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Clinical and Experimental Applications of NIR-LED Photobiomodulation

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ABSTRACT

This review presents current research on the use of far-red to near-infrared (NIR) light treatment in various *in vitro* and *in vivo* models. Low-intensity light therapy, commonly referred to as "photobiomodulation," uses light in the far-red to near-infrared region of the spectrum (630–1000 nm) and modulates numerous cellular functions. Positive effects of NIR–light-emitting diode (LED) light treatment include acceleration of wound healing, improved recovery from ischemic injury of the heart, and attenuated degeneration of injured optic nerves by improving mitochondrial energy metabolism and production. Various *in vitro* and *in vivo* models of mitochondrial dysfunction were treated with a variety of wavelengths of NIR-LED light. These studies were performed to determine the effect of NIR-LED light treatment on physiologic and pathologic processes. NIR-LED light treatment stimulates the photoacceptor cytochrome *c* oxidase, resulting in increased energy metabolism and production. NIR-LED light treatment accelerates wound healing in ischemic rat and murine diabetic wound healing models, attenuates the retinotoxic effects of methanol-derived formic acid in rat models, and attenuates the developmental toxicity of dioxin in chicken embryos. Furthermore, NIR-LED light treatment prevents the development of oral mucositis in pediatric bone marrow transplant patients. The experimental results demonstrate that NIR-LED light treatment stimulates mitochondrial oxidative metabolism *in vitro*, and accelerates cell and tissue repair *in vivo*. NIR-LED light represents a novel, noninvasive, therapeutic intervention for the treatment of numerous diseases linked to mitochondrial dysfunction.

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INTRODUCTION

Low-intensity light therapy, commonly referred to as "photobiomodulation," by light in the far-red to near-infrared (NIR) region of the spectrum (630–1000 nm) modulates numerous cellular functions. Clinical and experimental applications of photobiomodulation have expanded over the past 30 years.[1] Low-power lasers and light-emitting diodes (LED) are well-accepted therapeutic tools in the treatment of infected, ischemic, and hypoxic wounds, along with other soft tissue injuries.[2–5] Positive effects of photobiomodulation include acceleration of wound healing, improved recovery from ischemic injury in the heart, and attenuated degeneration in the injured optic nerve.[4,6,7]

At the cellular level, photobiomodulation can modulate fibroblast proliferation, attachment and synthesis of collagen and procollagen, promote angiogenesis, and stimulate macrophages and lymphocytes by improving energy metabolism within the mitochondria. In addition, photobiomodulation has demonstrated the ability to promote the production of growth factors, such as keratinocyte growth factor (KGF), transforming growth factor (TGF), and platelet-derived growth factor (PDGF).[5,8–10]

Optimal wavelengths and energy densities necessary for therapeutic interventions have been characterized. Wavelengths within the far-red to near-infrared range (630–1000 nm) along with a minimal energy density of 4 J/cm^2 have been proven effective at stimulating biological processes.[4,10–13]

Lasers are limited in their ability to deliver monochromatic far-red to -NIR light. Combined wavelengths cannot easily be reproduced with lasers, and the beam width makes it difficult to treat large areas. Moreover, lasers emit a fair amount of heat, which has the potential to produce tissue damage. An effective alternative to lasers are LED arrays, which were initially developed by NASA for experimental plant growth in space. LED arrays produce light in the far-red to NIR at optimal wavelengths and energy densities. The arrays can be constructed in various sizes to accommodate large areas and do not emit any heat, which eliminates the danger of additional tissue damage. Light emitted by LED arrays at optimal wavelengths penetrates skin and tissue to a depth of approximately 23 cm.[4,12–14] Further, NIR-LED light therapy has been deemed a nonsignificant risk by the FDA and has been approved for use in humans.

