Effect of NASA Light-Emitting Diode Irradiation on Wound Healing

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ABSTRACT

Objective: The purpose of this study was to assess the effects of hyperbaric oxygen (HBO) and near-infrared light therapy on wound healing. Background Data: Light-emitting diodes (LED), 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. Materials and Methods: In vitro and in vivo (animal and human) studies utilized a variety of LED wavelength, power intensity, and energy density parameters to begin to identify conditions for each biological tissue that are optimal for biostimulation. Results: LED produced in vitro increases of cell growth of 140–200% in mouse-derived fibroblasts, rat-derived osteoblasts, and rat-derived skeletal muscle cells, and increases in growth of 155–171% of normal human epithelial cells. Wound size decreased up to 36% in conjunction with HBO in ischemic rat models. LED produced improvement of greater than 40% in musculoskeletal training injuries in Navy SEAL team members, and decreased wound healing time in crew members aboard a U.S. Naval submarine. LED produced a 47% reduction in pain of children suffering from oral mucositis. Conclusion: We believe that the use of NASA LED for light therapy alone, and in conjunction with hyperbaric oxygen, will greatly enhance the natural wound healing process, and more quickly return the patient to a preinjury/illness level of activity. 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 (HBO) therapy and light therapy. HBO 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 HBO, 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 preinjury/illness level of activity.

Laser light and HBO have been widely acclaimed to speed
wound healing of ischemic, hypoxic, and infected wounds.\textsuperscript{1}
Lasers provide low-energy stimulation of tissues that results in increased cellular activity during wound healing.\textsuperscript{2,3}

These activities include collagen production and angiogenesis.\textsuperscript{2} HBO 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 from HBO is unable or unwilling to be treated in a high-pressure environment. These situations include lack of access to a facility equipped with HBO, claustrophobia, and certain current or chronic medical conditions that would make HBO therapy contraindicated. In these instances, light therapy provides an alternative for the patient.

Wound healing has three phases: first, a substrate is laid down, second, cells proliferate, and third, 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.\textsuperscript{2-5}
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,\textsuperscript{6} and greater rate of extracellular matrix production have been reported with laser light treatment.\textsuperscript{7-14} 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.\textsuperscript{15,16} Human studies with laser light have demonstrated greater amounts of epithelialization for wound closure and stimulation of skin graft healing.\textsuperscript{1,9} An excellent review of recent human experience with near-infrared light therapy for wound healing was published by Conlan et al. in 1996.\textsuperscript{1}

Lasers, however, have some inherent characteristics that make their 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 that 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 pinpoint beam of laser light can damage the eye. NASA develop-
oped LEDs to 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\textsuperscript{2,3,8,11–14,17,18} to speed wound healing include 680, 730, and 880 nm. These wavelengths can be produced accurately by NASA LEDs, which have a bandwidth of 25 nm. The depth of near-infrared light penetration into human tissue has been measured spectroscopically\textsuperscript{2,3,19} 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 of 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 are currently underway by the principle investigator.

**LED IN VITRO STUDIES**

In order to understand the effects of LEDs on cell growth and proliferation, we have measured radiolabeled thymidine incorporation \textit{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\textsuperscript{8,10–13,20,21,22} 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.\textsuperscript{23} With LED treatment, we have found that these cells demonstrated growth-phase specificity, responding only when cells are in the growth phase. In these experiments, fibroblasts seeded at a concentration of 500 cells/well and osteoblasts, seeded at a concentration of \(1 \times 10^3\) cells/well, were

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig3}
\caption{Growth phase specificity of L-6 skeletal muscle cells treated with individual wavelengths at 8 J/cm\(^2\); 50 mW/cm\(^2\).}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig4}
\caption{LED response at 4 J/cm\(^2\), 50 mW/cm\(^2\) using individual wavelengths of 670, 728, and 880 nm (percentage change from control versus number of hours after LED treatment).}
\end{figure}
seeded in 12-well plates with a well surface area of approximately 4 cm². DNA synthesis was determined on the second, third, and fourth days in culture for both fibroblasts (Fig. 1) and osteoblasts (Fig. 2). Exposure to LED irradiation accelerated the growth rate of fibroblasts and osteoblasts in culture for 2–3 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.

A series of experiments has 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 (670, 728, and 880 nm), energy densities of 4 and 8 J/cm², and an intensity of 50 mW/cm². Results demonstrated a cell growth increase of about 140% over untreated controls, particularly at 8 J/cm² energy, as shown in Figure 3.

In addition, experiments are now complete using a normal human epithelial cell line seeded in 12-well plates at a concentration of 500 cells/well in order to possibly explain the continued, dramatic results of LED light therapy in preventing oral mucositis in cancer patients. Cell growth increased 155% over untreated controls at 670 nm and 4 J/cm² energy density (50 mW/cm² power density), as shown in Figure 4. An increase of 171% over untreated controls was obtained with a wavelength of 880 nm and 8 J/cm² energy density (53 mW/cm² power density), as shown in Figure 5.

Collagen synthesis of the HaCAT epithelial cells was determined by measuring tritiated proline incorporation using a modified method described by Peterkofsky and Diegelmann. HaCAT epithelial cells were seeded in two 12-well tissue culture plates with 600 µL DMEM containing 10% FBS, 1% penicillin/streptomycin, and 6 µL L-proline [2,3-³H]. The media was free of nonessential amino acids. One plate was used as the control, and the other was treated with the 670-nm NASA LED

FIG. 5. LED response at 8 J/cm²; 50 mW/cm² using individual wavelengths of 670, 728, and 880 nm (percentage change from control versus number of hours after LED treatment).

