# 3D scaffolds via Multi-Photon Polymerization for the directed neurite development in co-culture systems

Antonis Kordas[1,2,*], Phanee Manganas[1], Anthi Ranella[1], Maria Farsari[1]

[1]*Institute of Electronic Structure and Laser, Foundation for Research and Technology-Hellas (FORTH-IESL), 100 Nikolaou Plastira Street, Heraklion, 70013, Greece*
[2]*Department of Materials Science and Technology, University of Crete, Vassilika Vouton, Heraklion, 71409, Greece*

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, 2]. Of great importance is the Peripheral Nervous System (PNS) Tissue Engineering which shows increasing potential as an alternative to established methods, namely surgery and grafts, that aim to counter PNS-related diseases and damage. A novel bridge-shaped 3D scaffold design was fabricated using a femtosecond fibre laser operating at 780nm (pulse duration: 120fs, repetition rate: 80MHz) using an already established organic/inorganic hybrid material [3]. The fabricated scaffolds had suitable dimensions (400μm x 400μm x 60μm) for the mono- and co-cultures of murine neuronal N2a and glial Schwann (SW10) cells at three timepoints (7, 14, 21 days). The cultures exhibited scaffold-dependent cell and neurite directionality compared to flat glass cultures which were used as controls and showed a completely random orientation. This highlighted the impact of scaffold topography in cell behavior which was significantly influenced in the presence of scaffolds. Additionally, longer neurites have been favored in scaffold co-culture systems compared to N2a scaffold mono-cultures after 21 days (a percentage of 31.4% ± 5.5% compared to a percentage of 15.4% ± 5.4% respectively), indicating a synergistic effect of scaffold topography and SW10 cells [4]. These findings support the ability of controlling neurite directionality and length, properties deemed crucial for practical applications such as treatment of PNS damage and are expected to contribute to the development of an *in vitro* model for the study of neurodegenerative diseases and understanding of key cellular responses such as myelination.

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

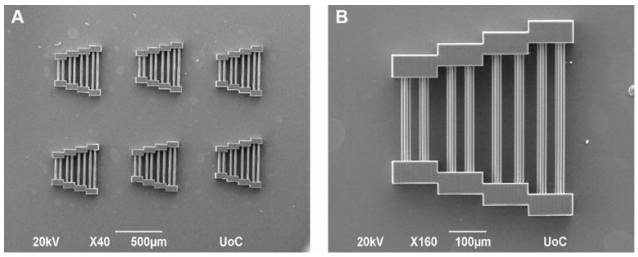


Figure 1: SEM image of 3D scaffolds for cell cultures. A: typical coverslip with 6 scaffolds for cell cultures. B: Magnification of a single scaffold [4].

## References

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* antkor89@iesl.forth.gr