NIR-LED PHOTOBIOMODULATION STIMULATES THE PHOTOACCEPTOR CYTOCHROME *C* OXIDASE

The mechanism by which far-red to NIR light produces its biological effects remains to be elucidated. There is a growing body of evidence that suggests that one primary effect is the stimulation of mitochondrial cytochromes, which in turn initiate secondary cell-signaling pathways.[1,11,16–18] The overall result of photobiomodulation is increased energy metabolism and improved cell viability.[18]

Within mammalian tissues, there are three major photoacceptor molecules: hemoglobin, myoglobin, and cytochrome *c* oxidase.[18] Of these three, cytochrome c oxidase is the only one that is involved in energy metabolism and production, as it comprises complex IV of the electron transport chain located within the mitochondria. Thus, cytochrome *c* oxidase has been postulated as the photoacceptor molecule for the biological effects of photobiomodulation.

The evidence to support cytochrome *c* oxidase as the primary photoacceptor has been steadily growing. Cellular proliferation studies comparing the action spectrum following laser irradiation compared to the absorption spectra of possible photoacceptor molecules have suggested cytochrome *c* oxidase as the primary photoacceptor.[16] In addition, it has been demonstrated that up to 50% of NIR light is absorbed by mitochondrial chromophores, including cytochrome *c* oxidase.[12,13] In studies using primary cultured neurons and tetrodotoxin (TTX)—a voltage-dependent sodium channel blocker that impedes neuronal impulses, decreases ATP demand, and down-regulates cytochrome *c* oxidase—NIR-LED light treatment has been shown to reverse the toxic effects of TTX. This is accomplished by reverting levels of cytochrome *c* oxidase back to control levels in TTX-exposed NIR-LED light–treated neurons and up-regulating the enzyme's activity in NIR-LED light–treated control neurons.[17] Furthermore, the action and absorption spectra in the far-red to NIR wavelengths, compared to the action and absorption spectra of cytochrome *c* oxidase activity and ATP content in neurons exposed to TTX that received NIR-LED light treatment, parallel each other[18] (Fig. 1). NIR-LED light treatment has also partially restored cytochrome *c* oxidase activity in primary cultured neurons exposed to 10–100 μM potassium cyanide (KCN), significantly reduced cell death in neurons exposed to 300 μM KCN, significantly restored ATP levels in neurons treated with 10 μM KCN, and enhanced the effect of photobiomodulation by pretreating neurons with NIR-LED light prior to exposure to 10–100 μM KCN *in vitro.*

NIR-LED PHOTOBIOMODULATION ACCELERATES WOUND HEALING *IN VITRO* AND *IN VIVO*

There is a growing need for safe and efficacious therapeutic intervention for the treatment of chronic wounds. Hyperbaric oxygen therapy (HBO) is a common treatment for ischemic, hypoxic, and infected wounds, but it is not appropriate for all patients.[4] HBO therapy is contraindicated in patients who have chronic medical conditions and are claustrophobic. Access to a facility equipped with HBO may also be a problem.[4] NIR-LED photobiomodulation can serve as an alternative to HBO.

The process of wound healing occurs in three phases: first, a substrate is laid down; second, cell proliferation occurs; and third, remodeling of the tissue takes place. Photobiomodulation exerts its biological effect during the proliferative phase of wound healing. *In vitro* experimentation utilizing NIR-LED light treatments at various wavelengths has shown to significantly increase cell growth in a variety of cell lines, including murine fibroblasts, rat osteoblasts, rat skeletal muscle cells, and normal human epithelial cells.[4] Accelerated wound healing following photobiomodulation has also been demonstrated in a

