

Effects of Surface Microgeometry on Fibroblast Shape and Cytoskeleton

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ABSTRACT

Introduction: Surface microgeometry influences tissue-implant interaction, although the interaction is poorly understood. Cellular contact guidance, a tissue response to surface microgeometry, profoundly influences cell growth and other behaviors. For example, on grooved surfaces, groove depth and width minima are required to affect cell shape and orientation and the direction of growth. Cytoskeletal organization reflects cell attachment, shape and orientation and likely contributes to these microgeometry-directed phenomena. We examined some properties of fibroblasts cultured on simulated biomaterials with various surface microgeometries. We studied rat tendon fibroblast (RTF) cells, because human fibrous tissue cells are among the first cells to contact implants. Fibrous implant encapsulation is influenced by surface roughness and microgeometry: roughening promotes thinner capsule development and, therefore, more intimate contact of bone cells and tissue with the implant and improved implant integration.

Methodology: RTFs from stock cultures derived from hind foot extensor tendons were grown on smooth (control) and patterned polystyrene substrates having parallel 2 or 12µm linear grooves or 8x50 or 80x50µm diamond-shaped islands separated by 3x3µm grooves. Substrates were solvent-cast on silicon molds and titanium oxide coated. Fifteen millimeter circular cutouts were fitted into 24-well plates, and wells containing inserts were seeded with 20,000 RTFs and fixed after 4 and 8 days in culture. Cell morphology was studied and recorded by scanning electron microscopy and by fluorescence microscopy of cultures stained with rhodamine phalloidin and anti-vinculin followed by a fluorescein-conjugated secondary antibody.

Results: The orientations and shapes of RTFs grown on control and patterned surfaces were consistently different. Cell orientation tended to be random in control cultures, but generally coincided with the directions of the linear grooves and the longer dimensions of the diamonds. RTFs grown on 2µm grooved and diamond-patterned substrates often bridged the grooves, attaching to elevated substrates. RTFs grown on 12µm grooves grew both within the grooves and on elevated surfaces, but rarely bridged grooves. RTFs grown on the larger diamond-patterned substrate often grew in clusters on elevated areas. RTFs grown on control surfaces were approximately round and symmetrical, extending short processes omnidirectionally from a central cell mass. RTFs grown on linear substrates more typically assumed a spindle shape, and extend processes perpendicular to the grooves only when spanning a narrow (2 or 3µm) groove or to establish lateral contact with groove walls (12µm grooves). Substrate microgeometry also affected the organization of microfilament bundles (stress fibers), which were aligned with the predominant direction of cellular orientation in cells grown on the patterned substrates. RTFs from control cultures typically showed microfilament bundles extending at miscellaneous angles throughout the cell cytoplasm. In all cells, vinculin was localized at microfilament bundle termini, as revealed by imunofluorescence microscopy, indicating points of cell-substrate attachment consistent with the presence of focal attachments.

Conclusions: This study showed that both the linear and diamond patterns are capable of influencing the orientation and cytoskeletal organization of fibroblast cells, extending previous observations of contact guidance effects based on substrate microgeometry on cell shape alteration and directional growth. RTFs, which vary from 3 to 10µm in width, frequently bridged 2 and 3µm grooves, suggesting that more pronounced surface features may be required to optimally control the growth of these cells. The results of this experiment differ from earlier reports of the growth of "dot" cultures prepared with cells suspended in a collagen gel. Seeded cultures appear to be less sensitive to microgeometry effects than dot cultures. Outgrowing dot culture cells probably migrate considerable distances across substrate surfaces. Grooves may thus serve as more substantial guides to migrating dot culture cells than to cells in seeded cultures, which settle and thereafter remain stationary. Continued experimentation and comparison of these models, particularly in cellular attachment to substrates, will yield additional insight into the behaviors of these cells on patterned substrates. For instance, it may prove possible to control the rate and direction of fibrous tissue growth at the tissue-implant interface, thereby optimizing the stability of these implants.

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