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Microfabrication and Non-Invasive Imaging of Porous Osteogenic Structures Using Laser-Assisted Technologies

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Abstract : A major concern in bone tissue engineering is to develop complex 3D architectures that mimic the natural cells environment, facilitate the cells growth in a defined manner and allow the flow transport of nutrients and metabolic waste. In particular, porous structures of controlled pore size and positioning are indispensable for growing human-like bone structures. Another concern is to monitor both the structures and the seeded cells with high spatial resolution and without interfering with the cells natural environment. The present approach relies on laser-based technologies employed for fabricating porous biomimetic structures that support the growth of osteoblast-like cells and for their non-invasive 3D imaging. Specifically, the porous structures were built by two photon polymerization –direct writing (2PP_DW) of the commercially available photoresists IL-L780, using the Photonic Professional 3D lithography system. The structures consist of vertical tubes with micrometer-sized heights and diameters, in a honeycomb-like spatial arrangement. These were fabricated by irradiating the IP-L780 photoresist with focused laser pulses with wavelength centered at 780 nm, 120 fs pulse duration and 80 MHz repetition rate. The samples were precisely scanned in 3D by piezo stages. The coarse positioning was done by XY motorized stages. The scanning path was programmed through a writing language (GWL) script developed by Nanoscribe. Following laser irradiation, the unexposed regions of the photoresist were washed out by immersing the samples in the Propylene Glycol Monomethyl Ether Acetate (PGMEA). The porous structures were seeded with osteoblast like MG-63 cells and their osteogenic potential was tested in vitro. The cell-seeded structures were analyzed in 3D using the digital holographic microscopy technique (DHM). DHM is a marker free and high spatial resolution imaging tool, where the hologram acquisition is performed non-invasively i.e. without interfering with the cells natural environment. Following hologram recording, a digital algorithm provided a 3D image of the sample, as well as information about its refractive index, which is correlated with the intracellular content. The axial resolution of the images went down to the nanoscale, while the temporal scales ranged from milliseconds up to hours. The hologram did not involve sample scanning and the whole image was available in one frame recorded going over 200µm field of view. The digital holograms processing provided 3D quantitative information on the porous structures and allowed a quantitative analysis of the cellular response in respect to the porous architectures. The cellular shape and dimensions were found to be influenced by the underlying micro relief. Furthermore, the intracellular content gave evidence on the beneficial role of the porous structures in promoting osteoblast differentiation. In all, the proposed laser-based protocol emerges as a promising tool for the fabrication and non-invasive imaging of porous constructs for bone tissue engineering. Acknowledgments: This work was supported by a grant of the Romanian Authority for Scientific Research and Innovation, CNCS-UEFISCDI, project PN-II-RU-TE-2014-4-2534 (contract 97 from 01/10/2015) and by UEFISCDI PN-II-PT-PCCA no. 6/2012. A part of this work was performed in the CETAL laser facility, supported by the National Program PN 16 47 - LAPLAS IV.

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