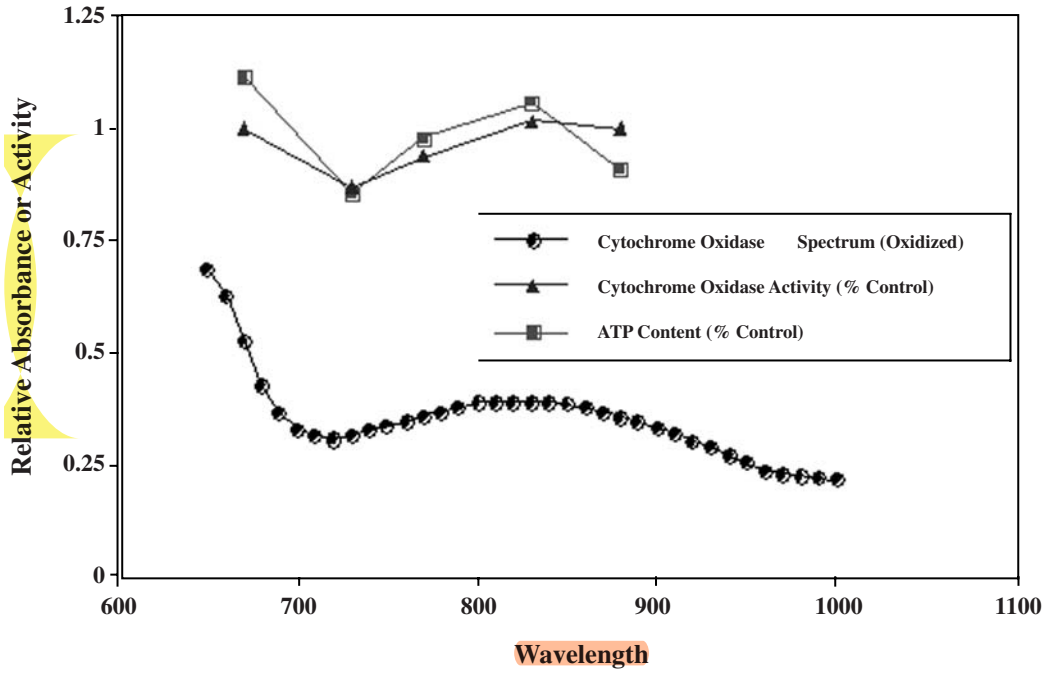

FIG. 1. Action and absorption spectra in the far-red to near-infrared (NIR) region of the spectrum for cytochrome *c* oxidase as compared to the relative cytochrome *c* oxidase activity and ATP content in tetrodotoxin (TTX)–exposed neurons treated with NIR–light-emitting diode (LED) light at varying wavelengths expressed as percent of controls.

number of *in vivo* models, including toads, mice, rats, guinea pigs, and swine.[19,20] In an *in vivo* rat model of ischemic wounds, a decrease in wound size and acceleration of wound closure has been demonstrated in rats treated with 880-nm NIR-LED light.[4] Human studies using NIR-LED light therapy have demonstrated greater amounts of epithelialization for wound closure and accelerated healing of skin grafts.[2,21]

To determine if NIR-LED light treatment can improve impaired healing, we used a murine model of diabetic healing, which is characterized by a delayed re-epithelialization.[22] Polyvinyl acetal (PVA) sponges were implanted subcutaneously in the dorsum of genetically diabetic mice (BKS.Cg-*m* +/+ *Lepr*^{db}). The mice were subsequently treated with 670-nm NIR-LED light, and wounds were harvested for RNA analysis.

Microarray analysis revealed that basement membrane and tissue regenerating genes were significantly up-regulated in mice that received NIR-LED light treatments as compared to controls. Integrins, nidogens, laminin, actin, and kinesin motor proteins were up-regulated. All of these proteins are necessary at specific time points for wound-induced epithelial cell migration and differentiation.[22] Up-regulation of these genes is one possible mechanism by which NIR-LED photobiomodulation can accelerate wound closure. Semaphorins/collapsins are another group of genes that were significantly up-regulated in mice receiving NIR-LED light treatments. Specifically, murine semaphorin H is involved in the inhibition of sensory peripheral nerve ingrowth. Murine semaphorin H, along with other semaphorin/collapsin proteins, is involved in pain management. Pain has been shown to slow the healing process by the recruitment of inflammatory cells to the site of injury.[22] Decreasing pain via NIR-LED light could aid in the acceleration of wound closure.

