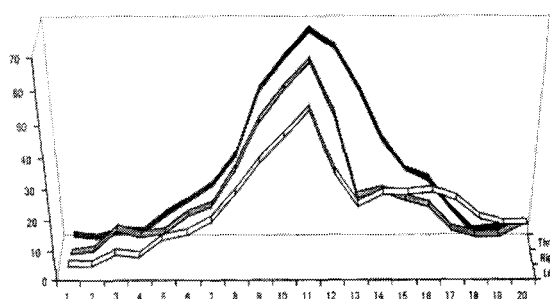
Patient #3

6 year old male with relapsed T-Cell ALL
Radiation therapy and ARA-C w/o Asparaginase, Daunomycin, Doxorubicin, Idarubicin.
This is this child's second Bone Marrow Transplant. He developed oral mucositis severe enough to require admission to the ICU and a trach with vent assisted respirations with his first BMT in another facility.
LED treatments began July 12, 2000, and were completed August 11, 2000.
He has minimal mouth sores and is able to tolerate some oral feeds. Mucositis continues in his throat and lower GI system as evidenced by nausea and diarrhea.

Further In Vitro LED Cell Growth Studies

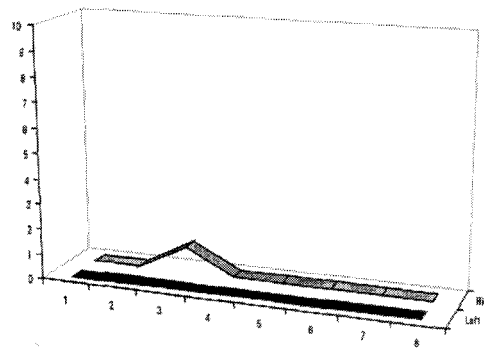
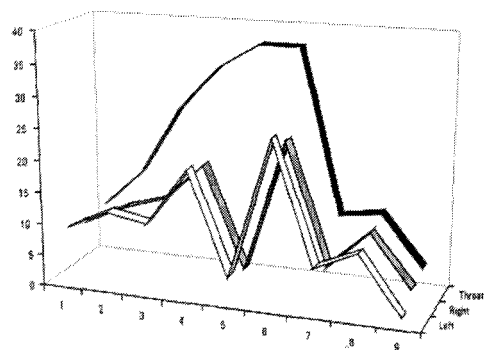
In order to better understand the effects of LEDs on cell growth and proliferation, we have measured radiolabeled thymidine incorporation *in vitro* in several cell lines treated with LEDs at various wavelengths and energy levels. As previously reported (Whelan, 2000), 3T3 fibroblasts (mouse derived skin cells) responded extremely well to LED exposure. Cell growth increased 150-200% over untreated controls. Additionally, we have treated osteoblasts (rat derived bone cells), and L6 rat skeletal muscle cells with LEDs and have found that both fibroblasts and particularly osteoblasts demonstrated a growth-phase specificity to LED treatment, 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-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 demonstrate that LED treatment accelerates this normal healing



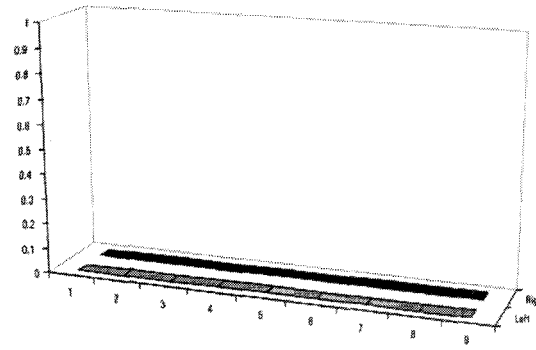
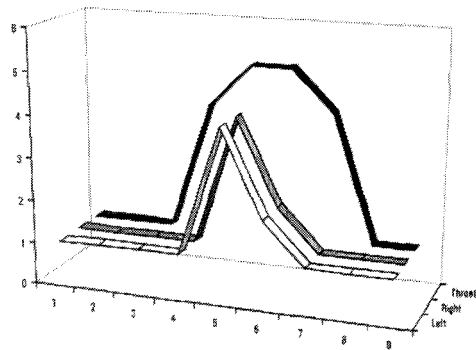
Patient 1 – Pain intensity scale vs. observation number reported during BMT. Light was applied to the left cheek. Even though the left cheek experienced the greatest degree of ulcerative mucositis, the patient reported the least pain in this area.

