Substrate Nanotopographic Regulation of Stem Cell Differentiation

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Statement of Purpose: It is of significant interest how to regulate stem cell differentiation and proliferation in vitro. Maintaining mesenchymal stem cell differentiation potential with continued expansion in vitro is usually a difficult task. As a potential substrate cue for controlling stem cell growth and differentiation, we have developed nanoscale topographies. Using nanotopographic film substrates, we have examined topographic regulation of osteoblastic and stem cell behavior including adhesion, proliferation, and differentiation. In this study, we examined the expression of multiple stem cell surface markers by bone marrow stromal cells to determine whether nanotopographic culture affects stromal cell differentiation. As a result, we clearly demonstrated that substrate physical cues from nanoscale topographies acts as important regulators of bone marrow stromal cell differentiation.

Methods: Nanotopographies filled with nanoscale islands were produced by polymer demixing. Polybromostyrene (PBrS, Aldrich, $M_w = 65\times10^3$) and polystyrene (PS, Aldrich, $M_w = 289\times10^3$) blend solutions were spin-cast onto glass slides at a polymer concentration of 0.5%, 2%, and 5% w/w to produce various topographic scales. Spin-cast films were annealed above the glass transition temperature (T_g) of PS but below the T_g of PBrS. Human bone marrow was obtained from the rimings of the femoral head of a 43 year old male patient undergoing hip surgery. The cells were washed and separated by using a ficoll gradient (Amersham). The cells at the interphase of the gradient were collected and plated at a density of 2×10^5 cells/cm^2 in growth medium composed of low glucose DMEM, 10% FBS, 1% P/S, and 1% L-glutamine. After 4 days of incubation, the non-adherent cells were removed and the adherent cells were maintained with media changes every 2-3 days. Bone marrow stromal cells (hBMSCs) were cultured onto the nanotopographic or flat surfaces at 4×10^3 cells/cm^2 with either growth medium or osteogenic differentiation medium further supplemented with 50 μg/ml ascorbic acid phosphate, 10 nM dexamethasone, and 10 nM β-glycerol phosphate. Cells were cultured for either 7 or 12 days before fluorescence-activated cell sorting (FACS) analysis. The cells were then tagged with SSEA-4, CD73, CD90, or CD105 primary antibodies and FACS was performed to determine the percentage of cells positive to each stem cell surface markers.

Results: PS/PBrS demixed films at varying spin-casting concentrations displayed randomly distributed nanoisland textures with varying island heights (11, 38, and 85 nm, Fig. 1). After annealing, PS segments segregate to the film surface (assessed by SIMS, not shown), and thus hBMSC response to nanotopographic scale could be assessed under the same surface chemistry of PS.

hBMSCs cultured on 11 nm substrates for 7 days displayed a significantly lower SSEA-4 positive cell percentage relative to cells on flat control and larger nanoisland surfaces (Fig. 2). After 12 days of culture, cells cultured in osteogenic differentiation media resulted in lower SSEA-4 positive percentages relative to cells in growth media, regardless of textured or flat surfaces (not shown).

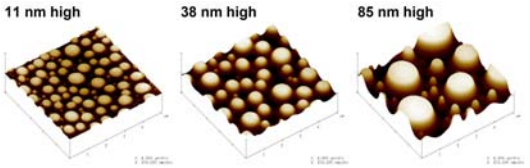

Fig. 1. Atomic force microscopy (AFM) images of nanoisland substrates having various island heights produced by PS/PBrS polymer demixing: 11 nm, 38 nm, and 85 nm average high island topographies.

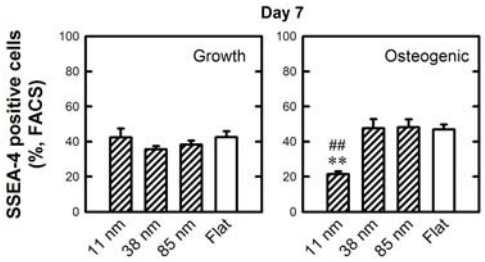

Fig. 2. SSEA-4 positive cell percentages on nanoscale substrates and flat control assessed by FACS analysis when hBMSCs are cultured for 7 days. ##: $p < 0.01$ compared with 38 and 85 nm; **: $p < 0.01$ with flat. ANOVA with $n = 3$.

Conclusions: We have previously shown that nanoscale topographies (10-100 nm height scale) can regulate osteoblastic cell morphology, integrin-mediated adhesion, proliferation, and differentiation (Lim JY. Biomaterials 2007;28:1787-1797). The data in this study demonstrate that nanoscale substrate topographies can also affect human bone marrow stromal cell differentiation in vitro. Stem cell surface markers, i.e., SSEA-4, would be down-regulated as the cells differentiate toward specific lineages. Thus, our data suggest that 11 nm high nanoisland surfaces can stimulate hBMSC osteoblastic differentiation and simultaneously stem cell markers such as SSEA-4 on 11 nm islands are down-regulated. Combined with our previous data that these substrates result in increased osteoblastic adhesion, proliferation, and bone cell differentiation, these data suggest that nanoscale substrate topographies can be exploited as a novel promoter of stem cell differentiation into the osteoblastic lineage.