Integrating Mechanical Cues and Biomolecular Patterns in a Collagen-Glycosaminoglycan Scaffold for Tendon-Bone Junction Repair

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Statement of Purpose: The tendon bone junction (TBJ) is a common site of injury that also displays poor healing properties. We are developing a collagen-glycosaminoglycan (CG) scaffold which mimics elements of the biophysical and biochemical heterogeneities of the native TBJ [1]. Our goal is to induce spatially-selective mesenchymal stem cell (MSC) differentiation as a precursor to generating a material to improve biological fixation between tendon and bone. Physical stress concentrations across the TBJ interface [2] impact device mechanical competence. We are employing biomimetic geometries found in the plates of turtle shells and in armored fish [3] to create single CG biomaterials containing distinct non-mineralized and mineralized compartments that display improved tensile competence. Using a benzophenone (BP) photolithography approach, we are exploring how spatial patterns of matrix mechanical stiffness and tethered biomolecules found across the TBJ [4] can drive selective MSC osteogenic vs. tenogenic lineage specification. Linking these two technologies, we propose to generate prototype CG scaffolds with improved mechanical and bioactivity properties to aid regenerative repair of the TBJ.

Methods: Three-dimensional CG scaffolds were created by lyophilizing a suspension of type I collagen and chondroitin sulfate. Interdigitated interfaces in scaffolds were created by placing a toothed divider in the mold with mineralized slurry on one side and non-mineralized slurry on the other, with the divider removed shortly before lyophilization. Scaffolds were characterized via Scanning Electron Microscope (SEM), microcomputer tomography (μCT), and mechanical tensile testing. Two-dimensional CG membranes are created via evaporation of the CG slurry. We monolithically immobilized biomolecules (bFGF) via carbodiimide chemistry and spatially patterned biomolecules (conA) via BP using methods previously published [4]. MSCs were cultured for up to 14 days for analysis of functional (proliferation, metabolic activity) and genomic metrics.

Results: The degree of interdigitation between scaffold compartments was found to depend on two parameters: the angle, and therefore number, of teeth across the interface of each sample, and the diffusion time before lyophilization. More teeth fit onto each sample as the angle between teeth is decreased; more interfacial area is created and the interfacial strength is expected to increase [5]. Similarly, the diffusion time impacts both the degree of intedigitation between compartments, initially increasing the interfacial strength, but eventually removing any distinction between cpompartments, suggesting optimal processing times. Tooth angle was varied by creating interdigitated scaffolds with flat, single-tooth, or double-toothed interfaces. Tensile tests

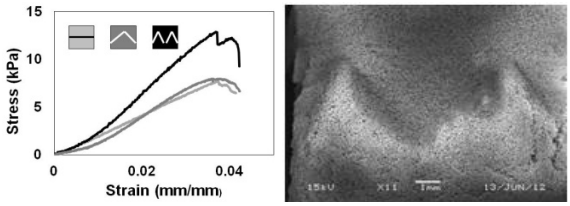

Figure 1: Tensile test and SEM image of interdigitated scaffolds

showed that failure load and elastic modulus increased with increasing interdigitation. Diffusion time before scaffold creation was varied and quantified using SEM imaging (Fig. 1), with diffusion time clearly impacting the degfree of interdigitation between scaffold compartments.

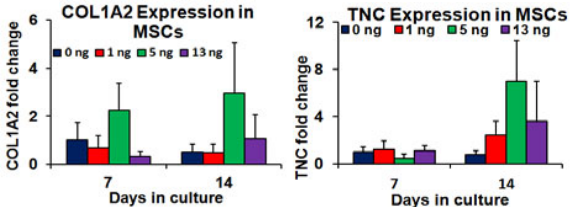

Figure 2: bFGF mediated changes in gene

BFGF was immobilized to a CG membrane monolithically via carbodiimide crosslinking to drive MSCs down a tenogenic pathway, suggested via expression of the tenogenic marker tenascin C (TNC) and increased collagen type I (COL1A2) synthesis. These tenogenic markers are particularly expressed at an immobilized concentration of 5 ng per membrane (Fig. 2). BP photolithography was subsequently used to create patterns of immobilized biomolecules (Fig. 3).

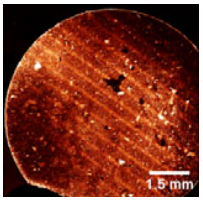

Figure 3: Photo-patterned ConA.

Conclusions: We have shown that an interdigitated scaffold interface can increase the mechanical competence of the interface within a multicompartment CG scaffold for TBJ applications. Furthermore, we have begun to identify optimal tethered growth factor densities for MSC culture and differentiation. Ongoing work is using BP patterning to immobilize biomolecules in a spatially selective manner to investigate the linked effects of biomaterial microstructural properties, tether growth factor density, and MSC specification.

References:

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