Fabrication of Stiffness Variant Thin Layer Substrate using Nanotopography Backbone for Cell Sensing

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Recent advances in nanoscale fabrication have allowed effective design of nanoscale substrates to direct cell behavior, such as stem cell differentiation. Studies in the last decade showed independently that substrates with nanotopography and specific stiffness can elicit stem cell differentiation. In native environment, nanotopography and stiffness of substrate are coupled. However, it is unclear how these two factors are related and the mechanism of action is still poorly understood. We hypothesized that NIH 3T3 fibroblast cells are able to sense stiffness patterns using contact guidance via focal adhesion complexes. To test this, a silicon wafer etched with nanogrooves (NG) was used as the "backbone substrate" (BBS). Ten percent poly(lactic-co-glycolic acid) (PLGA) dissolved in chloroform was spuncoat on the BBS at 1000 rotations per minute. This resulted in a thin, smooth coating of PLGA evenly filling the NG. The distance from the PLGA surface to the NG ridge and trough varied between 100nm and 2,100nm, respectively. The amount of PLGA covering the BBS created a stiffness pattern due to substrate effect of PLGA/silicon composite layer. PLGA smoothness and stiffness variation were verified using Atomic Force Microscopy. 3T3 cells were seeded on a blank silicon wafer and the BBS with and without the PLGA layer. DAPI was used to visualize cell location, phalloidin for morphology and vinculin for number and arrangement of focal adhesions. For analysis, cells were treated as ellipses in which the long and short axes were used to determine alignment and morphology. Alignment of the 3T3's on the BBS was compared as a negative control. 3T3 alignment occurred on the composite substrate with morphological differences similar with BBS alone. The control of cell genotype and phenotype using nanotopographies has broad applications in regenerative medicine.