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Enhanced neural stem cell differentiation in 3D printed scaffolds with light stimulation

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Introduction

Neural tissue can be damaged by a variety of degenerative diseases and trauma. Although repairing large nerve damage remains a challenging task, advances in cell-based therapeutics and tissue engineering are raising the possibility of replacing damaged nerves via artificial scaffolds to fulfill functional recovery. Recent studies focused on the use of advanced approaches to fabricate neural scaffolds with the aim of combining multiple stimuli to enhance cell functions on the tissue engineered scaffolds. 3D printing has shown promise for providing customized tissue scaffolds to structurally support and bridge the defect gap as well as deliver cells or various bioactive substances. Moreover, low-level light therapy (LLLT) presents positive effects on rehabilitation of degenerative nerves and neural disorders. Currently, LLLT is considered being a promising strategy to externally stimulate cells for improved function.

Materials and Methods

Our scaffold is composed of modified gelatin (gelatin methacrylate (GelMA)) and polyethylene glycol diacrylate (PEGDA), and printed with a stereolithography based 3D printer. Neural stem cells (NSCs) seeded on 3D printed scaffolds exposing to light stimulation were evaluated by detecting cell proliferation, and intracellular ROS synthesis. In addition, the differentiation of NSCs was examined by immunostaining and real-time quantitative reverse transcription polymerase chain reaction (qPCR).

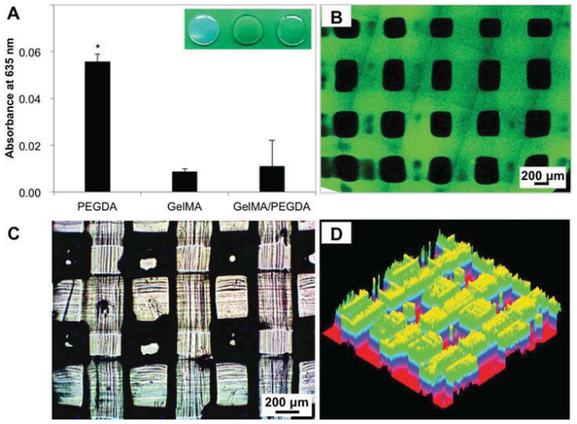

Figure 1. Characterization of 3D printed scaffolds. (A) Light absorbance at 635 nm on various materials, data are mean±standard deviation, n=3, *p<0.05 when compared to others. (B) Fluorescence dye stained scaffold. (C) Photograph of the printed scaffold, and, (D) 3D surface plotting image.

Neural stem cell growth under LLLT

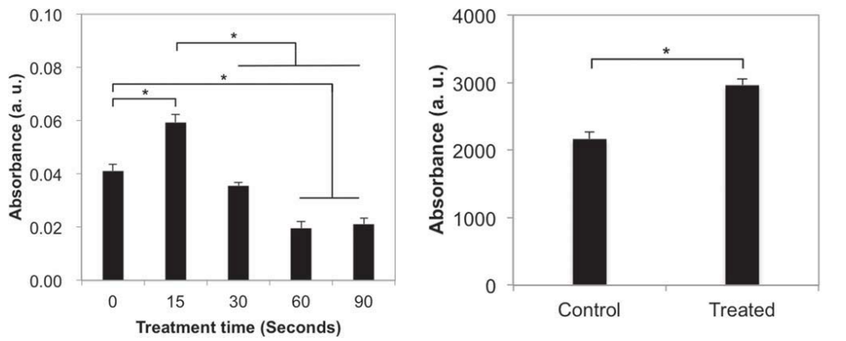

Figure 2. (A) NSC proliferation upon LLLT as a function of treatment (exposure) duration. (B) Light stimulation increased ROS synthesis in NSCs. Cells were treated with 15 s. Data are mean±standard deviation, n=9, *p<0.05.

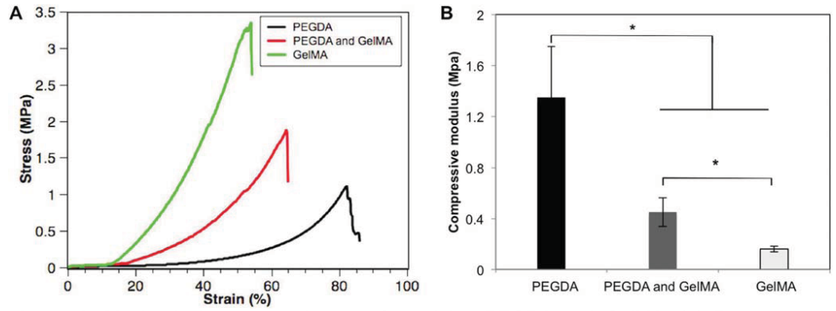

Figure 3. Mechanical property change after mixing PEGDA and GelMA. (a) Representative stress–strain curves of compression test, and, (b) relevant compressive moduli, data are mean±standard deviation, n=5, *p<0.05.