Genes that were down-regulated in NIR-LED light–treated mice include cytokine receptors, interleukin-1, interleukin-10, and macrophage inflammatory protein–2. A decrease in these genes encoding for proteins associated with the inflammatory response results in a decrease in pain, which in turn increases the ability of tissue-regenerating proteins to facilitate wound closure. Another group of genes that were down-regulated in response to NIR-LED light treatment were those encoding proapototic proteins. Activator of apoptosis harakiri (HRK), programmed cell death 1 protein precursor (PDCD-1; PD-1), and receptor-interacting protein (RIP) were all down-regulated.

NIR-LED PHOTOBIOMODULATION AS AN EFFECTIVE THERAPEUTIC TOOL FOR THE PREVENTION OF ORAL MUCOSITIS IN PEDIATRIC BONE MARROW TRANSPLANT PATIENTS

Chemotherapy and/or radiation therapy is administered prior to bone marrow transplant (BMT). Mucositis, especially oral mucositis (OM), is a common debilitating side effect of this treatment. The development of these ulcerations causes severe pain, compromises the ability of the patient to eat and drink independently, and can lead to infection and to increased morbidity.[23–25] Since NIR-LED photobiomodulation accelerates

wound healing and increases cell proliferation, this treatment was used in an attempt to treat pediatric BMT patients prophylactically to prevent the development of oral mucositis.[26]

The first clinical trial of NIR-LED light treatment as a preventative treatment for the development of OM was performed at the Children's Hospital of Wisconsin in Milwaukee, Wisconsin. Thirty-two pediatric patients receiving myeloablative therapy were treated with 670-nm NIR-LED light once a day for 14 days post-BMT at an energy density of 4 J/cm^2. Patients received NIR-LED light treatment on the left extraoral epithelium and sham treatment on the right. Subsequent to the light treatment, patients were asked to rate left and right buccal pain as compared to throat pain, which served as an untreated control. NIR-LED light treatment produced a significant reduction in left and right buccal pain (48% and 39%, respectively) when compared to throat pain. In addition, the incidence of OM in this patient population was decreased, with only 53% of patients developing OM, when compared to historical epidemiological data, which suggests that 70–90% of the patient population receiving BMT should have developed OM (Fig. 2). The results of this clinical trial demonstrate that NIR-LED light treatment may be an effective preventive countermeasure to the development of OM in cancer patients. This study served as the foundation for the current multi-centered, double-blinded trial underway.

NIR-LED PHOTOBIOMODULATION AS TREATMENT FOR RETINAL TOXICITY *IN VIVO*

Mitochondrial dysfunction plays a central role in the pathogenesis of numerous retinal and neurodegenerative diseases, including age-related macular degeneration, Leber's hereditary optic neuropathy, and Parkinson's and Alzheimer's disease.[27] Furthermore, mitochondrial dysfunction has been shown to play an integral role in the development of retinal toxicity resulting from methanol intoxication.[28,29] The neurotoxic agent in methanol intoxication is the metabolite formic acid. Formic acid is a mitochondrial toxin that specifically inhibits cytochrome *c* oxidase in the retina and optic nerve, resulting in blindness.[30,31]

To determine if exposure to monochromatic far-red to NIR light from LED arrays protects the retina against the toxic actions of methanol-derived formic acid, we employed a rat model of methanol toxicity.[32] Results from these studies demonstrate that three brief 670-nm NIR-LED light treatments of 2 min and 24 sec delivered at 5, 25, and 50 h of methanol intoxication significantly attenuated the retinotoxic effects of methanol-derived formate during intoxication (Fig. 3). In addition, NIR-LED light treatment protected the retina from the histopathologic changes induced by methanol-derived formate (Figs. 4 and 5). These findings provide a link between the actions of monochromatic far-red to NIR light on mitochondrial oxidative metabolism *in vitro* and retinoprotection *in vivo*. Moreover, they have provided the impetus for ongoing investigations of the therapeutic efficacy of far-red to NIR light therapy in other models of retinal disease.

