THE USE OF NASA LIGHT-EMITTING DIODE NEAR-INFRARED TECHNOLOGY FOR BIOSTIMULATION

Harry T. Whelan

Medical College of Wisconsin, Milwaukee, WI
NASA Marshall Space Flight Center, Alabama, USA

Introduction

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 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 spaceflight. 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. 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 spaceflight, 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.

Spaceflight 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 spaceflight. Enhancing the soldier's tissue responses to injury may lead to battlefield resilience and medical independence. Counter-measures to chemical, biological and radioactive weapons exposures, which are based on biostimulation of natural tissue regeneration mechanisms could be more universally safe and effective than conventional drugs and surgical modalities. Regeneration of wounded organs and limbs may also be possible if biostimulation could re-awaken molecular events leading to re-growth of tissue.

Central nervous system regeneration would be of particular benefit. Thus far, we have demonstrated that the best results for wound healing occur at wavelengths of 670 nm and 880 nm using energy densities 4-8 J/cm^2, applied at power intensities of approximately 50 mW/cm^2. However, studies to determine molecular mechanisms could lead to the optimization for current uses, as well as open up new applications.

Despite numerous reports on the benefits of near-IR on wound healing and rehabilitation over the last decade, the basic mechanisms of its action remain poorly understood. Britton Chance's group has reported that about 50% of near-IR light is absorbed by mitochondrial chromophores, such as cytochrome oxidase. However, the underlying cellular and molecular events are still unknown (Karu 1999, Sommer et al. 2001, Whelan et al. 1999, 2000, 2001).

Methods

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 and animals treated with LED light at various wavelengths and energy levels, including 670, 730, 880 nm, 50 mW/cm^2, 4-8 J/cm^2. 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. In addition, we have recently begun using NASA LEDs to promote healing of acute oral lesions in pediatric leukemia patients. A 4-J/cm^2, 50-mW/cm^2 dose of 670-nm light from LEDs was applied daily to the outside of each of 15 patients at the 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. Throat pain 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. 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.

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 (670, 720, and 880 nm) was delivered to Naval Special Warfare Group-2 (SEALS) in Norfolk, VA. Treatment was 4 J/cm^2, 10 mW/cm^2.

Results and Discussion

Near infrared (IR) light has documented benefits promoting wound healing in human and animal studies. Our preliminary results have also demonstrated two to five-fold increases in growth-phase-specific DNA synthesis in normal fibroblasts, muscle cells, osteoblasts, and mucosal epithelial cells in tissue cultures treated with near-IR light. Our animal models treated with near-IR have included wound healing in diabetic mice and ischemic bipedical skin flap in rats. Near-IR induced a thirty percent increase in the rate of wound closure in these animal models. Dose- and time-dependent increases in vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF-2) occurred in animals treated with near-IR. Human studies have included the use of near-IR to prevent ulcerative mucositis resulting from high doses of chemotherapy and radiation. Widely published reports, including those from our laboratory, described accelerated recovery from musculoskeletal injuries, hypoxic-ischemic wounds, burns, lacerations, radiation necrosis, and diabetic ulcers with the use of near-IR. Lasers 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 (LEDs) developed for NASA crewed spaceflight experiments 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.

Conclusion

We are now investigating new collaborations with the Defense Advanced Research Projects Agency (DARPA) for military applications of LED wound healing technology in military medicine. Several uniquely military situations and indications could be addressed, optimizing near-IR parameters for wound healing via LEDs during extended missions under conditions separated from medical personnel. These include burns, chemical agents, radiation, biological agents and highly infected flesh-eating wounds (with and without extended burns) typical for the hygienic conditions occurring in battle fields, also infectious diseases and external wounds occurring in environments with no solar irradiation, low oxygen and high carbon dioxide (submarines). The dramatic results with use of near-IR 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-IR light to treat U.S. troops exposed to chemical and radioactive warfare agents in the field. These examples illustrate the many possible military uses for this technology. These life-saving applications require especially accelerated wound healing, rapid reduction of infections and pain modulation. Regeneration of muscles in amphibians has also been produced by near-IR therapy. The potential for regeneration of human tissue also deserves study.

Lasers have some inherent characteristics, which make their use in a clinical setting problematic, including heat, 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 narrow 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. NASA's interest is dependent on chronic care due to tissue breakdown in microgravity for spaceflight. Military research with these LEDs, in contrast, will be directed to new LED technology aimed at rapid battlefield wound repair. 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 important to note that LED light therapy has been deemed to be a non-significant risk by the FDA.

