Changes in cytoskeletal structure and viscoelastic properties of *h*MSC induced by nanotopography

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Statement of Purpose: Both nanotopography *(1)* and substrate stiffness *(2)* influence various cell behaviors, as cells can sense and respond to both topographical cues and mechanical stimuli in their local environment. We have previously demonstrated that the morphology, proliferation, motility, and differentiation of smooth muscle cells and human mesenchymal stem cells (*h*MSCs) differ significantly when cultured on nanopatterned gratings than on plain surfaces *(3)*. To continue our attempt to understand the underlying mechanism of these nanotopography-induced changes, we examined the cytoskeleton arrangement and focal adhesion of *h*MSCs cultured on nanopatterns. We also measured the elastic and viscoelastic properties of these cells using a recently developed AFM technique *(4)*. Significant differences in both morphology and mechanical properties were revealed when comparing cells cultured on patterned and non-patterned surfaces.

Methods: Gratings of 500nm width on tissue-culture polystyrene (TCPS) were fabricated by nanoimprint lithography (NIL). The patterns were also replicated on poly(dimethylsiloxane) (PDMS) by soft lithography. Human MSCs (Cambrex) were cultured in MSCGM proliferation medium (Cambrex) on different patterns at a density of 2000 cells/cm^2. The cytoskeleton and structure of focal adhesion were studied by fluorescent and immuno-fluorescent staining. RNA was isolated from cells cultured on nanopatterned surfaces at day 3 and subject to RT-PCR and real-time PCR analyses. The biomechanical properties of *h*MSCs cultured on nanopatterned/non-patterned PDMS and TCPS were measured using AFM stress relaxation tests at day 3 (N=3, n>35 cells).

Results and Discussion: Fluorescent-staining of F-actin revealed a reduced amount of stress-fiber in the elongated hMSCs cultured on nano-gratings in comparison with that observed in the well-spread *h*MSCs on non-patterned substrates (Figure 1). Fewer filopodia were observed on the elongated cells compared to the well-spread cells. Focal adhesions were mainly observed at the polar regions of the elongated cells, and the area of focal adhesion was also reduced in the elongated cells. Expression of actin cytoskeletal components and actin cross-linkers such as α-actinin were reduced. These changes in F-actin arrangement and focal adhesions were observed on both nanopatterned PDMS and nanopatterned TCPS but not for non-patterned PDMS or TCPS.

Human MSCs cultured on nanopatterned TCPS showed significantly different ($p<0.05$) mechanical properties than cells cultured on non-patterned TCPS, with the former exhibiting smaller elastic ($E_{elastic}$), relaxed (E_R), and instantaneous moduli (E_0) (Figure 2). Human MSCs cultured on PDMS surfaces were less stiff than cells cultured on TCPS, with non-patterned PDMS supporting the lowest *h*MSC moduli. However, no significant differences were observed for the mechanical properties of cells cultured on nanopatterned and non-patterned PDMS.

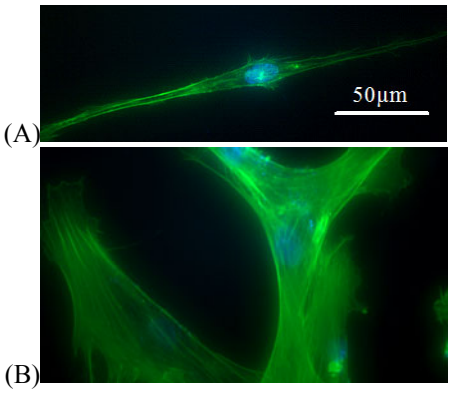

Figure 1. F-actin arrangement for *h*MSCs cultured on (A) nanopatterned and (B) non-patterned PDMS.

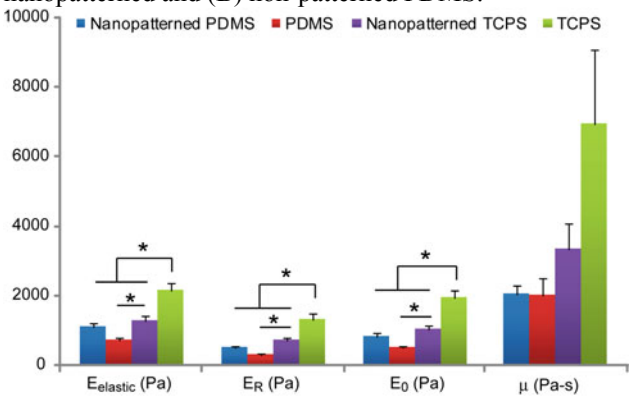

Figure 2. Mechanical properties of hMSCs cultured on various substrates (*$p<0.05$).

Summary: The results suggested that *h*MSCs respond to nano-topographical cues with focal adhesion and cytoskeleton rearrangement, independent of the two substrate compliances tested. The mechanical properties of *h*MSCs also changed in response to nanotopography; however, this response was only apparent for the rigid TCPS substrate. Further investigation is ongoing to determine if cells are more sensitive to the difference in substrate stiffness or nanotopography and to what extent these parameters can affect cell mechanical properties.

References:

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