Matrix mechanics and cytoskeletal function affect osteoblast differentiation in 3D scaffolds

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Statement of Purpose: Bone tissue regeneration following traumatic injury is a critical physiological process. However, bone transections may require use of scaffolds or grafts that promote bone generation and ossification to reproduce adequate load bearing strength and function. Recent advances in tissue engineering have lead to achievements in which osteoblastic lineage cells can be studied *in vitro* in 3-dimensional (3D) environments to begin understanding how cell-material interactions influence differentiation and ultimately bone formation. In this study, we use synthetic (poly)ethylene glycol (PEG) hydrogel scaffolds,[1] to encapsulate preosteoblastic MC3T3-E1 cells and demonstrate that mineralization is dependent on a complex interplay between cellular mechanical environment[2] and the cytoskeleton.

Methods: Linear PEG dimethacrylate (PEGDM) (~ 4 kDa) was prepared as described previously[3]. Disc gels were cast in 60 µL molds with dimensions of 5 mm (diameter) x 3 mm (height). For osteoblast experiments, low passage (< 7) MC3T3-E1 cells were mixed at a concentration of 2.5 x 10^6 cells/mL with 10% or 20% PEGDM (by mass) and 0.05% Irgacure (mass/volume in phosphate buffered saline). Gels were cured with 365 nm light for 15 min (2 mW/cm^2). The role of cytoskeletal components in osteoblast stiffness sensing was probed with a series of cytoskeletal inhibitors purchased from Sigma. Blebbistatin (50 µM) and nocodazole (2.5 µg/mL) were added fresh with media changes (twice/week). At 1 d, 14 d and 21 d, cell viability was assessed with Live/Dead staining and calcium deposits were stained with Alizarin red. Mineralization at 21 d was also quantified by Alizarin red extraction and absorbance at 405 nm.

Results: Osteoblasts cultured in growth media (GM) (without osteogenic supplements) mineralized stiff 20% PEGDM gels (compressive modulus 390 kPa) but not softer 10% PEGDM gels (46 kPa) consistent with previous results[2] (Figure 1). However, when the microtubule depolymerizing factor, nocodazole, was incubated with cells for 21 d, osteoblasts mineralized the softer 10% gels as well as in stiffer gels. When cells were cultured with the non-muscle myosin II inhibitor, blebbistatin, heavy mineralization was seen in 10% gels while 20% gels mineralized much less (Figure 2)

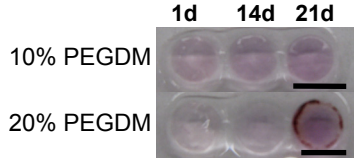

Figure 1. Alizarin Red staining shows mineralization of PEGDM scaffolds by encapsulated MC3T3-E1 osteoblasts in 20% gels. Scale bar = 5 mm.

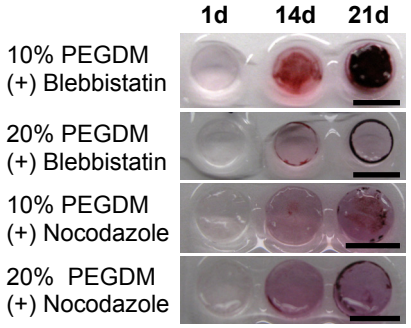

Figure 2. Alizarin Red staining shows mineralization of PEGDM scaffolds by encapsulated MC3T3-E1 osteoblasts in the presence of cytoskeletal inhibitors. Scale bar = 5 mm.

MC3T3-E1 osteoblasts did not mineralize during 2D culture on tissue culture polystyrene (21 d, without osteogenic supplements) (data not shown).

Conclusions: Our results show that microtubules and myosin II contraction are critical regulators of osteoblast stiffness sensing in 3D and that loss of either of these cytoskeletal elements is sufficient to trigger mineral deposition in softer matrices. This data also highlights differences between 2D and 3D culture in that no supplements are necessary to stimulate differentiation and subsequent mineralization in 3D hydrogel scaffold system. These results demonstrate that cytoskeletal assembly and integrity, specifically microtubule and myosin II activity, are critically involved in stiffness sensing by cells in 3D environments.

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References:

1. Bryant SJ. *J Biom Mat Res*. 2002; 59(63-72).
2. Chatterjee K. *in submission*, 2009.
3. Lin-Gibson S, Biomacromolecules 2004; 5(1280-7).