Developing Novel Dopamine Based Printing Ink for Promoting Neural Stem Cell Differentiation

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Introduction

Nerve repair and regeneration remain great challenging problem worldwide because of the extremely weak inherent regenerative capacity and fibrosis in native nerve. Inadequate and unsatisfied clinical therapeutics encourage the development of novel strategies to promote the nerve regeneration. The goal of our study is to fabricate 3D gelatin methacrylate(GelMA)- dopamine(DA)- neural scaffolds with hierarchical structures via our table-top stereolithography-based printer, and then investigate neural differentiation of neural stem cells (NSCs) in our designed scaffolds.

Overview and characterization

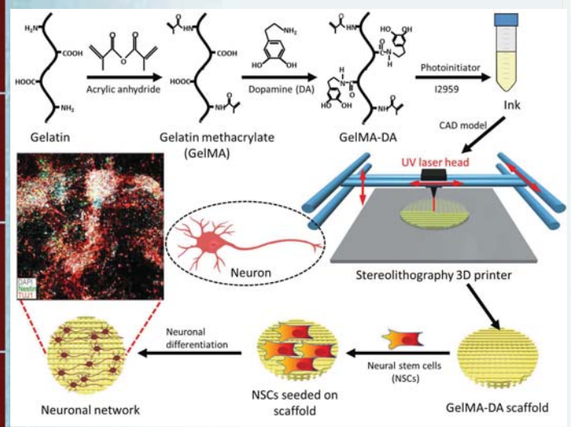

Figure 1. Schematic illustration of this study

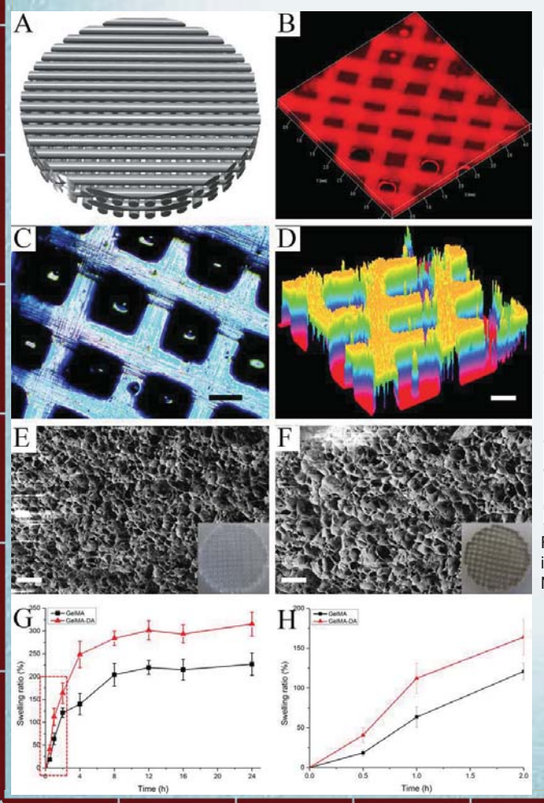

Figure 2. (A) Pre-designed CAD 3D scaffold model. (B) Fluorescence micrographs (Texas Red-X phalloidin), (C) light microscope image, and (D) surface plot of a 3D printed scaffold; Scale bar = 200 μm. Scanning electron micrographs (cross-sectional view) of (E) GelMA and (F) GelMA-DA porous matrices. Scale bar = 10 μm. The inset images are photographs of the corresponding scaffolds. (G) The swellability of GelMA and GelMA-DA matrices. (H) The magnifying plot of G in 0-2 h. Data are mean ± standard deviation; n = 5.