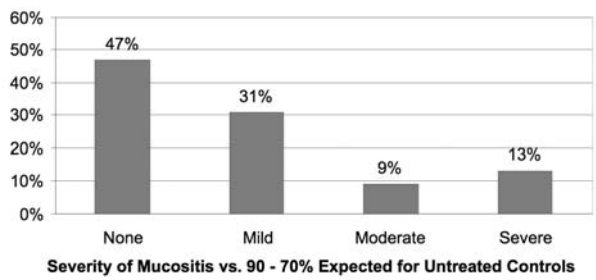

FIG. 2. Reduced incidence of oral mucositis in patients that received 670-nm near-infrared–light-emitting diode (NIR-LED) light treatments once daily for 14 days post–bone marrow transplant (BMT) as compared to historical epidemiological data.

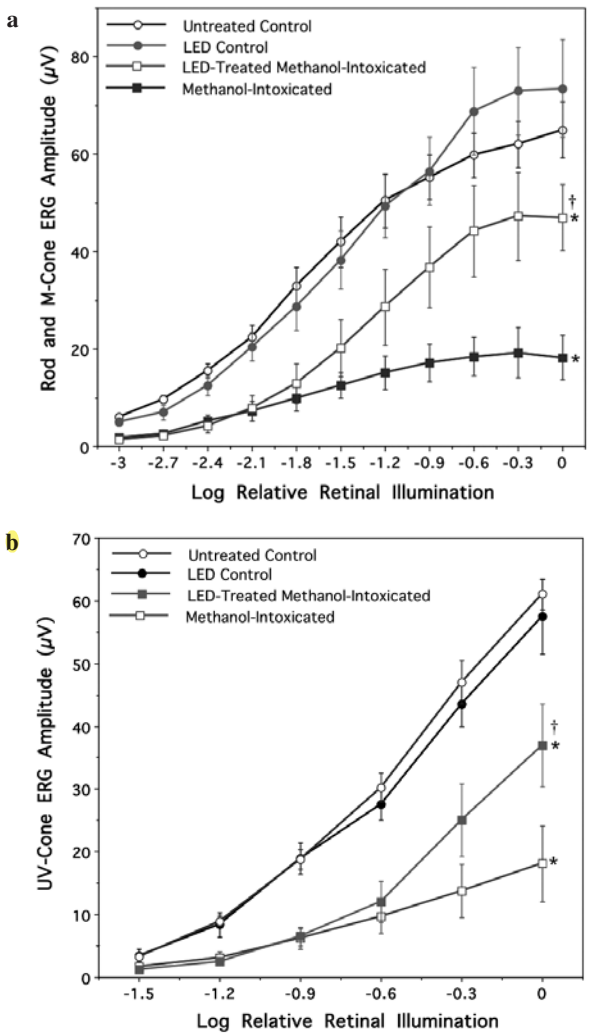

FIG. 3. (**a**) 670-nm near-infrared–light-emitting diode (NIR-LED) light treatment increases rod and M-cone ERG amplitude in LED-treated methanol-intoxicated rats as compared to methanol-intoxicated rats. (**b**) 670-nm near-infrared–light-emitting diode (NIR-LED) light treatment improves retinal function by increasing ultraviolet (UV)–cone ERG amplitude in LED-treated methanol-intoxicated rats as compared to methanol-intoxicated rats.