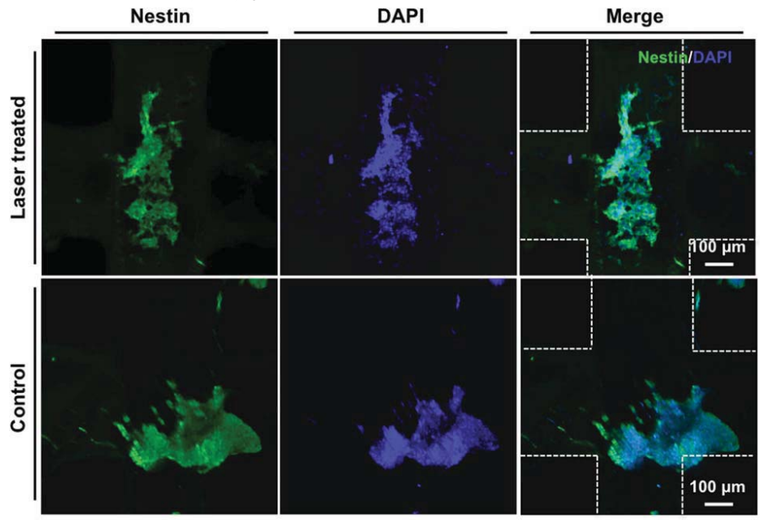

Figure 4. Distribution of nestin immunoprotein in NSCs with (top) and without (bottom) laser stimulation, green represents nestin, blue represents nuclei. Light stimulation maintained NSC functions as illustrated by normal nestin expression.

Cell differentiation

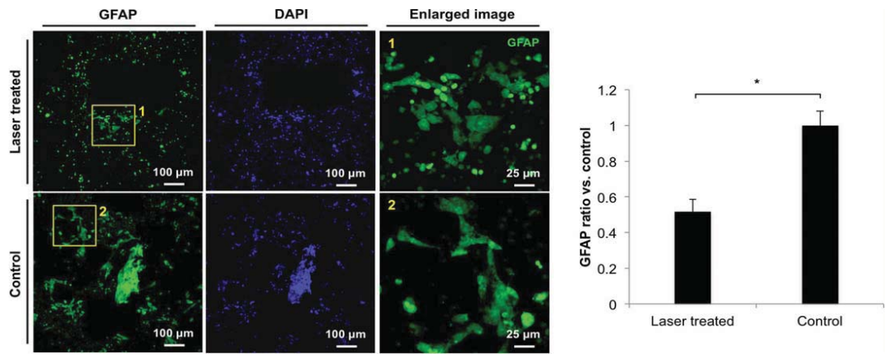

Figure 5. Differentiated NSC fluorescence images for the expression of GFAP after 14 d of culture and the quantification, data are mean±standard deviation, n=3 images each group *p<0.05. The GFAP expression of differentiated NSCs was inhibited by light stimulation.

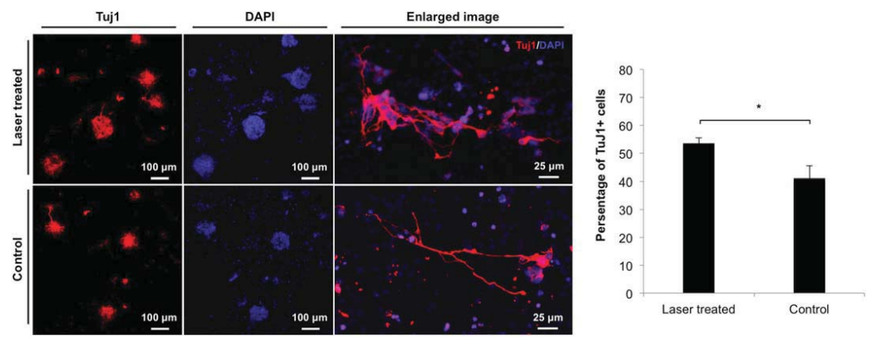

Figure 6. Immunocytochemical characterization of NSC-derived neurons at day 14, data are mean±standard deviation, n=3 images per group, *p<0.05. Cells are positive for neural maker Tuj1, and laser stimulation promoted neuronal differentiation.

Conclusions

This study shows that neural lineage of NSCs on 3D printed scaffold respond very favorably to LLLT, and can be coaxed to increase cell proliferation rate. ROS synthesis of NSCs was also promoted by laser stimulation. When NSCs were cultured on 3D printed scaffold and simulated by laser, it was demonstrated that the expression of glial cell marker was suppressed while positive neuronal population of cells was significantly enhanced. Gene expression levels of neuronal makers were also upregulated for the cells stimulated with laser, indicating the laser stimulation improved neuronal differentiation of NSCs. 3D printing technology is superior to many other conventional scaffold fabrication approaches regarding the design and controllability of architecture, and LLLT is a powerful adjunct strategy for improved cell function. Therefore, the integration of 3D printing and LLLT might provide a new methodology for neural regeneration.

Acknowledgements

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