# Mechanical Loading of 3D multi-Compartment Scaffolds for Spatial Control of hMSC Fate for Regeneration of Orthopedic Interfaces

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**Statement of Purpose:** Traditionally, efforts in musculoskeletal tissue engineering have concentrated on the regeneration of single tissue systems. However, injuries commonly occur at the interface between dissimilar tissues, such as the tendon-bone-junction (TBJ), where highly elastic and aligned tendinous tissue is integrated into the much stiffer and mineralized bone. Current clinical strategies to repair the TBJ post injury do not address the need to recapitulate the complex dynamics of this interface and, as a result, suffer from poor clinical outcomes and high re-failure rates. We have previously described the fabrication of a multi-compartment collagen-GAG (CG) scaffold with coincident gradients of microstructural architecture (pore size, geometric alignment) and mineral content to serve as a template for TBJ regeneration by eliciting spatially-dependent responses in human mesenchymal stem cells (hMSCs). Here, we show how the addition of uniaxial cyclic strain can further stimulate MSC responses through a variety of mechanotransduction pathways in a spatially-dependent manner on these multi-compartment scaffolds for the regeneration of hard-soft tissue interfaces such as the TBJ.

**Methods:** Multi-compartment TBJ scaffolds were fabricated by layering mineralized CG (CGCaP) and non-mineralized CG suspensions in a mold prior to freeze-drying. Directional solidification was used to form a geometrically anisotropic CG compartment. Scaffolds were either subjected to strain by immobilization onto Flexcell® membranes using a thin layer of PDMS, or PDMS was used to embed scaffold ends into plastic end blocks that were subsequently loaded into a custom built bioreactor. Scaffolds were then seeded with hMSCs and subsequently subjected to various levels of cyclic strain for up to 72 hours. Western blot, RT-PCR, ELISA, and histological analysis were used to evaluate to evaluate hMSC differentiation and activity.

**Results:** Short-term stretching of hMSC multi-compartment scaffolds immobilized onto Flexcell® membranes was sufficient to induce compartment specific mechanotransduction pathway activation and integrin subunit expression. Specifically, β1 and β3 integrins are preferentially expressed in the non-mineralized compartment and a general increase in integrin subunit expression is seen across both compartments with the addition of strain. Additionally, increased expression of osteogenic genes (BSP, OCN) is seen in the mineralized compartment while expression of COL1 and the tendon marker SCX in the aligned non-mineral compartment is upregulated with the addition of strain.

The custom bioreactor, adapted from the Levenston lab[1], utilizes a linear actuator to displace a rake. The end blocks at one end of each scaffold are hooked around the teeth of the rake while the other end block is placed around a loading post at the opposite end of the media well. The system consists of a modular design with individual wells so that it can be adapted for various scaffold sizes and shapes. The flexible programming also allows for the generation of custom strain paradigms with variable strain amplitude, frequency, and rest time between strain cycles. When loaded into the custom-built cyclic strain bioreactor, monolithic anisotropic scaffolds show consistent strain profiles between the two anchoring points.

Uniaxial stretching of hMSC seeded aligned scaffolds shows an immediate down-regulation in ROCK1 expression as well as a time-dependent relationship between the activation of ERK 1/2 and p38 MAP kinases. ERK 1/2, which has been linked to cyclic strain-induced collagen production[2], is maximally activated after 10 minutes of strain, with diminishing activation at further time points. The decrease in ERK 1/2 activation coincides with activation of p38 MAPK, which is known to inhibit ERK 1/2[3], after 30 minutes of strain.

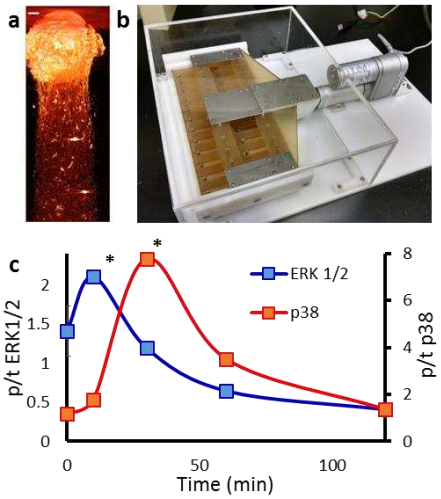


**Figure 1:** (a) Multi-compartment CG scaffold with distinct regions of varying mineral content and a continuous interfacial zone. (b) Custom built uniaxial cyclic strain bioreactor for use with porous 3D CG scaffolds. (c) Time-dependent activaiton of ERK 1/2 and p38 MAPK as a result of 10% cyclic strain.

**Conclusions:** We describe the use of uniaxial cyclic strain in two different bioreactor systems in order to elicit both spatially and strain-dependent hMSC responses on multi-compartment CG scaffolds with distinct, but continuous, regions of pore anisotropy and mineral content that mimic the native TBJ. Ongoing work involves the elucidation of both upstream effectors and downstream results (gene expression, growth factor secretion, ECM production) of these strain-dependent responses for the regeneration of a functional TBJ.

**References:**

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2. Papakrivopoulou J. Cardiovasc Res. 2004;61:736-744
3. Zhang H. J Bio Chem. 2001;276:6905-6908