# Cytological Characteristics of 3T3 Fibroblasts Cultured on Micropatterned Substrates

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**Introduction.** Implant surface geometry and microgeometry affect tissue responses, although the tissue-implant interaction is incompletely characterized. Physical and chemical properties of synthetic substrates affect the morphology, physiology and behavior of cultured cells of various types. Investigators are just now beginning to describe these in vitro effects in detail.

Shape, attachment, migration, orientation, and cytoskeletal organization differ between cells cultured on flat substrates and substrates having regular surface features of micrometric dimensions. We studied murine fibroblast shape, orientation, and microfilament and focal adhesion distribution—parameters relevant to contact guidance and to other factors influenced by substrate microgeometry. Microfilament organization reflects cell shape and orientation, but is also important in signal transduction schemes governing cell attachment, mitosis, migration and apoptosis. Microfilament bundles terminate in clusters of actin-associated proteins, adhesion proteins, and protein kinases having signal transduction functions. We employed assays that revealed the distribution of (1) microfilaments/stress fibers; (2) focal adhesion molecules; and (3) phosphotyrosine, the product of the major class of kinases associated with cell attachments.

**Methodology.** 3T3 fibroblasts (ATCC, Rockville, MD) from frozen stocks were grown in DMEM with 10% FBS in multiwell plates containing 1 cm square microtextured inserts. The inserts consisted of polystyrene solvent-cast on silicon molds and titanium-oxide-coated. The resultant surfaces had either 8-μm parallel grooves, 12-μm parallel grooves, 3-μm square posts (created by perpendicular 3-μm grooves), or no features (controls). Four thousand cells were seeded into wells containing the inserts and after 4 or 8 days were prepared for scanning electron microscopy (SEM) or stained with (1) rhodamine-phalloidin; (2) either mouse antitalin or mouse antivinculin followed by rhodamine-antimouse antibodies; or (3) fluorescein-antiphosphotyrosine antibody.

**Results.** By day 4, the 3T3 cells had adhered to all substrates, and by day 8 they showed considerable growth, in places approaching confluence. There was no predominant orientation or shape in cells grown on control surfaces. Their cytoplasms showed diffuse rhodamine staining; demonstrable stress fibers were absent. Focal adhesions and phosphotyrosine were diffusely distributed. Cells grown on 8- or 12-μm grooved substrates were nearly uniformly oriented in the direction of the grooves. Cells cultured on 8-μm grooves mostly grew atop the ridges, often bridging the troughs between ridges. Cells cultured on 12-μm grooves mostly grew either atop the ridges or within the troughs, only infrequently bridging the troughs between ridges. Some cells demonstrated limited evidence of stress fibers after 8 days in culture on the grooved surfaces. Focal adhesions and phosphotyrosine were limited to areas of cell-substrate contact; portions of cells spanning troughs lacked focal adhesions and phosphotyrosine. Cells grown on the posted surfaces showed orthogonal arrays of microfilaments that conformed to the intersecting troughs between posts; stress fibers, however, were not observed. These cells either rested atop the posts or settled down onto the posts, with the posts apparently displacing cytoplasm and limiting the distribution of microfilament bundles to areas of basal contact. SEM observations confirmed that the posts penetrated the basal cell membrane surface, with the cell contents settling around the posts. Focal attachments and phosphotyrosine were similarly distributed in these cultures.

**Discussion.** This experiment demonstrated that parallel and intersecting grooves are capable of affecting the shape, orientation, cytoskeletal organization, and distribution of focal adhesions in 3T3 fibroblasts, extending our previous findings of these effects in rat tendon fibroblasts. The role of extracellular matrix in guiding this process, while not characterized, is not discounted. The limitation of kinase activity by physical substrate features is a novel finding and could shed light on mechanisms by which cell types respond differentially to substrates. Ultimately, we hope to uncover phenotypic differences in these properties between cell types that will direct the tissue response to implants in ways that improve the incorporation of and extend the functional lifespans of the implants.

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