



LASERLAB-EUROPE

The Integrated Initiative of European Laser Research Infrastructures III

Grant Agreement number: 284464

Work package 30 –Laser and Photonics for Biology and Health (BIOPTICHAL)

Deliverable D30.3

Technologies for MP polymerization and biofunctionalization of polymer scaffolds

Lead Beneficiary: FORTH

Due date: Month 36

Date of delivery: Month 36

Project webpage: www.laserlab-europe.eu

<i>Deliverable Nature</i>	
R = Report, P = Prototype, D = Demonstrator, O = Other	R
<i>Dissemination Level</i>	
PU = Public PP = Restricted to other programme participants (incl. the Commission Services) RE = Restricted to a group specified by the consortium (incl. the Commission Services) CO = Confidential, only for members of the consortium (incl. the Commission Services)	PU

A. Abstract / Executive Summary

Deliverable 30.3 is related to the realization of workstations for multiphoton polymerization that enable the generation of appropriate micro- nano-structures and 3D scaffolds to be used in a variety of applications in basic cell biology studies, tissue engineering and regenerative medicine. Such microstructures can act as cell culture platforms enabling the study of real time live cell responses under different environment (e.g. topography & chemistry) or allowing the culturing or co-culturing of cells in an environment similar to *in vivo* conditions. Biomimetic 3D scaffolds can be created using Direct Laser Writing with different types of materials both conventional and self-assembling, organic-inorganic hybrid and/ or biodegradable photopolymers. These scaffolds can be used in a number of applications ranging from bone and hard tissue regeneration to amyloid plaques associated to neurodegenerative diseases.

B. Deliverable Report

1 Introduction

Within the Objective Nanobiophotonics FORTH has been working in close collaboration with VULRC and ILC towards the development of standalone workstations for multiphoton polymerization. VULRC has been working on developing a workstation for MP polymerization and methodologies to produce regular matrices of biological molecules. ILC built a prototype of the photopolymerization / micromachining workstation. At the same time, FORTH could generate laser-fabricated three-dimensional conical microstructures and biomimetic 3D scaffolds for tissue engineering and regenerative medicine applications. The work related to the completion of the Deliverable is described in detail in the following sections, while all relevant publications are listed at the References section.

2 Objectives

Objective 1: Nanobiophotonics

The objective is focused on the realization of workstations and techniques that will enable the manipulation and the analysis of biological samples at the level of a single molecule or cell, using pulsed laser sources. Within this framework FORTH has been involved in the development of technologies for multi-photon (MP) polymerization that enabled the generation of laser-fabricated three-dimensional conical microstructures and biomimetic 3D scaffolds for tissue engineering and regenerative medicine applications.

3 Work performed / results / description

FORTH has been developing and studying biomimetic 3D scaffolds for cell studies, tissue engineering and regenerative medicine applications. To this end, FORTH has been researching the fabrication by Direct Laser Writing of conventional and self-assembling scaffolds, particularly for bone regeneration (Figure 1 a and b, respectively), using pure proteins (such as BSA and Avidin), organic-inorganic hybrid and/ or biodegradable photopolymers. It has been shown that the scaffold topography and porosity can greatly influence the cell survival, proliferation, and differentiation. Such approaches are also based on the surface functionalization of peptide fibrils onto 3D polymeric scaffolds for hard tissue regeneration. For example amyloid fibrils with specific morphological and structural properties (self-assembled nanostructures, controllable sequence), associated with a large group of neurodegenerative diseases. The biomineralization of such amyloid peptides produce three-dimensional organic-inorganic hybrid material scaffolds presenting interweaving bundles of crystallites similar to the morphology of dental enamel.