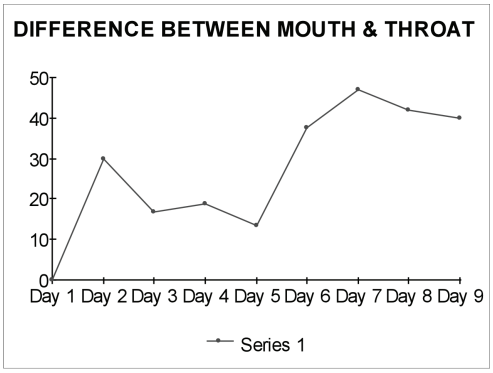


Figure 1: The difference between LED-treated (mouth) and untreated control (throat) becomes more dramatic over time, with daily treatment using NASA LED at 670 nm, 50 mW/cm^2, 4 J/cm^2.

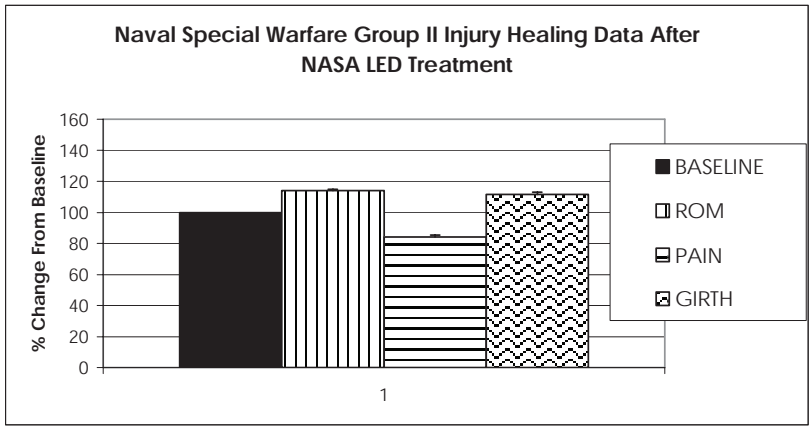
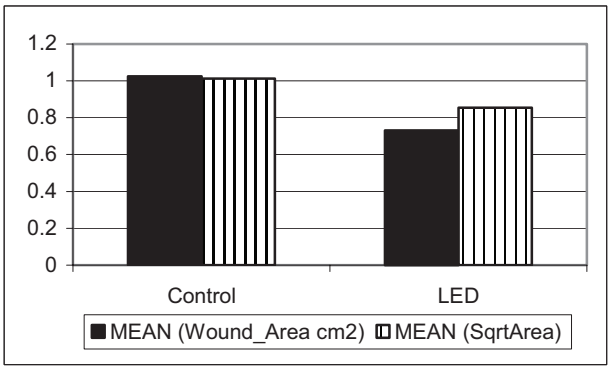


Figure 2: Cumulative results of data from 11 patients (SEALS) showing improvement in range of motion, pain, and girth reported as % change from chronic, unimproving injured baseline after LED treatment at 4 J/cm^2, 10mW/cm^2

Group	Day 1	Day 3	Day 7	Day 12	Day 17
Control	100	73.5 ± 7.9	41.4 ± 8.4	20.4 ± 3.8	12.4 ± 2.9
LED only	100	69.2 ± 5.7	33.2 ± 6.2	14.1 ± 3.7	8.1 ± 2.0

Figure 3: Percent of original wound area in experimental controls and LED treated rats.

Figure 4: Type II Diabetic Mice with excisional skin wounds treated with 3 LED wavelengths, 50 mW/cm^2, 4 J/cm^2. 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.



