**Multi-Compartment Collagen-Glycosaminoglycan Scaffolds for Engineering the Tendon-Bone Junction**

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**Statement of Purpose:** While most tissue engineering approaches focus on the repair or replacement of a single tissue, orthopedic injuries usually occur at the interface between soft tissue and bone, highlighting the need for multi-tissue regenerative strategies. Tendon-bone junctions (TBJs) are a commonly injured class of orthopedic interfaces. Current clinical approaches for repair of TBJs such as the rotator cuff are often inadequate, providing rudimentary mechanical fixation instead of biological re-integration. Biomaterial solutions for multi-tissue repair must present a well-defined set of instructive cues to guide spatially-stratified regeneration. To this end, we have utilized a regulatory compliant collagen-glycosaminoglycan (CG) scaffold system to construct a material with regionally-specific microstructural (pore size and alignment), mechanical, and biochemical (mineral content, biomolecule presentation) properties. The goal of this work was to show that this material, when seeded with human mesenchymal stem cells (MSCs) and subjected to intermittent uniaxial strain, could support the *in vitro* formation of neo-TBJ tissue with regionally-specific differences in cell phenotype and matrix deposition.

**Methods:** Multi-compartment CG scaffolds were fabricated by a combined layering and directional freeze-drying process [1, 2]. Briefly, precursor CGCaP suspension (type I collagen, chondroitin sulfate, calcium salts) was layered on top of CG suspension (type I collagen, chondroitin sulfate), allowed to interdiffuse for 20 min, and then freeze-dried at -10°C. Scaffold microstructure was evaluated using stereology, SEM, and microCT. Scaffolds were seeded with human bone marrow-derived MSCs (Lonza) and maintained in growth media for 2-8 weeks. For mechanical stimulation experiments, scaffolds were immobilized onto plates using PDMS and were stretched under uniaxial strain (0.5 Hz, 5% max strain) for 6 h/day using a FlexCell system (*Figure 1a*). Cell metabolic activity was quantified via alamarBlue fluorescence. Expression of tenogenic (collagen I, scleraxis, tenascin-C) and osteogenic (osteocalcin, bone sialoprotein, RUNX2) genes was determined using real-time PCR. Cell distribution and cytoskeletal organization were visualized using histology and confocal microscopy. Mineral and GAG content were evaluated using Alizarin red and Alcian blue staining respectively. Collagen organization was measured using second harmonic generation imaging.

**Results:** Scaffolds were fabricated with a distinct zonal structure containing spatially-graded mineral content and pore geometric anisotropy mimicking the native TBJ. Pores were significantly more aligned and elongated in the tendon compartment than the bone compartment (n = 3). Additionally, microCT revealed a linear gradient of mineral content at the compartment interface and SEM confirmed the continuity of collagen fibers at this interface (n = 3). Scaffolds supported long-term MSC viability

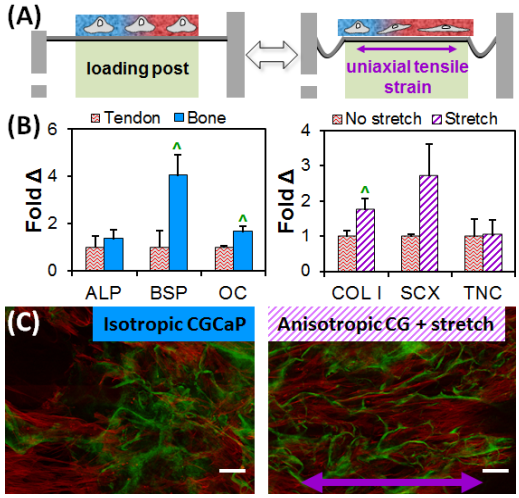


**Figure 1.** (A) Schematic of multi-compartment scaffold in FlexCell bioreactor. (B) Enhanced osteogenesis in CGCaP, tenogenesis in anisotropic CG compartments with cyclic tensile loading. (C) Second harmonic image of remodeled scaffold compartments (collagen is colored green, actin red). Scale bar: 50 µm. ^: $p < 0.05$.

for up to 8 weeks (n = 6). After 6 weeks culture, multi-compartment scaffold displayed zonally-distinct genomic profiles with significant up-regulation of osteogenic markers bone sialoprotein and osteocalcin in the bone compartment compared to the tendon compartment (*Figure 1b*, n = 3). Mechanical stimulation resulted in significant up-regulation of tenogenic markers collagen I and scleraxis in the tendon compartment (*Figure 1b*, n = 3) as well as increased cell elongation as evidenced by actin and nuclear staining. Second harmonic generation imaging revealed that collagen organization was more compact and isotropic in the bone compartment compared to the tendon compartment (*Figure 1c*). Alizarin red staining showed that mineral deposition was restricted to the bone side of the scaffold.

**Conclusions:** We describe a CG system where scaffold microstructure, mechanics, and biochemistry can be tailored in a spatially-defined manner for TBJ tissue engineering. Scaffold microstructure and composition successfully mimicked the native TBJ. Tensile stimulation had positive effects on the induction of a tenogenic phenotype. An increased osteogenic response was observed in the bone compartment with mineralization restricted to this region. Ongoing work is optimizing mechanical stimulation with biomolecule-immobilized CG scaffolds to more efficiently drive multi-lineage MSC differentiation and long-term phenotypic maintenance across the graded scaffold, both *in vitro* and in subsequent animal models.

**References:** 1. Harley BA. J Biomed Mater Res A. 2010;92:1078-93. 2. Caliari SR. Biomaterials. 2011;32:5330-40.