The Dimension of nano-grooves modulates the adhesion of osteoblast-like cells, MG-63, on silicon surfaces

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Statement of Purpose:

The interactions between cells and biomaterial surfaces are crucial to many biomedical fields. Cells seem to be sensitive to the scales much less than their size and responsive to many nanoscale signals, both chemical and topographic. Anisotropic topographic features of a surface have been shown to induce many cell types to align and to migrate along the direction of the anisotropy, a phenomenon called contact guidance. Microgrooves have been found to enhance cell orientation and spreading[1]. Furthermore, the width of the contact adhesions was determined by the width of the ridges on the underlying substrate. Nevertheless, the literature lacks the studies regarding the dependence of cell alignment and elongation on the dimension of nano-groove scales. In this study, we investigated the behavior of an osteoblast-like cell line, MG-63, on a series of nano-grooved silicon surface with different groove width. Cell orientation, elongation and spreading area are expressed in a time-dependent manner.

Methods:

Silicon wafers were patterned with grooves, 116.4, 215.4, 295.5, 380.6 or 460.0 nm in width and 80 nm in depth, while the width of ridges was 380 nm. The patterned silicon wafers were immersed in piranha solution (7/3 (v/v) of 98% H_2SO_4/30% H_2O_2) at 90°C for 20 min and then rinsed with deionized water prior to sterilization with 70% ethanol. MG-63 cells were seeded in MEM with 10% FBS and incubated for 4, 8, 24 and 72 hours. The adherent cells were observed by using SEM. Six areas were captured randomly in every sample. The outlines of the cells were traced manually and analyzed by using NIH Image J software. Elongation is defined by dividing the length of the major axis of the fitted ellipse to the length of the minor axis. Orientation is determined by measuring the angle between the directions of the long axis of the fitted ellipse and nano-grooves.

Results / Discussion:

After 4 h incubation, cells were found to align along the nano-grooved surfaces, while cells did not show any direction preference on the non-patterned surface. Figure 1 shows a SEM image of aligned cells on the surfaces with 380-nm-wide grooves. After one-day incubation, more than 90% of cells adhered on the surfaces with 295.5, 380.6 or 460.0 nm grooves extended along the direction of grooves (angles < 10°), while approximately 80% of cells adhered on the surfaces with 116.4 or 215.4 nm grooves aligned along the groove direction (Fig. 2). On the other hand, cells cultured on the non-patterned surface did not show any preferred orientations. Cells cultured on the nano-grooved surfaces also showed larger elongation in the order of 460.0 nm ≈ 380.6 nm ≈ 295.5 nm > 215.4 nm > 116.4 nm >> non-patterned surfaces (Fig. 3). However, we did not find any enhancement in cell spreading area on the nano-grooved surfaces. After 3 day culture, cells grew confluent. The cells grown on all the nano-grooved surfaces followed the direction of grooves, while cells grew randomly on the non-patterned surfaces.

We showed here that the alignment and elongation of the cells adhered to the nano-grooved surfaces depend on the width of grooves.

Conclusions:

This study shows that cells aligned along the direction of nano-grooved surfaces. The fact that cells grow along the grooves provides a mechanism for the biomaterial applications which needs cell alignment.

References:

1. B. Wojciak-Stothard, et. al., Exp Cell Res 223, 426 (1996).

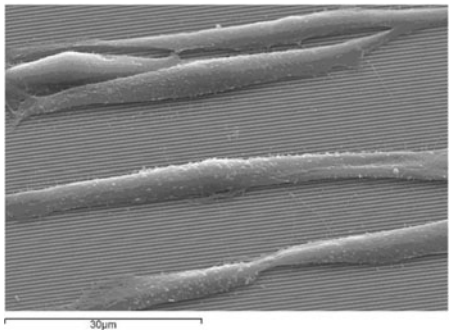

Fig. 1. SEM images of the aligned MG-63 cells.

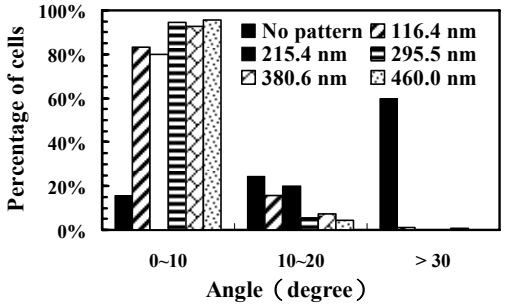

Fig. 2. Orientation of MG-63 cells on different surfaces after 1 day incubation

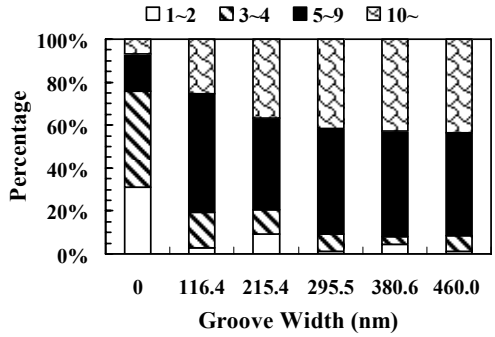

Fig. 3. Elongation of MG-63 cells on different surfaces after 1 day incubation