Auxetic Scaffolds via Multiphoton Lithography for Neuroregeneration

Andreas Parlanis[1,2,*], Maria Farsari[1], Anthi Ranella[1]

[1] Foundation for Research and Technology - Hellas (FORTH), Institute of Electronic Structure and Laser (IESL), Heraklion, Greece
[2] Department of Biology, University of Crete, Heraklion, Greece
*Corresponding author email: andreasparlanis@iesl.forth.gr

Introduction

Multiphoton lithography is an additive manufacturing technology that can generate 3D structures. The beam's high intensity can cause the absorption of two or more photons within the focal volume of a photocurable material, leading the material to crosslink locally. Auxetic materials are materials with a negative Poisson ratio that possess exceptional mechanical characteristics and have been highlighted as potential candidates for the fabrication of porous scaffolds [1]. The objective of this work is to investigate the auxetic effect on the differentiation of neural stem cells.

Materials and Methods

The design of the auxetic and non-auxetic concentric cylinders was carried out using SolidWorks and fabricated via MPL. The material that was employed was the biocompatible hybrid organic-inorganic photoresist material SZ2080, and the auxetic mechanical properties of the scaffolds were determined via MicroTester LT. Neuronal stem cell lines (NE-4C) were employed, and SEM was performed for their morphological characterization as well as immunocytochemical assays that assessed the ability of the cells to perceive and convert mechanical stimuli into biochemical signals that result in intracellular changes, focusing on vinculin activity, TAZ translocation, and actomyosin contractility.

Results and discussion

Auxetic concentric cylinder scaffolds with a bowtie design for unit cell, and non-auxetic scaffolds with a feature size of 5μm were successfully fabricated. The Poisson's ratio exhibited by the auxetic scaffolds differed from that of the non-auxetic scaffolds, as evidenced by the presence of negative values. Moreover, regarding the SEM images, it can be observed that the cells have successfully infiltrated the scaffolds. In addition, utilizing confocal microscopy images, we observed the maturation of the focal adhesions via vinculin detection, the TAZ nuclear/cytoplasm translocation, and the phosphorylation of myosin. The results suggest that there were distinct differences in the behavior of cells within auxetic scaffolds compared to the cells within non-auxetic scaffolds.

Conclusions

The auxetic concentric cylinder scaffolds fabricated via MPL have shown encouraging indications for enhancing the biological performance of NE-4C stem cells, inducing their differentiation into neuronal lineages, and also for their use as models to study neuroregeneration as well as future implants.

References: [1] Flamourakis G et al. Macromol Mater Eng. 2020