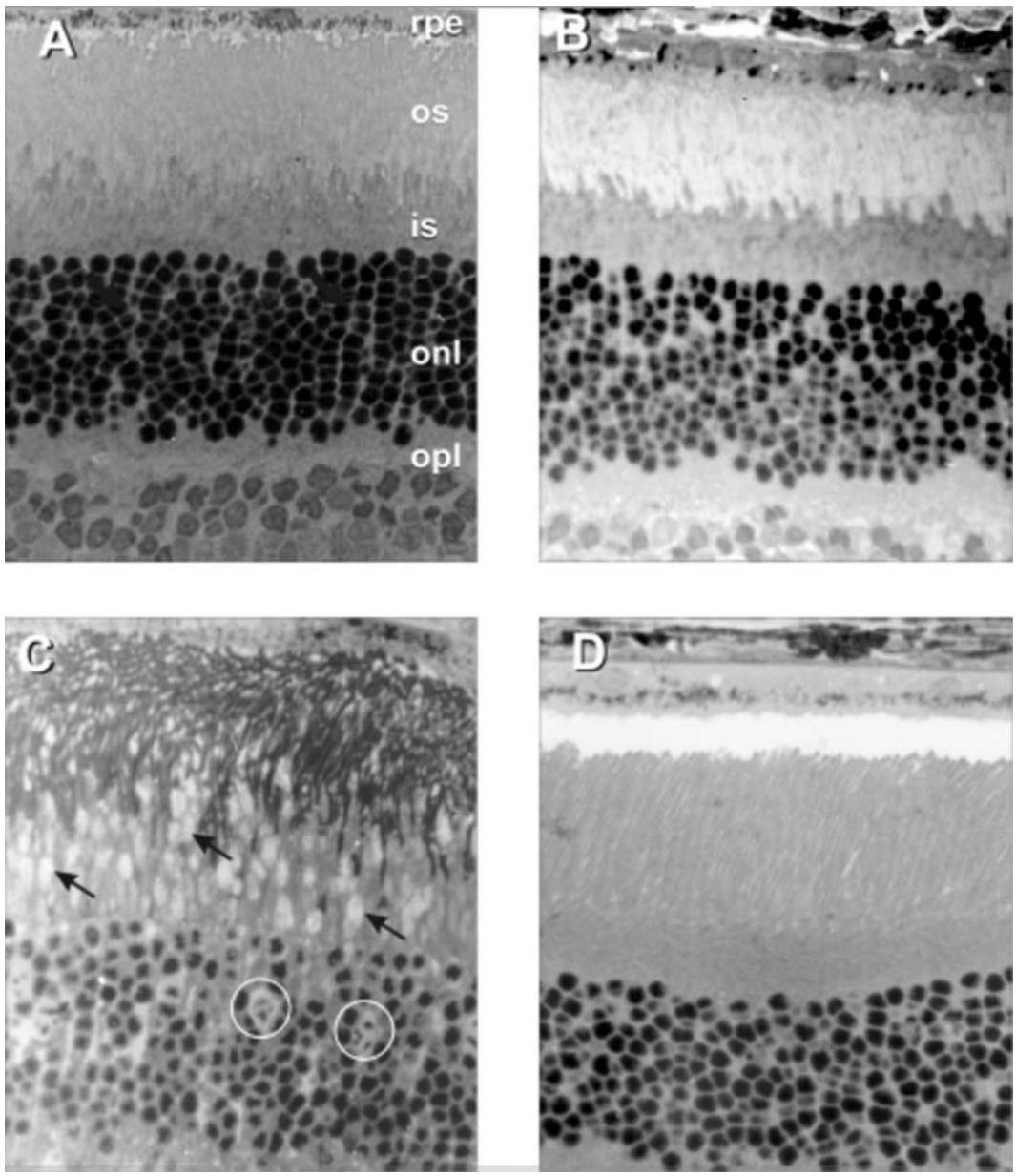

FIG. 4. Near-infrared–light-emitting diode (NIR-LED) light treatment protects the retina from morphologic changes resulting from methanol intoxication. Untreated control (**A**), LED control (**B**), methanol-intoxicated (**C**), and LED-treated methanol-intoxicated (**D**) rats.