Patient 1 – Degree of ulcerative mucositis changes vs. observation number during BMT. (Scale: 0=no change; 1=mild; 2=moderate; 3=severe change). The light was applied to the left mucosa.



Patient 2 – Pain intensity scale (100mm) vs. observation number reported during BMT.

Patient 2 – Degree of ulcerative mucositis changes vs. observation number during BMT.



Patient 3 – Pain intensity scale (1-6) vs. observation number reported during BMT.

Patient 3 – Degree of ulcerative mucositis changes vs. observation number during BMT.

FIGURE 4. LED-treatment results in three patients with mucositis.

and tissue regeneration without producing overgrowth or neoplastic transformation. Similar data is also currently under study using skeletal muscle cells A human gingival fibroblast cell line and a human epithelial cell line have been acquired for further study and both cell lines will be cultured and treated with LED light at 688nm and doses of 4 and 8J/cm².

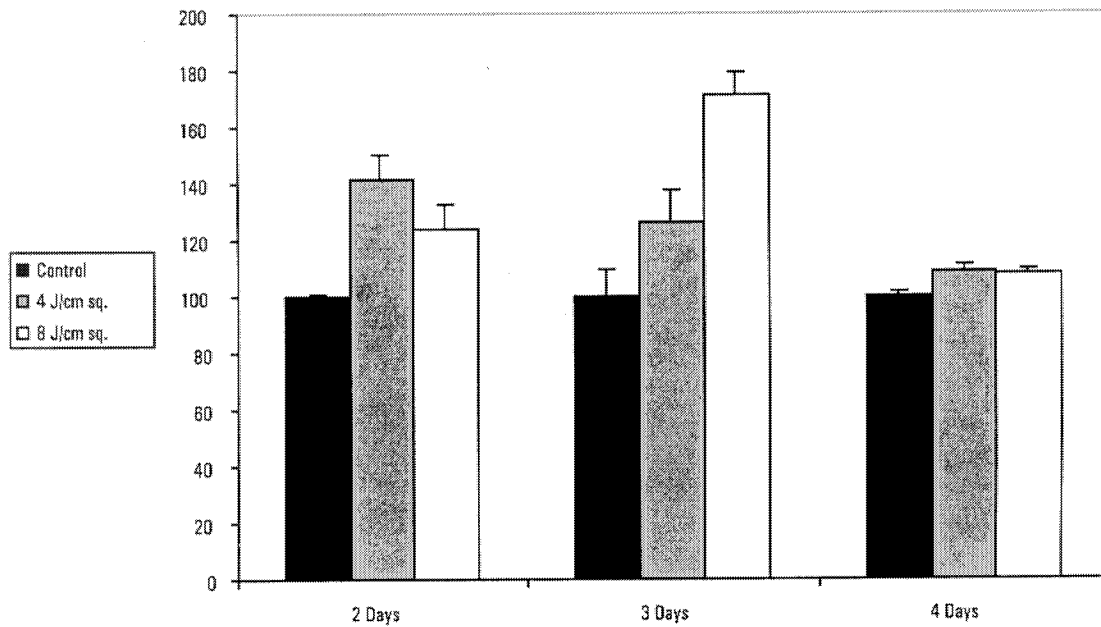


FIGURE 5. 3T3 Fibroblast DNA Synthesis - LED-response, 4 & 8J energy/cm² using combined wavelengths of 670nm,728nm & 880nm showing growth phase specificity (% change from Control vs. # of days in culture).