Cell proliferation

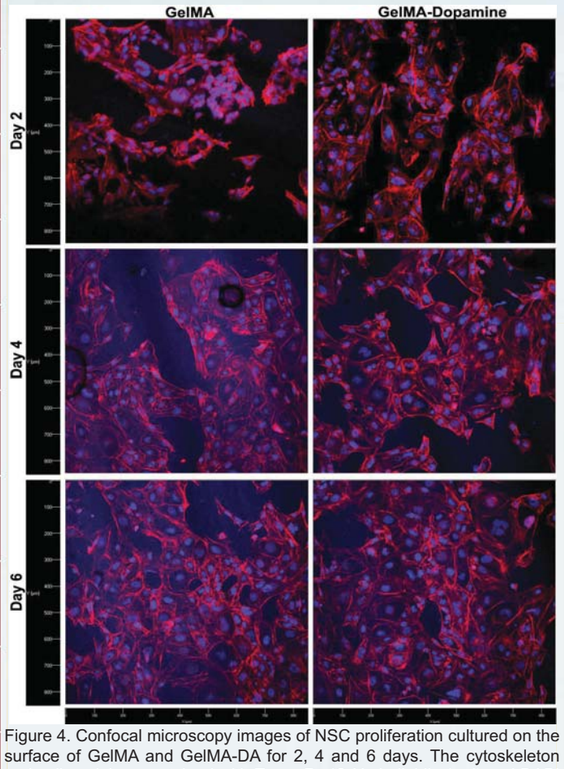

Figure 4. Confocal microscopy images of NSC proliferation cultured on the surface of GelMA and GelMA-DA for 2, 4 and 6 days. The cytoskeleton and cell nuclei were stained with Texas Red®-X phalloidin (red) and DAPI (blue), respectively.

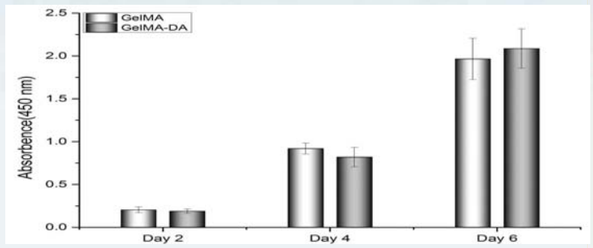

Figure 5. Proliferation of NSC cultured on the GelMA and GelMA-DA scaffold for 2, 4, and 6 days. The cells were quantified by CCK-8 assay. Data are a mean ± standard deviation, n = 8.

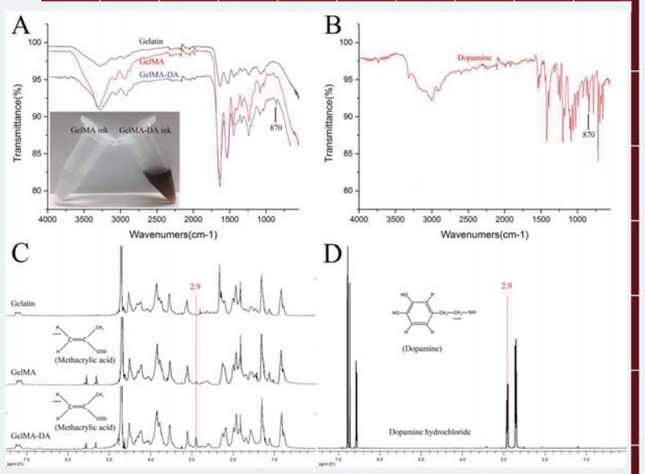

Figure 3. (A) FTIR and (C)^{1}H-NMR spectra of gelatin, GelMA, GelMA-DA. The inset images are the GelMA and GelMA inks, respectively. (B) FTIR and (D) 1H NMR spectra of Dopamine (DA).

