Effect of 3D Hydrogel Scaffold Stiffness on Human Bone Marrow Stromal Cell Differentiation

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Introduction: There is growing recognition that cells can sense and respond to the mechanical properties of tissue scaffolds and that these interactions are critical in optimizing scaffold design [1-4]. Previous studies in planar 2D culture format have shown that human bone marrow stromal cell (hBMSC) differentiation and proliferation change in response to change in stiffness of the underlying substrate. Herein, the effect of matrix modulus on differentiation of hBMSC within three-dimensional (3D) polyethylene glycol tetramethacrylate (PEGTM) hydrogel scaffolds is examined.

Methods: hBMSCs (29 yr. old female, Texas A&M Stem Cell Center) were suspended (10^6 cells/mL) in pre-polymer solutions containing different mass fractions (2%, 3%, 5% and 10%) of PEGTM in PBS (with 0.05 mass % Irgacure 2959). Gels were crosslinked with 365 nm light (15 min, 2 mW/cm^2), transferred to growth medium (α-MEM with 16.5 % FBS, 2 mM L-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin) and cultured up to 6 weeks. Compressive modulus of 2%, 3%, 5% and 10% gels ranged 300-fold from 0.2 to 59 kPa (Fig. 1). Osteogenic differentiation of hBMSCs was assessed with a calcium stain for mineralized matrix and (Alizarin Red S) and X-ray microcomputed tomography (μCT) for mineral volume. Role of cytoskeletal elements in hBMSC differentiation was examined with inhibitors: latrunculin A (disrupt actin filaments), colchicine (disrupt microtubules), ML-7 (inhibit myosin light chain kinase), blebbistatin (inhibit non-muscle myosin II ATPase activity), and Y-27632 (inhibit ROCK activity).

Results & Discussion: Increasing modulus of PEGTM gels enhanced deposition of calcium phosphates by hBMSCs (Fig. 1). Fluorescence cytoskeletal staining/imaging indicated lack of pronounced actin fibers or microtubules and hBMSCs had a rounded morphology at all times and moduli (not shown). Inhibitors did not affect hBMSC mineralization, except for latrunculin A which caused a 2-fold mineralization increase compared to controls (not shown).

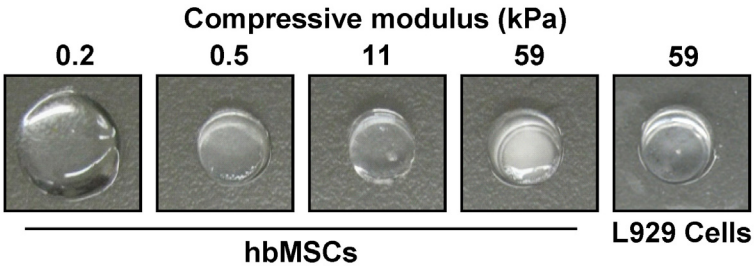

Fig. 1. Increasing modulus of PEGTM gels enhanced deposition of calcium phosphates (white deposits) by hBMSCs (21 d). As a control, non-mineralizing L929 cells did not mineralize high modulus gels (21 d). Gels are 5 mm in dia.

Previous work in 2D systems (flat surfaces) have established myosin-generated tension and ROCK signalling as the canonical pathways used by cells for mechano-sensing. However, the current data indicate that myosin-tension and ROCK signalling are not required for hBMSCs to sense differences in the modulus of their matrix during culture in hydrogel scaffolds in 3D. These results suggest that cells may use alternative pathways for mechanosensing in 3D.

Acknowledgements: KC and SHP acknowledge NRC fellowships. This work was supported by NIST, NIH-NIBIB R21 EB006497-01 and the NIH-NIDCR Intramural Program. The content is solely the responsibility of the authors and does not necessarily represent the official views of NIH, NIBIB, NIDCR or NIST. The "standard deviation" (S.D.) is the same as the "combined standard uncertainty of the mean" for the purposes of this work. Certain equipment and instruments or materials are identified in the paper to adequately specify the experimental details. Such identification does not imply recommendation by NIST, nor does it imply the materials are necessarily the best available for the purpose.

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