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In Vitro and In Vivo Optimization of Infrared Laser Treatment for Injured Peripheral Nerves

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Background and Objective: Repair of peripheral nerve injuries remains a major challenge in restorative medicine. Effective therapies that can be used in conjunction with surgical nerve repair to improve nerve regeneration and functional recovery are being actively investigated. It has been demonstrated by a number of peer reviewed publications that photobiomodulation (PBM) supports nerve regeneration, reinnervation of the denervated muscle, and functional recovery after peripheral nerve injury. However, a key issue in the use of PBM as a treatment for peripheral nerve injury is the lack of parameter optimization for any given wavelength. The objective of this study was to demonstrate that for a selected wavelength effective *in vitro* dosing parameters could be translated to effective *in vivo* parameters.

Materials and Methods: Comparison of infra-red (810 and 980 nm wavelengths) laser treatment parameters for injured peripheral nerves was done beginning with a series of *in vitro* experiments using primary human fibroblasts and primary rat cortical neurons. The primary rat cortical neurons were used for further optimization of energy density for 980 nm wavelength light using measurement of total neurite length as the bioassay. For these experiments, the parameters included a 1 W output power, power density of 10 mW/cm^2, and energy densities of 0.01, 0.1, 0.5, 2, 10, 50, 200, 1,000, and 5,000 mJ/cm^2. For translation of the *in vitro* data for use *in vivo* it was necessary to determine the transcutaneous penetration of 980 nm wavelength light to the level of the peroneal nerve. Two anesthetized, male White New Zealand rabbits were used for these experiments. The output power of the laser was set at 1.0 or 4.0 W. Power density measurements were taken at the surface of the skin, sub-dermally, and at the level of the nerve. Laser parameters used in the *in vivo* studies were calculated based on data from the *in vitro* studies and the light penetration measurements. For the *in vivo* experiments, a total of 22 White New Zealand rabbits (2.34–2.89 kg) were used. Translated dosing parameters were refined in a pilot study using a transection model of the peroneal nerve in rabbits. Output powers of 2 and 4 W were tested. For the final set of *in vivo* experiments, the same transection nerve injury model was used. An energy density of 10 mW/cm^2 at the level of the peroneal nerve was selected and the laser parameters were further refined. The dosing parameters used were: 1.5 W output power, 43 seconds exposure, 8 cm^2 area and a total energy of 65 J.

Results: *In vitro*, 980 nm wavelength light at 10 mW/cm^2 significantly improved neurite elongation at energy densities between 2 and 200 mJ/cm^2. *In vivo* penetration of the infrared light measured in anesthetized rabbits showed that on average, 2.45% of the light applied to the skin reached the depth of the peroneal nerve. The *in vivo* pilot study data revealed that the 4 W parameters inhibited nerve regeneration while the 2 W parameters significantly improved axonal regrowth. For the final set of experiments, the irradiated group performed significantly better in the toe spread reflex test compared to the control group from week 7 post-injury, and the average length of motor endplates returned to uninjured levels.

Conclusion: The results of this study demonstrate that treatment parameters can be determined initially using *in vitro* models and then translated to *in vivo* research and clinical practice. Furthermore, this study establishes that infrared light with optimized parameters promotes accelerated nerve regeneration and improved functional recovery in a surgically repaired peripheral nerve.

Key words: immunolabeling; light therapy; motor end plates; peripheral nerve injury; photobiomodulation; regeneration; re-innervation; toe spread reflex