FIG. 6. HaCAT epithelial cell collagen synthesis at 8 J/cm², 50 mW/cm², 670 nm. Shown in 24-h ³H proline incorporation.
at 8 J/cm². After 24 h of incubation in 5% CO₂ at 37°C, two 200-µL 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 min 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.

**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, nonhealing 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 HBO therapy, 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 min, and the LED was delivered at a fluence of 4 J/cm² and 50 mW/cm² for 14 consecutive days. A future study

![FIG. 7. Change in wound size in rat ischemic wound model versus time (days).](image1)

![FIG. 8. Change in vascular endothelial growth factor (VEGF) concentration (µg/mg protein) versus time (days).](image2)
will incorporate the combination of three wavelengths (670, 728, and 880 nm) 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) proved 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 (880 nm) group were 36% smaller than those of the control group. That size discrepancy remained by day 10. The LED (880 nm) alone also speeds wound closure. On day 7, the LED (880 nm) treated wounds was 20% smaller than the control wounds. By day 10, the difference between these two groups dropped to 12%. This was 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 at day 14 (Fig. 7), when the points are converging due to the fact that the wounds are healing.

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

![Figure 9](image-url)  
**FIG. 9.** Change in basic fibroblast growth factor (FGF-2) concentration (µg/mg protein) versus time (days).

![Figure 10](image-url)  
**FIG. 10.** Type 2 diabetic mice with excisional skin wounds treated with combined LED wavelengths, 4 J/cm², 50 mW/cm². The square root of wound area is used in the dependent variable in the analysis. This transformation was needed to correct for nonconstant error in the general linear model. SqrtArea could be interpreted as being proportional to the radius of a circular wound.
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.

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 (880 nm) 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.

A wound healing impaired type 2 diabetic mouse model has also been studied. As previously reported, genetically diabetic mice treated with low level laser irradiation demonstrated significantly enhanced wound closure grossly, and improved wound epithelialization, cellular content, granulation tissue formation, collagen deposition, and extensive neovascularization on histological evaluation.21 In our study, type 2 diabetic mice with excisional skin wounds were treated with LEDs at individual wavelengths of 680, 730, and 880 nm at 4 J/cm² and 50 mW/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 (Fig. 10). This analysis was carried out using the SAS statistical software package, published by The SAS Institute, Inc.

### Table 1. Pain Intensity in Mucositis Patients Is Reduced by LED Therapy in Cheeks Compared to Throat (Control = 100%)

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
<th>Day 8</th>
<th>Day 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (throat)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Right cheek</td>
<td>98.56</td>
<td>67.81</td>
<td>84.78</td>
<td>79.78</td>
<td>86.16</td>
<td>63.79</td>
<td>52.73</td>
<td>58.65</td>
<td>61.48</td>
</tr>
<tr>
<td>Left cheek</td>
<td>104.1</td>
<td>71.21</td>
<td>81.48</td>
<td>82.32</td>
<td>86.82</td>
<td>60.93</td>
<td>53.27</td>
<td>57.48</td>
<td>58.65</td>
</tr>
<tr>
<td>Control SEM</td>
<td>33.2</td>
<td>22.9</td>
<td>20.7</td>
<td>18.1</td>
<td>17.8</td>
<td>18.9</td>
<td>21.7</td>
<td>22.6</td>
<td>22.8</td>
</tr>
<tr>
<td>Right SEM</td>
<td>34.2</td>
<td>25.2</td>
<td>20.4</td>
<td>23.5</td>
<td>24.5</td>
<td>24.1</td>
<td>17.9</td>
<td>11.1</td>
<td>18.1</td>
</tr>
<tr>
<td>Left SEM</td>
<td>33.8</td>
<td>24.1</td>
<td>20.6</td>
<td>24.1</td>
<td>24.3</td>
<td>25.9</td>
<td>17.7</td>
<td>11.1</td>
<td>15.8</td>
</tr>
</tbody>
</table>

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

Clinical LED wound healing studies have been reported previously,\textsuperscript{11–13} 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 three wavelengths combined in a single unit (670, 720, and 880 nm) was delivered to Naval Special Warfare Group–2 (SEALS) in Norfolk, Virginia. Treatment was with 4 J/cm\textsuperscript{2}. 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 centimeters, 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.

In collaboration with U.S. Navy Submarine Squadron ELEVEN, data have 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 three wavelengths combined in a single unit (670, 720, 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 6-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 or 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 gastrointestinal effects, greater than one-third 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, debride, and comfort are recommended, and prophylactic oral antiviral and antifungal agents have been used to minimize infections. Because lasers have been shown to speed healing of oral mucositis we have recently expanded the wound-healing abilities of LED light therapy to include these oral lesions.

A 4 J/cm², 50 mW/cm² dose of 670-nm 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 was 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 were photographed, and mouth and throat pain were assessed using a 1–10 Visual Analog scale. 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 7 (Table 1, Figures 12 and 13). The greatest difference between throat and mouth pain was reported on day 7, when, surprisingly, oral mucosal ulceration is believed to be worst in untreated patients.

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 requirements 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 three more academic medical centers, in addition to our own, to be supplied with NASA LED arrays by Quantum Devices, Inc. A multisite 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. These life-saving applications require especially accelerated wound healing, rapid reduction of infections, and pain modulation.

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