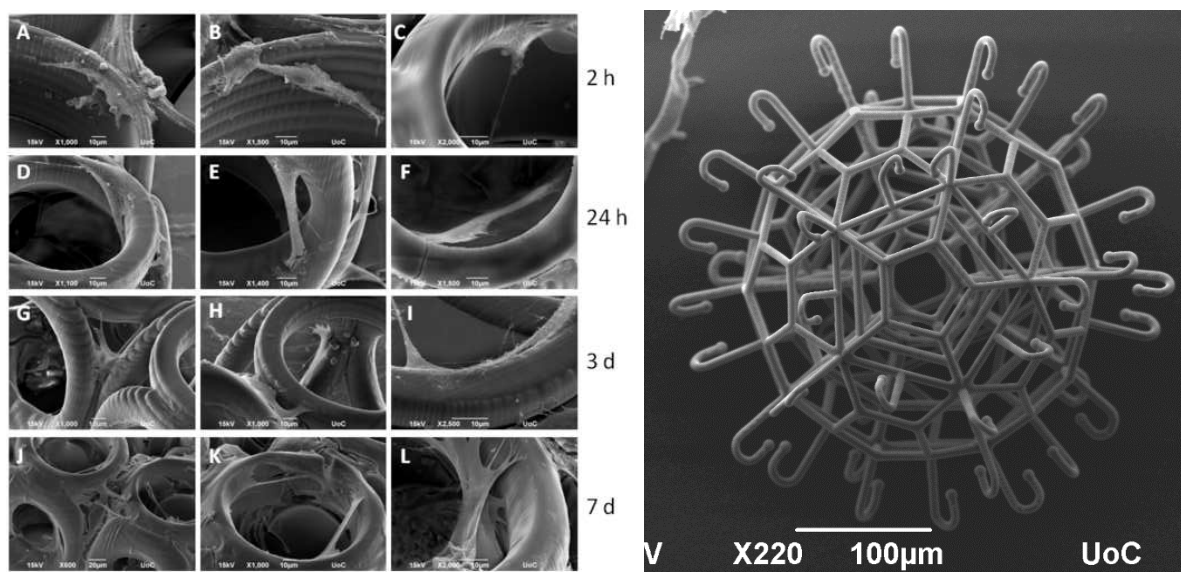


Figure 1: a) A conventional ring scaffold with human bone marrow mesenchymal stem cells and b) a self-assembling scaffold.

4 Conclusions

Laser-fabricated scaffolds exhibiting different geometrical characteristics were used as cell culture substrates. It was shown that topography could influence the adhesion, morphology and differentiation of cultured cells. These results define a useful experimental approach to influencing cell responses by proper selection of surface micro-texture. The patterned substrates presented here could potentially be used as model scaffolds for the systematic exploration of the role of 3D micro-topography on cell differentiation and neural network outgrowth.

5 References/Publications

1. R. Nazir, E. Balčiūnas, D. Buczyńska, F. Bourquard, D. Kowalska, D. Gray, S. Maćkowski, M. Farsari, D.T. Gryko, Donor–Acceptor Type Thioxanones: Synthesis, Optical Properties, and Two-Photon Induced Polymerization. *Macromolecules*, 48 (8), 2466-2472 (2015).
2. M. Chatzinikolaidou, S. Rekstyle, P. Danilevicius, C. Pontikoglou, H. Papadaki, M. Farsari, M. Vamvakaki, Adhesion and growth of human bone marrow mesenchymal stem cells on precise-geometry 3D organic–inorganic composite scaffolds for bone repair, *Materials Science and Engineering: C*, 48 (2015) 301-309.
3. A. Selimis, V. Mironov, M. Farsari, Direct laser writing: Principles and materials for scaffold 3D printing, *Microelectronic Engineering*, 132 (2015) 83-89.
4. P. Danilevicius, L. Georgiadi, C.J. Pateman, F. Claeysens, M. Chatzinikolaidou, M. Farsari, The effect of porosity on cell ingrowth into accurately defined, laser-made, polylactide-based 3D scaffolds. *Applied Surface Science*, 336, 2-10 (2015).
5. R. Nazir, P. Danilevicius, A.I. Ciuciu, M. Chatzinikolaidou, D. Gray, L. Flamigni, M. Farsari, D.T. Gryko, π -Expanded Ketocoumarins as Efficient, Biocompatible Initiators for Two-Photon-Induced Polymerization, *Chemistry of Materials*, 26 (2014) 3175-3184.
6. K. Terzaki, M. Kissamitaki, A. Skarmoutsou, C. Fotakis, C.A. Charitidis, M. Farsari, M. Vamvakaki, M. Chatzinikolaidou, Pre-osteoblastic cell response on three-dimensional, organic-inorganic hybrid material scaffolds for bone tissue engineering. *Journal of Biomedical Materials Research Part A*, 101A (8), 2283-2294 (2013).

7. K. Terzaki, E. Kalloudi, E. Mossou, E.P. Mitchell, V.T. Forsyth, E. Rosseeva, P. Simon, M. Vamvakaki, M. Chatzinikolaïdou, A. Mitraki, M. Farsari, Mineralized self-assembled peptides on 3D laser-made scaffolds: a new route toward 'scaffold on scaffold' hard tissue engineering. *Biofabrication*, 5 (4), 045002 (2013).
8. A. Skarmoutsou, G. Lolas, C.A. Charitidis, M. Chatzinikolaïdou, M. Vamvakaki, M. Farsari, Nanomechanical properties of hybrid coatings for bone tissue engineering. *Journal of the Mechanical Behavior of Biomedical Materials*, 25, 48-62 (2013).