Effect of Micropattern Dimensions on Adhesion, and Nuclear Shape of Osteosarcoma Cells

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Statement of Purpose: Lithography techniques enable engineering of topographies with precision and reproducibility allowing production of surfaces decorated with geometric nano- and microstructures[1]. This kind of designed surfaces could be used to study cell-material interactions at nano and micro level about how cells sense and respond to their physical environment. Cells attach to substrates through their focal adhesions[2]. During adhesion, forces are generated at the perimeter of the cells and then transmitted to the cytosol and finally to the nucleus via cytoskeletal elements and certain nuclear membrane proteins[3]. Aim of this study was to show the effect of pillar dimensions and interpillar gap size of surface micropatterns on adhesion, and nuclear shape of Saos2 (human osteosarcoma) cells.

Methods: A silicon wafer array comprising of 9 different topographies with pillar widths in the range of 4-16 µm and interpillar gaps in the range of 4-16 µm (P: pillar size, G: gap size. P4G4 represents 4 µm pillars, 4 µm gaps) were produced using photolithography. PDMS copies were made and used as molds for solvent casting poly(lactic acid-co-glycolic acid) (PLGA) films. SEM shows that the surfaces were produced with high fidelity (Fig. 1A) and seeded with Saos2 cells. Alamar Blue assay was used to determine cell numbers on Day 1. Cells were fixed and stained with Alexa 532 Phalloidin and DRAQ-5 for confocal laser scanning microscopy (CLSM). Shapes of the nuclei were analyzed using MATLAB for extent and circle variance descriptors to study the influence of the surface decorations on cell nuclei morphology. Principal component analysis were conducted and statistical analysis were made using Welch's ANOVA and Games-Howell test ($p<0.05$).

Results: CLSM images of Saos2 cells show large oval nuclei when on unpatterned surfaces but on P4G4 they presented deformed, lobulated nuclei (Fig. 1B). Significantly higher number of cells adhered to P8G16 and P8G8 than P8G4, P16G16 than P16G8 ($p<0.05$) (Fig. 1C). Nucleus deformations observed on 9 surfaces were different from the smooth control with statistical significance ($p<0.05$) with P4G4 showing the most distinct deformation (Fig. 1D).

Conclusions: Gap size appears to have a more distinct effect on cell adhesion and nuclear shape than pillar size. As the gap size got smaller, cell adhesion also decreased. Nuclei deformed on every surface but smaller gap sizes have stronger deforming capacity. Micropatterned surfaces are good tools to study cell responses to the surfaces of the substrates which is important in developing better implants and also for studying inherent mechanisms of cell adhesion.

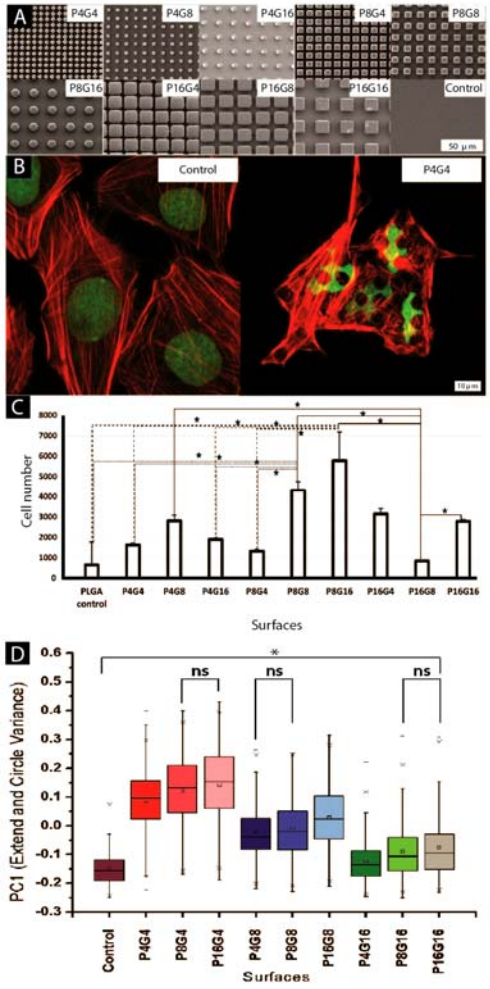

Figure 1. Attachment and deformation of nuclear shape of Saos2 cells on micropatterned substrates. (A) SEM images of the PLGA micropillar array. (B) CLSM images of Saos2 cells on unpatterned control and P4G4 surface (Green: nucleus, DRAQ5, red: cytoskeleton, Alexa 532). (C) Alamar Blue cell viability assay results for the surfaces on Day 1 showing optimum cell attachment on P8G16 ($p<0.05$). (D) Nuclear shape deformations analyzed by principal component analysis of extent and circle variance descriptors.

References:

1- Hasirci, V. Nanomedicine. 2006;1(1):73-90.
2- Ingber, D. E. The FASEB journal. 2006; 20(7), 811-827.
3- Starr, D. A. Annu. Rev. Cell Dev. Biol. 2010; 26, 421-444.