Supplementary Materials: Bioinstructive Micro-Nanotextured Zirconia Ceramic Interfaces for Guiding and Stimulating an Osteogenic Response In Vitro

Livia Elena Sima ¹, Anca Bonciu ^{2,3}, Madalina Baciu ⁴, Iulia Anghel ², Luminita Nicoleta Dumitrescu ², Laurentiu Rusen ^{2,*} and Valentina Dinca ^{2,5,*}



Figure S1. SEM and AFM images of the lasers textured surfaces, depicting profiles and depths.

Sample type	Sample name	Topography	Rq 3d [µm]	Rq profil [µm]	Center-to- center 2 point distance- lenghts	Average trench height	Average top width
non- irradiated control	flat		0.123 (± 0.03)	0.22 (± 0.10)	NA	NA	NA
anisotropic structures	24 µm	wavy ridges	2(± 0.042)	3.03 (± 0.16)	23.3 (± 0.46)	3.39 (±0.797)	0.99 (± 0.22)
	33 µm ∏	rectangular ridge	1.4(± 0.15)	4.08 (± 0.28)	34.87 (± 0.19)	4.3 (± 0.01)	10 (± 0.89)
isotropic structures	24/24 μm #	wavy grating	2.3(± 0.2)	3.14 (± 0.44)	23.5 (± 0.6)	3.46 (± 0.025)	0.95 (± 0.1)
	33/33 µm #	square pillar	2.8(± 0.18)	2.48 (± 0.25)	35.2 (± 0.63)	4.53 (± 0.02)	11.09 (± 0.68)
	24/33 μm #	square pillar	2	1.92 (± 0.4)	34.8 (± 0.19) and 23.3 (± 0.55)	4.37 (± 0. 31)	5.14 (± 0.87)

Table S1. Average surfaces parameters as measured by profilometry, SEM and AFM.



Figure S2. Early attachment of hMSCs onto Zirconia microtopografies. Immunofluorescence microscopy depicting hMSCs onto each of the structured Zirconia substratum (**a**) upon labeling with antibodies for vinculin (red), and staining of actin filaments with Alexa Fluor 488-Phalloidin (green) and nuclei with Hoechst (blue) (scale bar = $50 \,\mu$ m). Representative cells are showed in detail (**b**) to depict the vinculin-labeled focal adhesions (scale bar = $20 \,\mu$ m). Image acquisition was performed using the TissueFAXS iPlus automatic system.



Figure S3. Cell morphology and mineralization granules analysis upon osteogenic differentiation of hMSCs onto Zirconia microtopografies. SEM images of cells cultured for 28 days on Zirconia bio-interfaces in osteoinductive media conditions. Low- and high-magnification SEM images are presented representing each of the structured Zirconia substratum along with granule size measurements before and after hMSCs culture and differentiation.