The Effects of Fibronectin on Cell Adhesion to the Substrata with Nano-grooves/ridges structure

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Introduction:

Cells are known to respond to chemical and topographic cues in their *in vivo* nanometrical environment. Previously, we found that osteoblast-like cells (MG-63) aligned along nanoscale grooves/ridges structures on silicon substrates[1]. However, serum adhesion proteins also influence cell adhesion onto artificial materials[2]. In this study, we investigated the behavior of MG-63 cells, on a series of nano-ridge surface pre-adsorbed with fibronectin (FN).

Methods:

Silicon wafers were patterned with nano-ridges, of which width is 90, 250, or 500 nm, separated by equal width of grooves with 215 nm in depth, and the ridges had the same width as the ridges. 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. Prior to cell culture, silicon chips were dipped in 0.1 mg/mL FN solution for 60 minutes and then rinsed with PBS twice. Control groups were the patterned substrata without FN-preadsorption. MG-63 cells were seeded in MEM with 10% FBS and incubated for 2, 4, 8 and 24 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. Cell width is represented by the length of minor axis obtained from fittest ellipse. Orientation is determined by the angle between the directions of the major axis of the fitted ellipse and nano-grooves.

Results / Discussion:

Preadsorption of FN onto the silicon substrata enhanced cell spreading. However, the alignment of cells along the direction of nanogrooves was decreased by FN pre-adsorption. As shown in Fig. 1, after 4-hr incubation, cell alignment on the FN-preadsorbed substrata was less than that on the control groups. Fig. 2 shows an example image of MG-63 cells on the FN-coated 90-nm substrata. The cell morphology was less elongated and showed polygonal appearance compared to the control surface. Similarly, the proportion of aligned nuclei was also decreased on the FN-coated surfaces. Nevertheless, after 24 hrs the cells on the FN-coated substrata became more elongated, comparable to the control groups. After 24 hrs incubation, the alignment of the cells on the FN-coated surfaces was also increased and compared to that on the surface without preadsorbed with FN on the 250-nm and 500-nm surfaces, but the cells on the 90-nm surface pre-adsorbed with FN was still much less oriented than the control.

Our results suggest that an increase in surface FN concentration enhance cell lateral expansion across grooves, resulting increased cell spreading area and decreased cell orientation. The phenomenon is especially profound on the substrata with the smallest feature, 90-nm grooves/ridges.

Conclusions:

The alignment and elongation of the cells adhered to the nano-grooved surfaces are influenced by the pre-adsorption of FN. The results of this study provide an assessment for the relative importance of surface nano-topography and adhesion proteins in cell adhesion behavior.

References:

1. W.-B. Tsai, et al., abstract at the 31st annual meeting of SFB, Pittsburg, Penn, April 26-29, 2006.
2. DiMilla, P.A., et al., J Cell Biol.1993;122(3): 729-737.

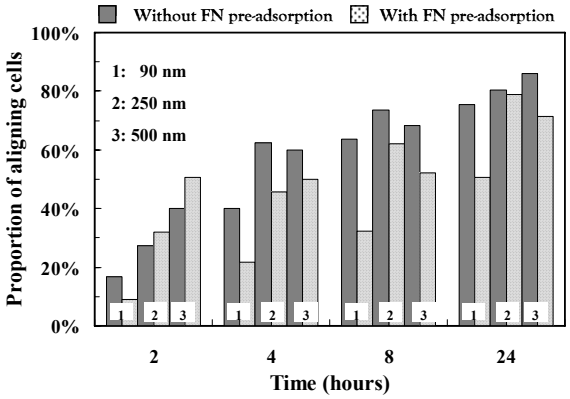

Fig. 1. Proportion of aligned cells (orientation angle < 10°) on different surfaces.

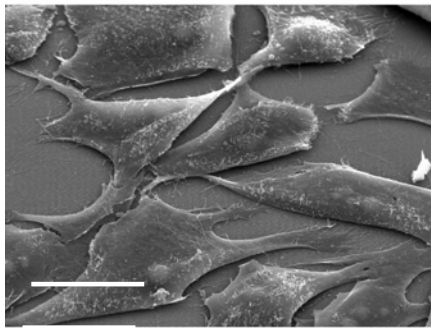

Fig. 2. SEM images of cells on the 90-nm-wide ridges pre-coated with FN. Bar = 20 μm.