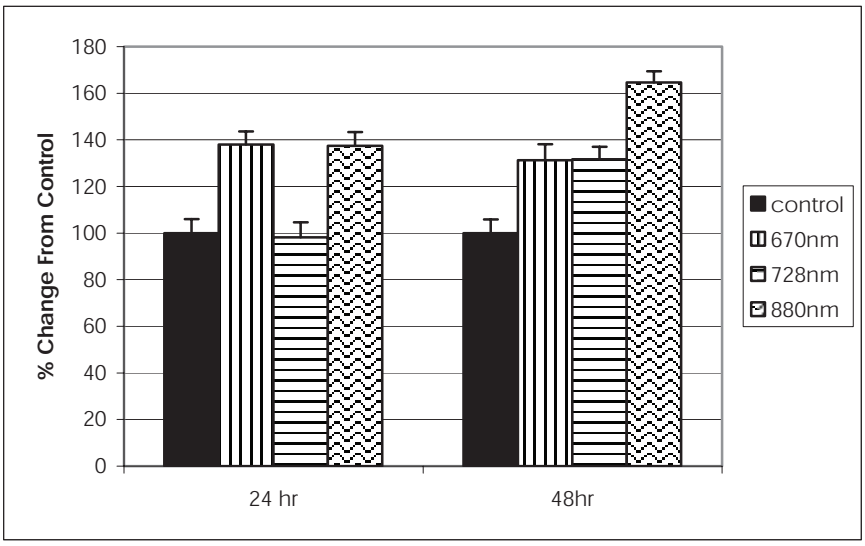
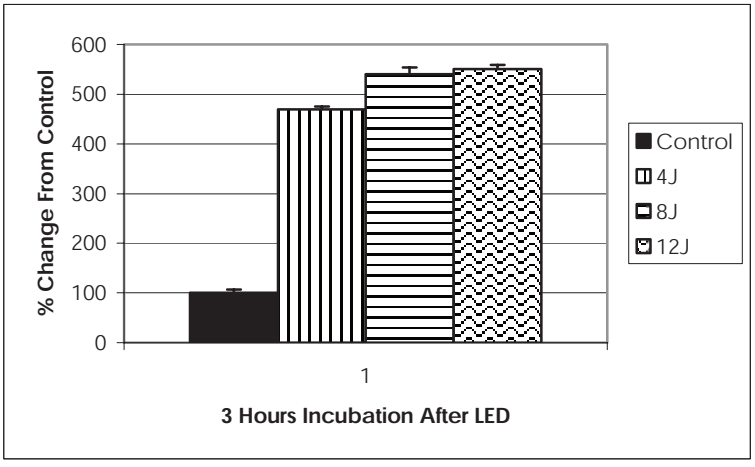


Figure 5. 3T3 Fibroblast DNA Synthesis 8 J/cm^2, 50 mW/cm^2, individual wavelengths. 24- and 48-hour ^{3}H thymidine incorporation.

Figure 6. 3T3 Fibroblast DNA synthesis 3-hour incubation; LED 50 mW/cm^2; 4, 8, 12 J/cm^2.



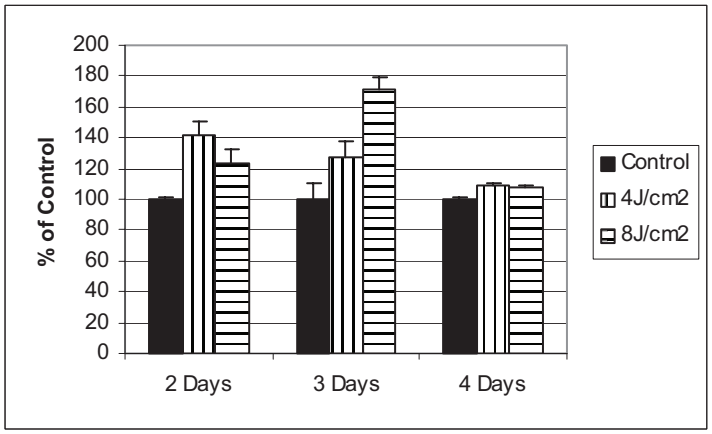
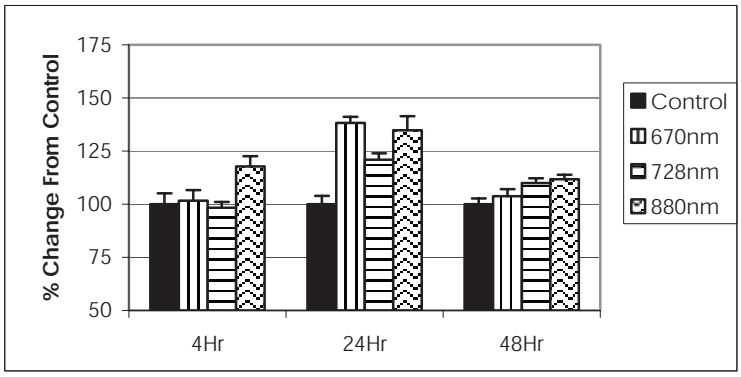


Figure 7: Growth phase specificity of 3T3 fibroblasts; combined wavelengths; 50 mW/cm^2; 4 J/cm^2 vs. 8 J/cm^2.

Figure 8: Growth phase specificity of L-6 cells treated at 50 mW/cm^2; 8 J/cm^2.



