Controlling Stem Cell Mechanosensitivity by Nanotopographic Culture

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Statement of Purpose: Enhancing mechanical sensitivity of human mesenchymal stem cells (hMSCs) is recognized as a novel tool to achieve successful musculoskeletal tissue engineering. We have developed biophysical extracellular cues, both static (substrate nanoscale topographies) and dynamic (fluid flow), as potential regulators of stem cell mechanosensitivity. An intriguing possibility is that substrate topography and fluid flow stimulation work synergistically to regulate stem cell behavior. To explore this possibility, we examined the hypothesis that mechanosensitivity of hMSCs in response to fluid flow induced shear stress is enhanced when cells are cultured on nanoscale topographies relative to cells cultured on flat surfaces.

Methods: Nanotopographies filled with randomly distributed nanoscale islands were produced by using the polymer demixing technique. Poly(L-lactic acid) (PLLA, Polysciences, $M_w = 50\times10^3$) and polystyrene (PS, Aldrich, $M_w = 289\times10^3$) blend solutions (PLLA/PS at 70/30 w/w) were spin-cast onto quartz slides at a total polymer concentration of 0.5%, 1%, 2%, and 3% w/w to produce various topographic height. hMSCs (Cambrex, PT-2501) were cultured at 4×10^3 cells/cm^2 using growth media composed of Dulbecco's modified Eagle's medium, 10% fetal bovine serum, 1% penicillin-streptomycin, and 1% L-glutamine. The media were changed with serum starvation media (0.5% serum) 24 h before fluid flow. On day 3 hMSCs cultured on test surfaces were placed within a fluid flow chamber, and shear stresses of 5, 10, and 20 dyne/cm^2 were applied to the cells by using oscillating fluid flow at 1 Hz. Fura Red AM was used to measure intracellular calcium levels, $[Ca^{2+}]_i$, with an initial 180 s static period followed by 180 s of fluid flow. The average (λ) and standard deviation (σ) of fluorescence for the static period was obtained, and a calcium concentration greater than $\lambda+5\sigma$ during the flow period was defined as a response. Statistical significance was determined through ANOVA and a post hoc test.

Results: PLLA/PS (70/30 w/w) demixed films at varying spin-casting concentrations displayed randomly distributed nanoisland textures with varying island heights (12, 21, 45, and 80 nm, Fig. 1). Secondary ion mass spectroscopy analysis revealed that the topmost film layer of demixed, textured films was predominantly filled with PLLA (> 96% at 20 Å depth, not shown), and thus the topography effect could be examined under similar surface chemistry. hMSCs cultured on all test surfaces displayed a robust, relative to the static period, cytosolic calcium response to fluid flows at all shear stress levels. At a shear stress of 5 dyne/cm^2 hMSCs cultured on 12 nm high nanoislands displayed a significantly greater $[Ca^{2+}]_i$ response to fluid flow, in both percentage of cells responding to fluid flow and absolute $[Ca^{2+}]_i$ increase in responding cells, relative to hMSCs cultured on flat control and 45-80 nm nanoisland surfaces (Fig. 2). At higher shear stresses of 10 and 20 dyne/cm^2, these trends were also observed but no significant differences among the test substrates were obtained (not shown).

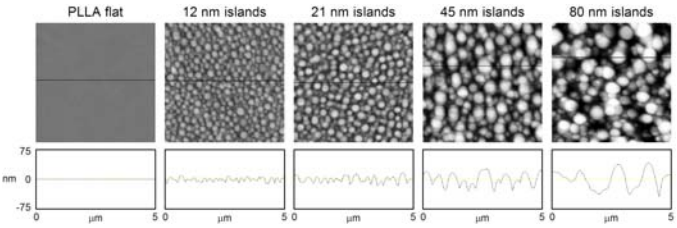

Fig. 1. Atomic force microscopy (AFM) images of nanoisland substrates having various island heights produced by PLLA/PS (70/30 w/w) demixing: 12 nm, 21 nm, 45 nm, and 80 nm average high island topographies and flat control films.

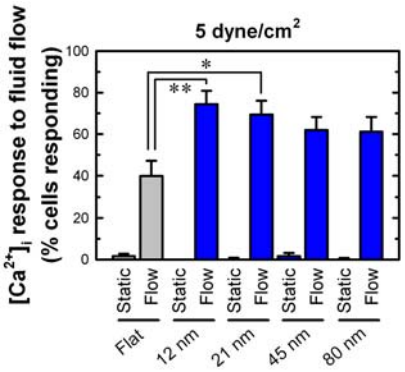

Fig. 2. Stem cell mechanosensitivity as assessed by intracellular calcium, $[Ca^{2+}]_i$, response to fluid flow. Percentage of hMSCs responding to oscillatory fluid flow inducing shear stress (5 dyne/cm^2) was greater for cells on 12 nm nanoisland surfaces. *: $p < 0.05$; ** $p < 0.01$ (ANOVA with n = 7-9).

Conclusions: We previously demonstrated that nanoscale topographies significantly affect osteoblastic and stem cell adhesion, proliferation, and differentiation (Lim JY. Biomaterials 2007;28:1787-1797). We have also shown that fluid flow induced shear stresses can increase stem cell proliferation and differentiation (Riddle RC. Am J Physiol Cell Physiol 2006;290:C776-84). Our current data clearly demonstrate that static (nanotopography) and dynamic (flow induced shear stress) physical signals may synergistically regulate stem cell behavior. As $[Ca^{2+}]_i$ mediates ERK expression, proliferation, and differentiation of hMSCs, our data suggest that 12 nm high nanoislands may facilitate optimal environments for promoting stem cell proliferation and potentially differentiation.