Effect of topography and statin loaded micropatterned polymeric replicas on osteogenic differentiation

E. Kanakousaki*, P. Kavatzikidou, A. Manousaki, E. Stratakis, A. Ranella
Foundation for Research and Technology - Hellas (FORTH), Institute of Electronic Structure and Laser (IESL)
E. Kanakousaki
Department of Biology, University of Crete, Greece
E. Stratakis
Department of Physics, University of Crete, Greece

Introduction: Engineered microenvironments are offering mechanistic insights into how the extracellular matrix (ECM) and physical forces regulate stem cells, revealing how these control self-renewal, proliferation and differentiation potentials. The cells sense the ECM mechanics and spread via transcription regulator proteins. YAP/TAZ are considered as nuclear relays of mechanical signals exerted by ECM rigidity and cell shape and as master regulator of cell–ECM interaction. Statins are inhibitors of cholesterol biosynthesis and studies demonstrated their effect on stimulation of new bone formation [1]. Ultrafast pulsed laser irradiation is considered as a simple microfabrication method to produce structures controlling the structure geometry and pattern regularity [2]. Such structures with an anisotropy discontinuous topographical nature could enhance cellular growth and alignment (eg neuronal [3,4]). Soft lithography has been successfully used to transfer micro-sized patterns from silicon (Si) to polymeric surfaces allowing the in-depth study on cell behavior [5]. The aim of this study is to investigate the effect of topography and statins on osteogenic differentiation.

Experimental Methods: A series of micro-patterned Si structures were fabricated by ultrafast laser irradiation. Positive replicas of polymers have been successfully reproduced from the Si structures via soft lithography (Figure 1). Statin-loaded replicas were then produced and characterized by Scanning Electron Microscopy. Finally, the cytocompatibility and cytotoxicity of the statin-loaded replicas was investigated with mouse Mesenchymal Stem Cells (MSCs) C57BL/6 was evaluated.

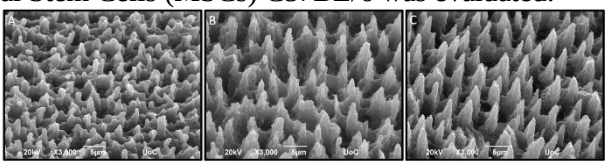

Figure 1:10% cellulose acetate micropatterned polymeric replicas (A) Low Roughness, (B) Medium Roughness and (C) High Roughness and magnification (x3000).

Results and Discussion: Cell mechanotransduction was analyzed via the cytoskeleton/nuclear organization and YAP localization, on the replicas. The effect of the replicas on MSCs fate was also studied. The surface roughness had an effect on the MSCs mechanotransduction and differentiation. The chemical composition and degradation rate influenced cell morphology and cell nuclear mechanics. The ability of our technique to control the cellular behavior could be potentially useful in understanding disease pathogenesis and for the development of patient-specific applications.

Acknowledgments: The authors would like to thank the European Project NFFA-Europe-Pilot (n. 101007417 from 1/03/2021 to 28/02/2026) for providing financial support to this project.

References

[1] Lee, T.-C. et al. J Bioact Compat Polym. **33(2)**, 160–177 (2018).
[2] Ranella A. et al. Acta Biomaterialia **6**, 2711 (2010).
[3] Simitzi C. et al. Biomaterials. **67**, 115-128 (2015).
[4] D Angelaki D. et al., Mater. Sci. Eng. C. **115**, 111144 (2020).
[5] Babaliari E. et al. Int. J. Mol. Sci. **19(7)**, 2053 (2018).

* ekanakousaki@iesl.forth.gr