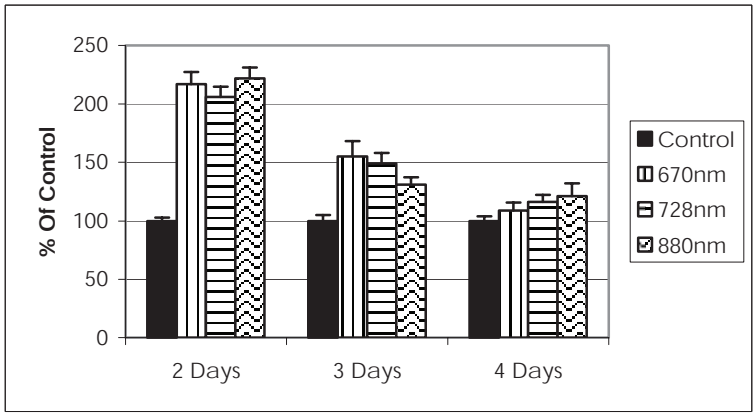
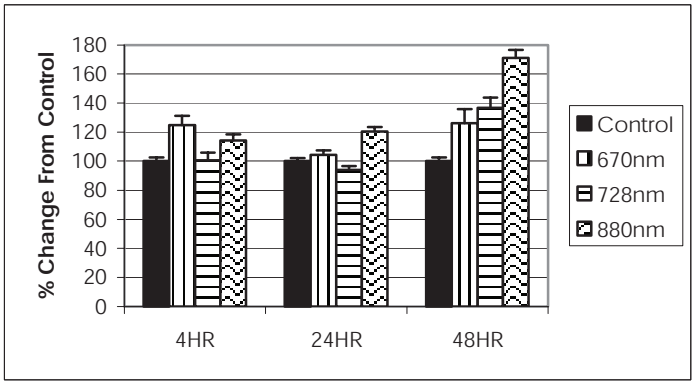


Figure 9: Growth phase specificity of osteoblasts; individual wavelengths; 50 mW/cm^2, 8 J/cm^2.

Figure 10: Growth phase specificity of HaCAT epithelial cells treated with individual wavelengths at 50 mW/cm^2, 8 J/cm^2.



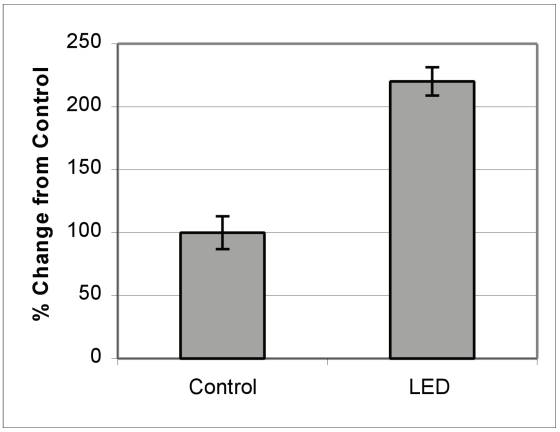


Figure 11: HaCAT epithelial cell collagen synthesis 50 mW/cm^2, 8 J/cm^2, 670 nm. 24-hour ^{3}H proline incorporation.

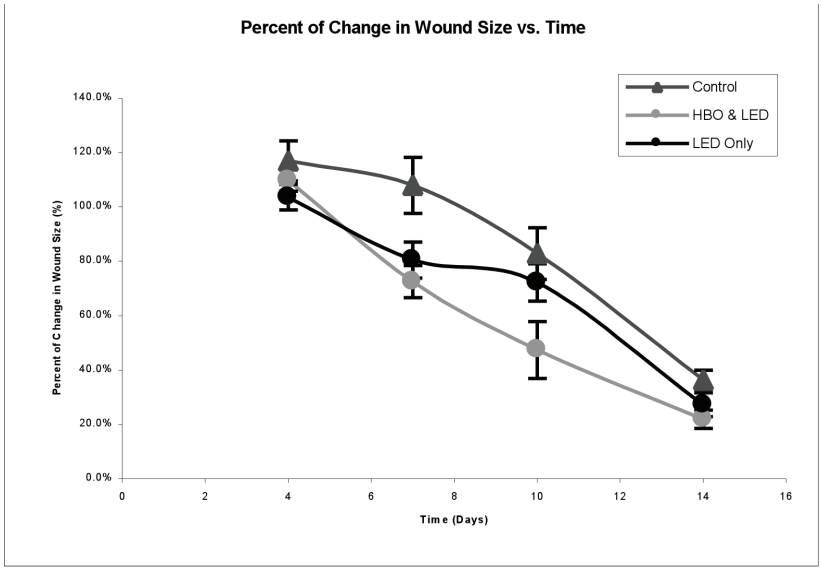


Figure 12: Change in wound size in rat ischemic wound model vs. time (days).

