3D scaffolds via Multi-Photon Polymerization as a co-culture system for application in peripheral nervous system regeneration.

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Multi-Photon Polymerization (MPP), a Direct Laser Writing (DLW) technique, has found application in the field of Tissue Engineering (TE), due to the ability of fabrication of high precision scaffolds that can be used as a cell culture substrate [1]. Of great importance is the Peripheral Nervous System (PNS) Tissue Engineering and Regeneration which shows increasing potential as an alternative to established methods, namely surgery and grafts, that aim to counter PNS-related diseases and damage. A femtosecond fiber laser operating at 780nm (pulse duration:120fs, repetition rate:80MHz) was utilized to fabricate a novel pyramid-shaped scaffold geometry (400μm×400μm×60μm) using an organic/inorganic hybrid material [2]. The scaffolds were used as a substrate for the mono- and co-culture of murine neuronal N2a and glial Schwann (SW10) cells for 7, 14 and 21 days with flat glasses as controls. Comparison between scaffolds and controls revealed cell and neurite directionality that was highly influenced by the presence of scaffold topography vs the random orientation controls exhibited, due to the cell responses to the topographical cues provided. In addition, the co-culture system provided a favorable environment for longer neurite formation after 21 days compared to mono-cultures, showing a 2-fold increase in neurites longer than 40μm (31.4%±5.5% vs 15.4%±5.4% of total neurites respectively), indicating a possible synergistic effect of co-cultures and scaffold topography [3]. These findings suggest the ability to control neurite length and directionality, which are crucial parameters in PNS TE, and could form the basis for the development of an *in vitro* model for the study of PNS-related diseases.

Keywords: Multi-photon polymerization (MPP), Tissue regeneration, Peripheral Nervous System (PNS), Co-culture system, Scaffold Topography, Cell Orientation, Neurite Directionality

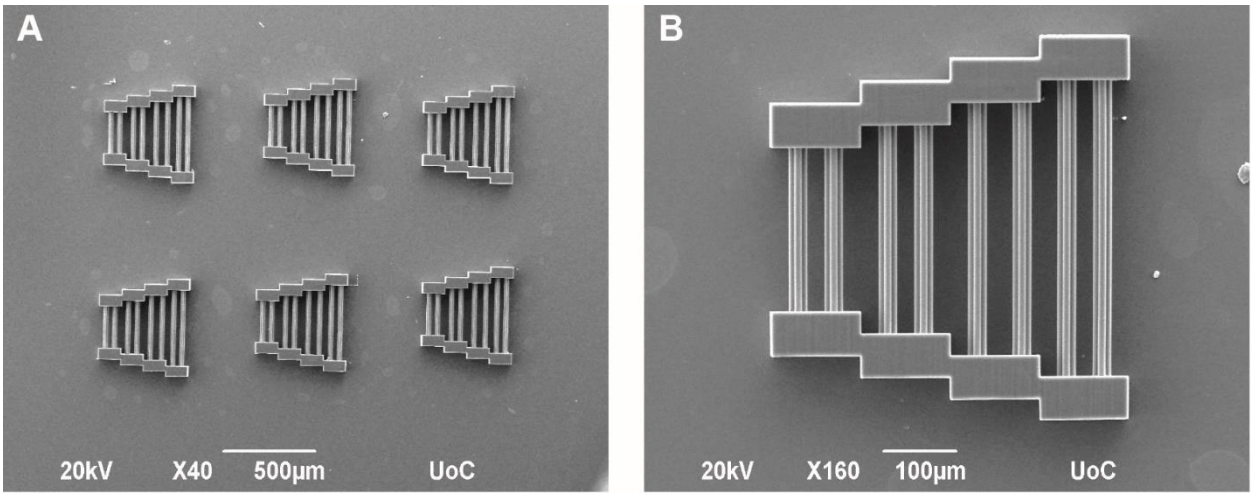

Figure 1: SEM image of 3D scaffolds for cell cultures. A: typical coverslip with 6 scaffolds for cell cultures. B: Magnification of a single scaffold.

References

[1] Nguyen, A. K. and Narayan, R. J., (2017), Materials Today, 20, (6):314-322

[2] Ovsianikov A. et al., (2008), ACS Nano., 2:2257-2262

[3] Kordas A., Manganas P. et al., (2022), Materials, 15: 4349-4365