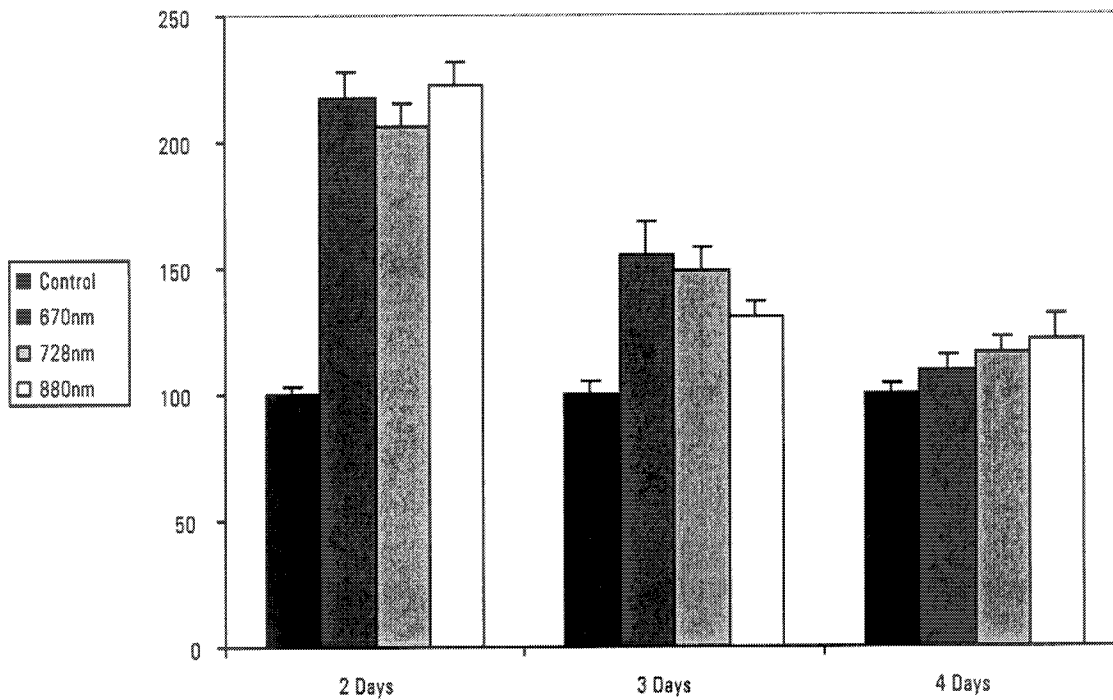


FIGURE 6. Osteoblast murine MC3T3-E1 cells DNA Synthesis- LED-response at 4J energy /cm² using individual wavelengths of 670nm,728nm & 880nm showing growth phase specificity (% change from Control vs. # of days in culture).

We have begun collaborating with Dr. Neal Pellis and Dr. Dennis Morrison at Johnson Space Center in Houston. Their experiments consist of: (1) Using LEDs for Photodynamic Therapy (PDT) with the PDT drugs "microencapsulated" using microgravity technology, which will further improve the selectivity of PDT because "microcapsules" lodge selectively in cancer-tumor capillaries. (2) Using LEDs to stimulate cell healing mechanisms in "simulated" microgravity, using the Johnson Space Center "Rotating Bioreactor" and then proceed to Bioreactor experiments with the LEDs treating cells in the actual microgravity environment of space flight. This addresses the need for "countermeasures" under the new NASA Bioastronautics Initiative. The effects of simulated microgravity on cellular damage and repair mechanisms using bioreactors and using near-IR light (630-880nm) to stimulate wound repair at the cellular level as a possible countermeasure for wound healing and cellular repair during space flight is also being studied. Dr. Whelan has the methods and the lab at Johnson Space Center has the bioreactors and the related research background that could be used for an Earth-based analogue to determine whether or not the near-IR light affects key mechanisms in microgravity. This is an important topic that we should study under the new Bioastronautics Initiative.

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