Femtosecond Laser Micropatterning of Chitosan Thin Films For Surface Functionalization

A. Daskalova[1], I. Bliznakova[1], A. Trifonov[2], A. Popatanasov[2], H. Declercq[3], C. Nathala[4,5], A. Ajami[4], W. Husinsky[4], I. Buchvarov[2]

1. Institute of Electronics, Bulgarian Academy of Sciences, 72, Tsarigradsko Chaussee blvd., 1784 Sofia, Bulgaria
2. Department of Physics, Sofia University, "St. Kliment Ohridski", 5, J. Bourchier Blvd., BG-1164 Sofia, Bulgaria
3. Department of Basic Medical Sciences, Ghent University, De Pintelaan 185 6B3, 9000 Gent, Belgium
4. Institute of General Physics, Vienna University of Technology, Wiedner Hauptstr. 8-10/134, A-1040 Wien, Austria
5. Spectra-Physics Vienna, Fernkorngasse 10, 1100 Wien, Austria

The possibility to control surface properties of materials and to tailor behaviour of cells and biomolecules are the basic requirements in the development of a new generation biomaterials for applications in tissue engineering. Surface patterning on micro and nano- scale is critical to distinguish the effects of cell shape, focal adhesion, and ligand input for cell functions. Recently, much attention has been paid to laser-assisted micro and nanofabrication technologies to pattern surfaces with different topographies for providing valuable inside on cell-substrate junction [1]. Laser modification by pulses in the femtosecond time domain, provide a quality of modification of thin films of biopolymers that is unobtainable with longer pulses in the range of nanoseconds [2].
The interaction of femtosecond pulses with thin biopolymer films results in “foam formation”. The created foam simulates the natural environment of the fibrilar network of the extracellular matrix and permits the adhesion and growth of cells for fabrication of tissue scaffolds [3]. The typical morphology of microfoam consists of network of interconnected nanofibers simulating the complex structure of ECM Fig. 1 (a).

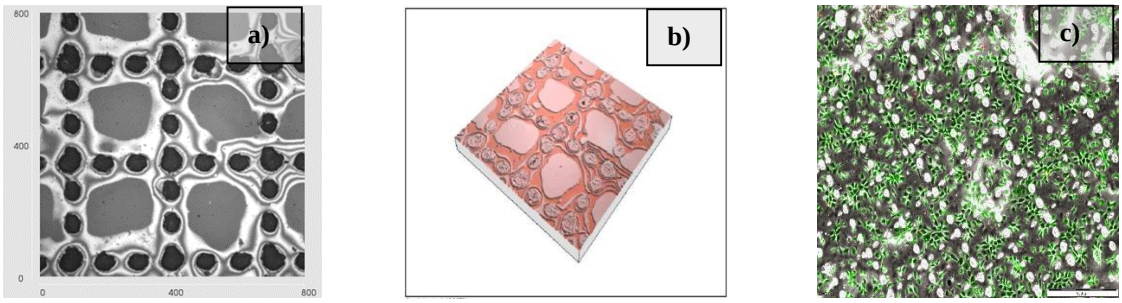

Fig. 1 Confocal microscope image of the laser induced modification of chitosan thin film ;(a-b) topography and 3D reconstructed image of chitosan grid after laser irradiation (N=1; τ=30fs, F= 0.17J/cm^2) ; (c) MC3T3 osteoblasts cell adhesion on laser treated chitosan film after 1 day culturing.

The basic cellular activities with biopolymer surfaces are related to different protein adsorption that takes place on topographically patterned scaffolds. For this purpose, chitosan substrates were treated by using an amplified Ti:sapphire laser system at λ=800 nm central wavelength with τ=30 fs and τ=150 fs pulse duration and repetition rate 1 kHz and 50 Hz, respectively. The structured surface of a biomaterial into arrays with different micro-geometries and architectures is analysed by means of SEM and confocal microscopy Fig. 1 (a-b). The size and shape as well as morphological forms occurring in the resulted areas of interaction were analyzed as a function of irradiation fluence, number of applied laser pulses and repetition frequency.The modified, on a micrometer scale, surface array is employed for cell-culture experiments for testing cell’s responses to substrate morphology. MC3T3 cells migration was monitored after 1 and 3days cultivation period using fluorescence microscopy Fig. 1 (c). The performed research proved that the processed chitosan based biofilms suite as a template for successful MC3T3 cell guidance and orientation. Femtosecond laser induced morphological modification of biomimetic materials exhibit direct control over cells behaviour due to induced change in their wettability state.

References

[1] T. Jiang, M. Deng, R. James, L. Nair, C. Laurencin, ’’Micro- and nanofabrication of chitosan structures for regenerative engineering’’ Acta Biomaterialia **10** 1632–1645 (2014).
[2] A. Sionkowska, H. Kaczmarek, M. Wisniewski, J. Skopinska, S. Lazare, V. Tokarev, „The influence of UV irradiation on the surface of chitosan films”, Surface Science **600** 3775–3779 (2006)
[3] Wen-Chuan Hsieh, Jiun-Jia Liau, and Yi-Jhong Li, „Characterization and Cell Culture of a Grafted Chitosan Scaffold for Tissue Engineering”, International Journal of Polymer Science **2015** 1-7 (2015)

Acknowledgements

This work was financially supported by the Bulgarian National Science Fund (NSF) under project № DH08/5-„Bioactivity improvement of biomimetic materials by texturing with ultra-short laser pulses" (2016-2018); EU project COST MP1301 NEWGEN and project FWO/BAS - “Functionalization of biomaterials modified by femtosecond pulses for cell adhesion and guidance improvement”(2016-2019).