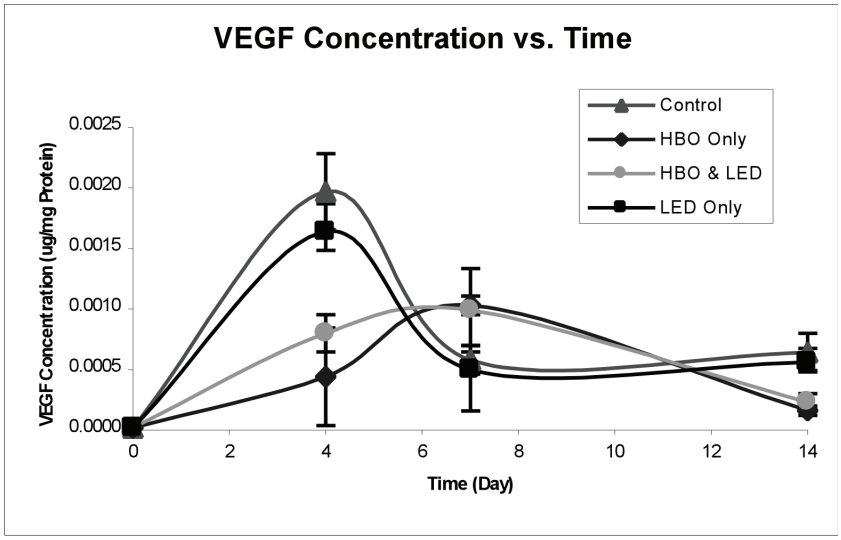


Figure 13: Change in vascular endothelial growth factor (VEGF) concentration (µg/mg protein) vs. time (days) in rat ischemic wound model.

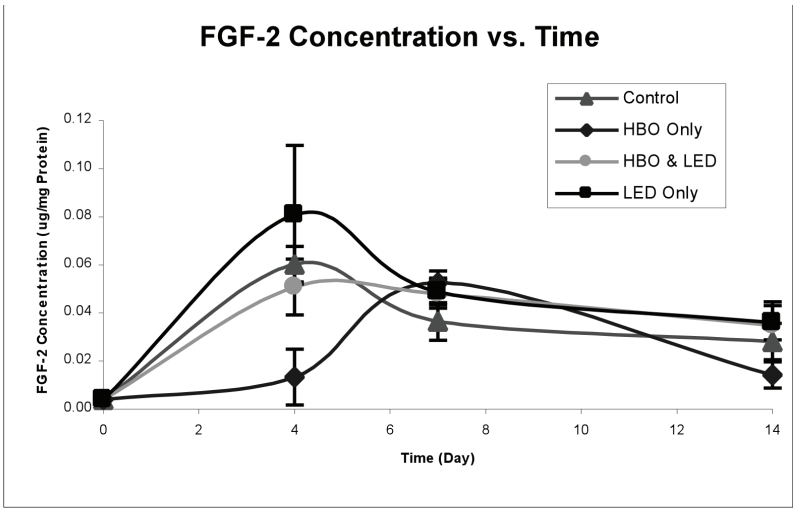


Figure 14: Change in basic fibroblast growth factor (FGF-2) concentration (μg/mg protein) vs. time (days) in rat ischemic wound model.

References

Karu T., "Primary and secondary mechanisms of action of visible to near-IR radiation on cells." *J. Photochem. Photobiol. B. Biol*. 49,1-7 (1999*).*

Sommer A.P., Pinheiro A.L., Mester A.R., Franke R.P., Whelan H.T. "Biostimulatory windows in low intensity laser activation: Lasers, scanners and NASA's light emitting diode array system." *Journal of Clinical Laser Medicine & Surgery* 19, 27-33 (2001).

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., and Stinson, 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).

Whelan HT, Smits RL, Buchmann EV, Whelan NT, Turner SG, Margolis DA, Cevenini V, Stinson H, Ignatius R, Martin T, Cwiklinski J, Philippi AF, Graf WR, Hodgson B, Gould L, Kane M, Chen G, Caviness J: Effect of NASA Light-Emitting Diode (LED) Irradiation on Wound Healing. *Journal of Clinical Laser Medicine and Surgery*. 19, 305-314 (2001).

Acknowledgments

The authors wish to thank Quantum Devices and NASA Marshall Space Flight Center SBIR Program for providing us with the LED array. This work was supported by grants from NASA Marshall Space Flight Center NAS8-01166 and NASA NAS8-99015.