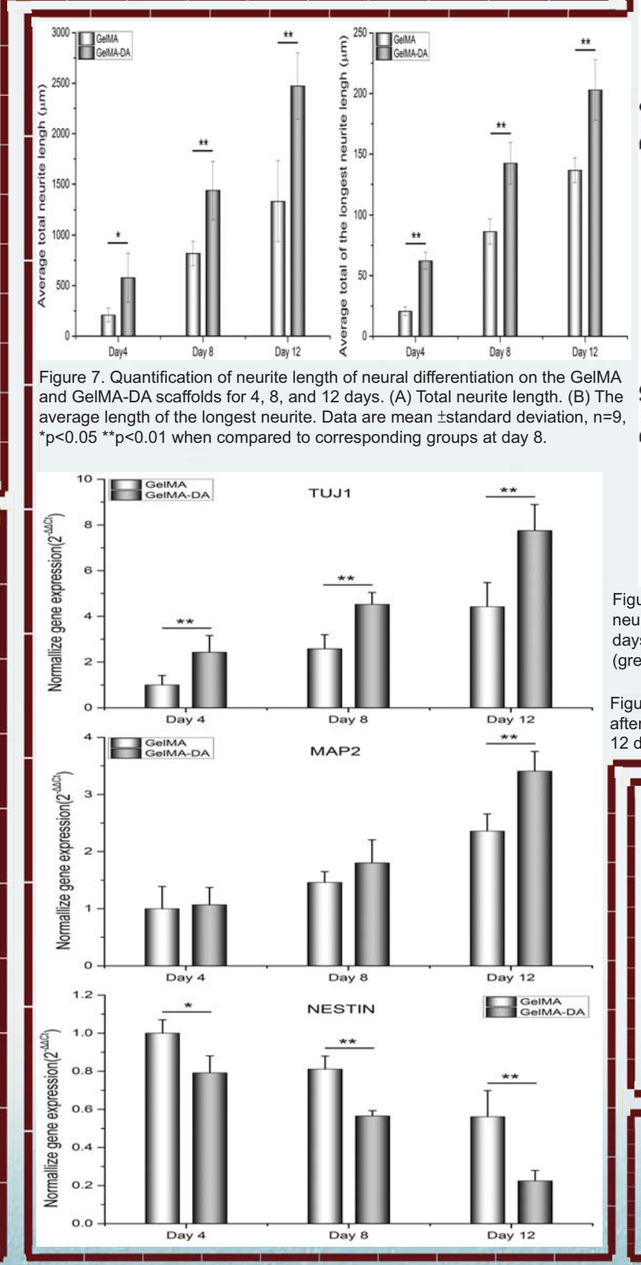

Figure 7. Quantification of neurite length of neural differentiation on the GelMA and GelMA-DA scaffolds for 4, 8, and 12 days. (A) Total neurite length. (B) The average length of the longest neurite. Data are mean ±standard deviation, n=9, *p<0.05 **p<0.01 when compared to corresponding groups at day 8.

Neural differentiation

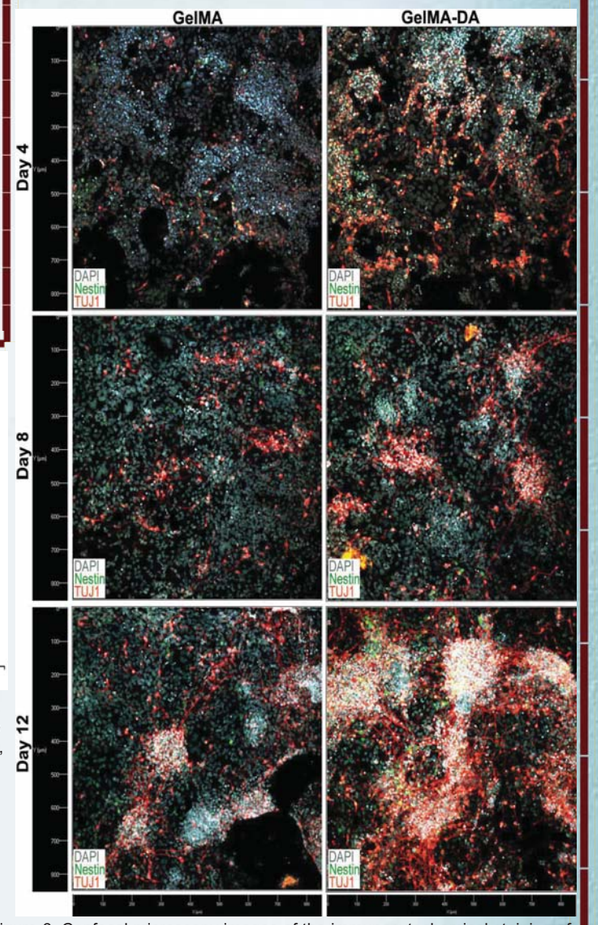

Figure 6. Confocal microscopy images of the immunocytochemical staining of neural differentiation on the GelMA and GelMA-DA scaffold for 4, 8, and 12 days. The neuron, NSCs and cell nuclei were stained by TUJ1 (red), Nestin (green) and DAPI (gray), respectively.

Figure 8. Normalized gene expression (TUJ1, MAP2, and NESTIN) of NSCs after neural differentiation on the GelMA and GelMA-DA scaffold for 4, 8 and 12 days. Data are mean ± standard deviation, n = 6. *p < 0.05 and **p < 0.01.

Conclusion

A customizable 3D printed dopamine-modified GelMA scaffold supporting stem cell growth and synergistically improving neural differentiation of NSCs. The resultant analysis demonstrated that a significant neural network was conducted on 3D printed GelMA-DA scaffold after 12 days. In particular, the neuron related TUJ1 and MAP2 genes expression upregulated while the NSCs related NESTIN gene expression downregulated during the neural differentiation process. These findings illustrates customizable 3D printed GelMA-DA scaffolds can promote neural differentiation and show great promise for neural tissue engineering.

Acknowledgments

The authors would like to thank the financial support from March of Dimes Foundation's Gene Discovery and Translational Research Grant and the George Washington University Center for Microscopy and Image Analysis for imaging support.