The prolonged effect of three brief NIR-LED light treatments in mediating the retinoprotective actions in methanol intoxication suggests that 670-nm NIR-LED light treatment induces a cascade of signaling events, which is initiated by the initial absorption of light by cytochrome *c* oxidase. These signaling events may include the activation of immediate early genes, transcription factors, cytochrome oxidase subunit gene expression, and a host of other pathways related to increased oxidative metabolism. Preliminary gene expression studies in control untreated, methanol intoxicated, and NIR-LED light–treated methanol-intoxicated rodents were performed. At least 80 genes are involved in subsequent biological processes resulting from methanol intoxication and NIR-LED light treatment. Of these, at least 26 genes are up-regulated in methanol-intoxicated rats. These same genes are down-regulated in NIR-LED light–treated methanol intoxicated rats, as compared to methanol-intoxicated rats. NIR-LED light regulates the expression of a number of genes that control important cellular functions and include DNA repair proteins, antioxidant enzymes, molecular chaperones, protein biosynthesis enzymes, trafficking and degradation proteins, along with cell growth and maintenance proteins.

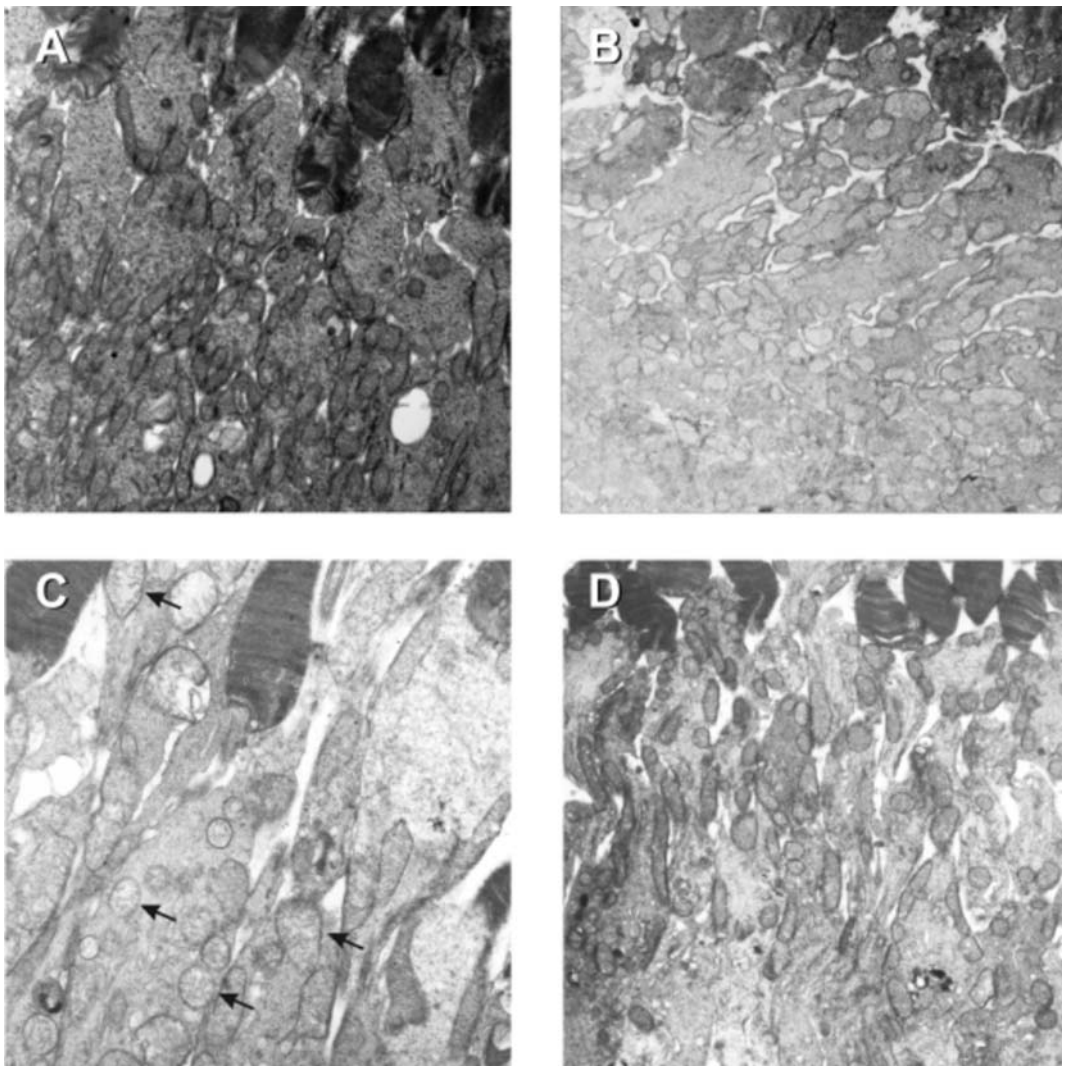

FIG. 5. Near-infrared–light-emitting diode (NIR-LED) light treatment protects the photoreceptor ultrastructure from the retinotoxic effects of methanol intoxication. Untreated control (**A**), LED control (**B**), methanol-intoxicated (**C**), and LED-treated methanol-intoxicated (**D**) rats.

NIR-LED PHOTOBIOMODULATION ATTENUATES DIOXIN-INDUCED DEVELOPMENTAL TOXICITY

Dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) is the most acutely toxic of a group of chemicals known collectively as polycyclic halogenated aromatic hydrocarbons (PHAHs), and is used as the model chemical to investigate the mechanism of action of the larger chemical class. The PHAHs are potent developmental toxins that cause increased embryo mortality as well as sub-lethal changes in the morphological patterning of the skeleton and of multiple organs, including the heart and the brain.[33,34] Dioxin, acting in part through activation of a transcription factor (ARNT), is known to affect the expression of a number of genes. These genes encode for proteins that play a role in cell-cell and cell-extracellular matrix interactions, cell signaling, cytoskeleton-related proteins, proteins associated with cell cycle regulation, and the homeostasis and metabolism of many xenobiotics and hormones.