CELL INTERACTION WITH MICROTEXTURED SURFACES

Ricci JL, Rose R, Charvet JK, Alexander H, Naiman CS*

Department of Bioengineering, Hospital for Joint Diseases Orthopaedic Institute, New York
*Orthogen Corporation, New York, NY

It has long been recognized that implant surface microtexture can influence tissue interaction. In previous studies we have examined the in vitro interaction of connective tissue fibroblasts with a variety of defined surface microgeometries, including microgrooved [1], roughened [2], and more complex surfaces [2]. In most cases, these surfaces, while having similar composition, have different (and pronounced) effects on the rate and direction of growth of fibroblast cell colonies. The mechanism of surface microgeometry's effect on cell colony growth rate is unknown. This study investigates the effect of defined surface microgeometry on connective tissue cell colony density, cell attachment area (spreading), and cell shape. The results suggest a possible basic mechanism of surface microgeometry control of attached cell growth.

Materials and Methods Rat tendon fibroblast (RTF) cells were grown as stock cultures from hindfoot extensor tendons from 14-day-old Sprague-Dawley rats. Second- to fourth-passage cells, grown in Dulbecco's Modified Eagle's Medium containing penicillin-streptomycin and 10% fetal bovine serum were used for all experiments. Cell colonies were grown on these surfaces using a "dot" culture model similar to explant culture models. These cells were suspended in solubilized collagen (Vitrogen, Celltrix, Palo Alto, CA) and 2-μL droplets containing 20,000 cells each were polymerized on the experimental surfaces, where they acted as sources of radiating cell colony growth. Light microscopy and image analysis methods were used to measure rate and direction of growth as well as cell density (cells/mm^2), cell attachment area (μm2), cell orientation (relative to substrate orientation), and cell elongation (eccentricity, the ratio of cell length to cell width). For individual cell measurements, 30 cells of each experimental group were measured. Experimental substrates consisted of solvent-cast polystyrene surfaces, vapor-deposited with 60 nm of TiO_2, molded from silicon wafer templates produced by optical lithography methods at the National Nanofabrication Facility at Cornell University (Ithaca, NY). Substrates consisted of mirror-smooth surfaces (controls) and square-wave microgrooves with ridges and grooves 1.75, 6.5, and 12 μm in size. Results were analyzed for statistical significance using t-tests.

Results All three microgrooved surfaces had a pronounced effect on cell colony growth, cell attachment area, cell eccentricity, cell density, and cell orientation (Table 1): they reduced cell colony growth and cell spreading, increased cell eccentricity (elongation), and effectively oriented the cells parallel to the surface. Cell density on all experimental surfaces was reduced relative to controls.

Table 1 Cell growth and shape parameters

	Surface			
	Control (smooth)	1.75-μm	6.5-μm	12.0-μm
Cell colony area after 8 days, mm^2 (S.D.)	83.8 (11.0)	85.6 (5.6)	36.6* (4.7)	39.3* (6.1)
Cell attachment area, μm^2 (S.D.)	2666.4 (1534.9)	604.4* (247.5)	621.1* (250.0)	934.0* (493.1)
Cell eccentricity, length/width (S.D.)	2.1 (0.9)	6.0* (2.6)	6.7* (3.4)	7.3* (3.4)
Cell density, cells/mm^2 (S.D.)	912.8* (135.5)	667.6* (115.8)	715.3* (114.9)	676.0* (134.6)
Cell orientation (relative to grooves)	Random	>90% within ±40°	100% within ±20°	>95% within ±20°

*$p < .05$

Discussion Well-defined surface microgrometries with the tested dimensions are effective at orienting cells, changing cell shape, and reducing cell growth rates. It is well known that attachment-dependent cells must attach and spread to trigger cell division [3]. The present results suggest that the growth inhibition effect demonstrated by these surfaces may be based on reduction of cell spreading by the surface microgrooves. These experiments suggest that observed differences in fibrous encapsulation of smooth vs. microtextured surfaces may be based on direct suppression of fibro- blast spreading and growth by microtextures. These microgeometries have potential application as implant surfaces for control of tissue integration.

References

1. Ricci JL, Charvet J, Chang R, Howard C, Green WS, Weiser L, Alexander H: Trans Orthop Res Soc, 19(2):302, 1994.

2. Ricci JL, Charvet J, Sealey R, Biton I, Green WS, Stuchin SA, Alexander H: Trans Soc Biomater, 18:114, 1995.

3. Folkman J, Moscona A, Nature 273:345 (1978)

Acknowledgment This work was funded by Orthogen Corporation through NSF SBIR Phase I award 9160684.

This work is dedicated to the memory of Dr. Charles S. Naiman.

LIBRARY.pdf