Cytoskeletal Organization in Three Fibroblast Variants Cultured on Micropatterned Surfaces

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Introduction. Implant surface geometry and microgeometry influence tissue responses to implants. The physical and chemical properties of synthetic substrates affect the morphology, physiology and behavior of cultured cells of various types. To date, studies of tissue-implant interaction have emphasized cell attachment, signalling and other cellular response mechanisms. Cellular attributes influenced by micrometric substrate features include cell shape, attachment, migration, orientation, and cytoskeletal organization.

We studied three phenotypic variants of a murine fibroblast cell line to explore the influence of substrate microgeometry on cell shape, orientation, and microfilament distribution. Microfilament organization reflects cell shape and orientation, attendant to cell signalling events that also regulate cell attachment, mitosis, migration and apoptosis. Microfilament bundles (stress fibers) terminate at clusters of actin-associated proteins, adhesion molecules, and protein kinases which contribute to in vitro cell responses to culture substrates.

Methodology. NIH-3T3 fibroblasts, 3T3-L1 fibroblasts (ATCC, Manassas, VA) and MC-3T3 fibroblasts (gift of JP O'Connor) were grown in DMEM with 10% NCS and 1% antibiotics in 24-well plates containing TiO_2-coated, microtextured polystyrene inserts. Culture substrates had either 8-μm parallel grooves, 12-μm parallel grooves, 3×3-μm square posts separated by 3-μm perpendicular grooves, or no features (controls). Ten thousand cells were seeded into wells containing the inserts, and after 1 day, were prepared for scanning electron microscopy (SEM) or stained with rhodamine-phalloidin.

Results. All three variants of 3T3 fibroblasts adhered to all substrates within 1 day. There was no predominant orientation or shape in cells grown on control surfaces. The cytoplasms of some cells grown on control surfaces showed random arrays of stress fiber, apparently terminating at focal adhesions.

Nearly all cells of all types grown on 8- or 12-μm grooved substrates were elongated and oriented parallel to the grooves, growing atop the ridges or within the troughs (Figure 1). Cells cultured on 8-μm grooves bridged grooves more frequently than cells cultured on 8-μm grooves. Few cells of any type demonstrated evidence of stress fibers formation after 1 day in culture on grooved surfaces.

Many cells grown on posted substrates displayed stress fibers terminating on posts. These assumed a stellate conformation, with processes extending orthogonally from a central cytoplasmic mass and terminating atop the elevated posts (Figure 1). This finding is similar to our previous observations of NIH-3T3 cells grown on posted substrates (Grew, et al., Trans. Soc. Biomater., 24:40).

SEM observations confirmed the shape and orientation effects of the substrates on the 3T3 variants.

Discussion. This experiment demonstrated that parallel and intersecting grooves dictate the cell shape, orientation and cytoskeletal organization of three phenotypic variants of 3T3 fibroblasts. The NIH-3T3 variant is a fibrogenic line, while the 3T3-L1 variant is lipogenic and the MC-3T3 variant is osteogenic. The phenotypes of these cells were assessed by alkaline phosphatase assay (MC-3T3 cells are alkaline phosphatase positive) and by Sudan Black B staining (for lipid inclusions in 3T3-L1 cells).

The roles of extracellular matrix and cell adhesion molecules in the contact guidance events described above were not characterized, but are not discounted. We have previously demonstrated that integrin distribution and tyrosine kinase activity is physically constrained by micrometric substrate features. We hypothesize that the same constraint has occurred in the cells described herein. Elucidation of phenotypic differences between cell types that direct the tissue response to implants may yield information leading to improved implant integration and extended implant lifespan.

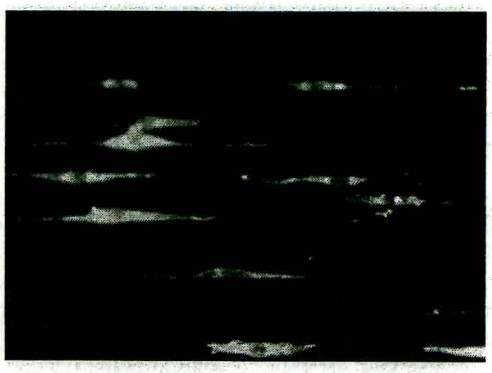

Figure 1. 3T3-L1 fibroblasts cultured on 12-μm grooves. Note the uniform orientation of the elongated cells. (100×)

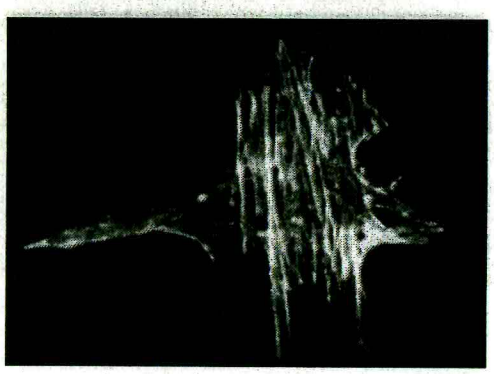

Figure 2. MC-3T3 fibroblasts cultured on 3-μm posts. Note the many cytoplasmic processes oriented at right anlges and the stress fibers extending orthogonally and diagonally between posts. (400×)

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