[35–37] Further, dioxin has long been known to induce cellular oxidative stress and increase production of free radicals.[38] It is through a combination of these mechanisms that dioxin and the PHAHs are believed to increase the incidence of birth defects, and a variety of cancers and hormonally linked dysfunctions in humans and wildlife. In addition, late embryo mortality, which is typical of birds exposed to higher levels of dioxin, has long been hypothesized to be due to cellular stress decreasing available energy needed for the animal to peck out of the shell.[39]

To determine the effect of 670-nm NIR-LED light therapy on dioxin-induced developmental toxicity, a chicken (*Gallus gallus*) embryo model was employed. Domestic chickens have been investigated as an animal model for vertebrate embryonic development for over a century.[40] The embryonic development of chicken is well characterized anatomically, physiologically, biochemically, and in terms of the molecular cues that control the developmental process. Moreover, chicken embryos are

sensitive to many developmental toxins and are therefore an ideal laboratory model. For this study, domestic chicken eggs were divided into the following treatment groups: no-inject, sunflower oil vehicle, and 2,20,200 ppt dioxin. All of these groups contained untreated control eggs and 670-nm NIR-LED light–treated eggs resulting in an energy density of 4 J/cm^2 at 24-h intervals. Results from these experiments indicate that daily light treatment throughout embryonic development is not detrimental to the health of the embryo.[41] Further, daily NIR-LED light treatment reduced dioxin-induced mortality of chick embryos by 40% as well as the incubation time before the embryo start to hatch (initial pip time).[42,43] Thus, NIR-LED light treatment obviates at least some of the adverse developmental impacts of a model xenobiotic.

CONCLUSION

Experimental results demonstrate that NIR-LED light treatment stimulates mitochondrial oxidative metabolism *in vitro*, and accelerates cell and tissue repair *in vivo*. NIR-LED light represents a novel, noninvasive, therapeutic intervention for the treatment of numerous diseases linked to mitochondrial dysfunction, including age-related macular degeneration, Leber's hereditary optic neuropathy, and Parkinson's and Alzheimer's disease.

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