Integrating Low Level Light Therapy with 3D Printed Scaffolds for Improved Neural Tissue Regeneration

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Statement of Purpose: Neural tissue damage is common amongst injuries in emergency rooms and clinics worldwide. Unfortunately, current approaches to nerve repair do not promote full recovery of previous function in all cases, and fall prey to Wallerian degeneration. Scientists seek an appropriate method that can improve neural regeneration and promote full functional recovery. As an emerging 3D biomaterial scaffold fabrication technique, 3D printing is attracting increased interest in neural tissue regeneration. By using a patient specific design, researchers can tailor 3D printed implants to specific injuries, further increasing probability of complete recovery. On the other side, low-level light therapy (LLLT) is a powerful method for externally stimulating cell populations to grow faster than normal. Chen et al explored the effect of LLLT on different cell types [1] and found that wavelengths in the red and near infra-red range can upregulate NF-κB, while others reported additional explanations for increased cell proliferation [2]. To leverage both advantages of the 3D printing and LLLT, we created and 3D printed a transparent poly (ethylene glycol) diacrylate (PEG-DA)/PEG scaffolds and explored the response of PC-12 neural cells in the scaffolds to LLLT.

Methods: Scaffolds were first designed in computer aided design (CAD), and fabricated from PEG-DA/PEG using an in-house developed stereolithography based 3D printer. Briefly, PEG-DA and PEG were mixed in a 60:40 ratio, and supplemented with 1% by weight (of the PEG-DA) Irgacure 819 photoinitiator. Known volumes of resin were added serially as the printer cured each layer via UV laser radiation (Figure 1). Scaffolds measured approximately 2 mm in height after printing.

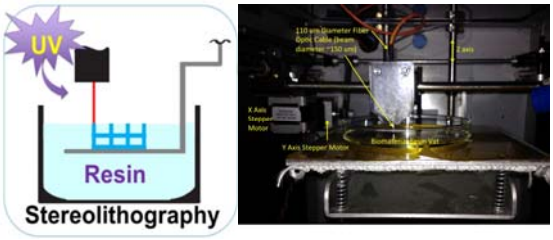

Figure 1: Schematic illustration of stereolithography based bioprinting process and picture of existing printer.

Before cell studies were conducted, scaffolds were sterilized in 70% ethanol and pre-wet in culture media for 24 hours. PC-12 cells were seeded at 30,000 cells per scaffold and irradiated with LED or laser LLLT once per day for 6 seconds. Cell response was quantified via MTS assay after 48 hours, and DNA content measured via PicoGreen assay at 2, 4, and 6 days of culture.

Results: This study showed significant increases in cell proliferation 24 hours after treatment with LLLT (Figure 2), which persisted after 6 days of culture (Figure 3). PC-12 cells rapidly proliferated when stimulated with LLLT, eventually reaching ~2-3 times that of non-stimulated controls.

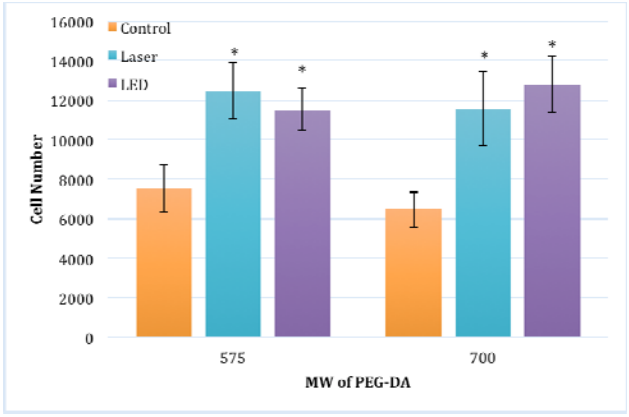

Figure 2: Cell response 24 hours after exposure to LLLT on scaffolds of different molecular weight. Data are mean ± standard error of the mean, n=9; * $p<0.05$.

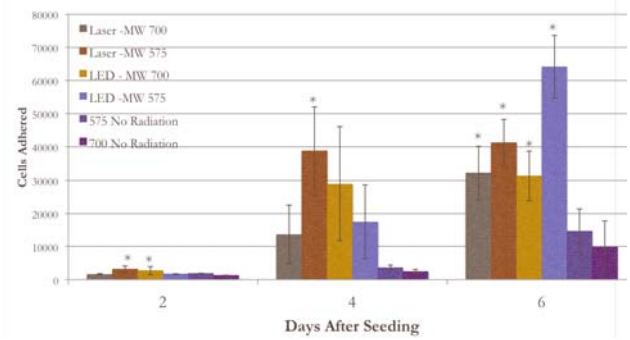

Figure 3: PC-12 cell proliferation on 3D printed scaffolds exposed to LLLT from laser and LED light sources. Data are mean ± standard error of the mean, n=9; * $p<0.05$.

Conclusion: This study shows that cells of neural lineage respond very favorably to LLLT, and can be coaxed to increase proliferation rate. Future work to be conducted will verify that this stimulation will still prove beneficial in a co-cultured environment with astrocytes, and explore whether or not LLLT has an effect on axon length or number of neurites when seeded on transparent 3D printed neural scaffolds.

References: 1. Chen AC-H, et al. (2011) Low-Level Laser Therapy Activates NF-kB via Generation of Reactive Oxygen Species in Mouse Embryonic Fibroblasts. *PLoS ONE*
2. Alghamdi, Khalid M, et al. (2012) "Low-level Laser Therapy: A Useful Technique for Enhancing the Proliferation of Various Cultured Cells." *Lasers in Medical Science* 27.1: 237-49.