Mechanoregulation of Stem Cell Activity across an Osteotendinous Insertion Biomaterial

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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-GAG (CG) scaffold that replicates elements of the biophysical and biochemical heterogeneities of the native TBJ as a platform to improve healing post-injury [1]. Our goal is to develop a biomaterial approach to induce spatially-selective mesenchymal stem cell (MSC) differentiation towards osteotendinous lineages in order to improve biological re-integration of tendon and bone. Physical stress concentrations across the TBJ interface impact device mechanical competence. We have recently developed a lyophilization approach to CG scaffolds containing coincident gradients of matrix anisotropy and mineral content. We also employ biomimetic interdigitated geometries found in the plates of turtle shells and in armored fish [2] to create interfacial zones that display improved tensile competence. While not surprising that MSCs exhibit a range of responses across a graded biomaterial, this range may be highly dependent on intrinsic properties of the interface. Notably, does decreasing the width of the interface zone to increase the severity of gradient between compartments compress or fundamentally alter MSC response? Here we report characterization of the mechanical microenvironment across the interface as well as differential cellular response with applied strain.

Methods: CG scaffolds were created by lyophilizing a suspension of type I collagen and chondroitin sulfate. *Osteotendinous* scaffolds were created by previously established directional solidification techniques [3], such that the non-mineral compartment contains aligned pores while the mineralized compartment contains isotropic pores. Interdigitated interfaces (*layered* scaffolds) were created using a toothed divider to initially separate mineralized non-mineralized CG slurries prior to lyophilization. CG suspensions were allowed to interdiffuse for 0 – 2 hours to increase the degree of interpenetration between mineralized and non-mineralized scaffold compartments. Scaffolds were characterized via SEM, microcomputer tomography (μCT), mechanical tensile testing, and microindenation. MSCs were cultured on the scaffolds for up to 18 hours under strain to observe nuclear response and actin arrangement.

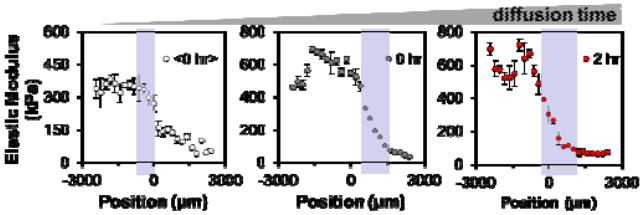

Figure 1: Mapping elastic moduli across interfaces of varying diffusion times via microindentation

Results: The degree of interdigitation between scaffold compartments depends on the angle, hence total number, of interdigitations, and the diffusion time before lyophilization. We characterized the mechanical properties across a flat interface with varying diffusion times via serial microindentation and found a gradient in scaffold stiffness across the interface (Fig. 1).

The impact of suspension diffusion time prior to lyophilization was quantified via μCT (Fig. 2); increasing diffusion was found to modify the degree of interdigitation between scaffold compartments. We found a significant effect of the inclusion of an interdigitated interface, and on the degree of interdigitation (flat, single-tooth, double-tooth), on the overall stiffness and failure stress of the layered scaffolds (Fig 2)..

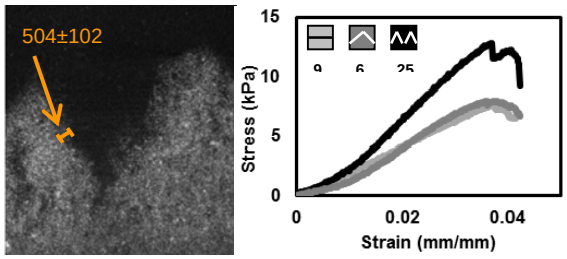

Figure 2: Tensile test and μCT image of layered scaffolds

We used confocal analysis of nuclear aspect ratio and orientation as well as actin arrangement for MSCs seeded on the layered and osteotendinous scaffolds as a function of applied strain (Fig. 3). Notably, we observed an increase in nuclear aspect ratio and orientation with strain in the osteotendinous vs. layered scaffolds (Fig. 3).

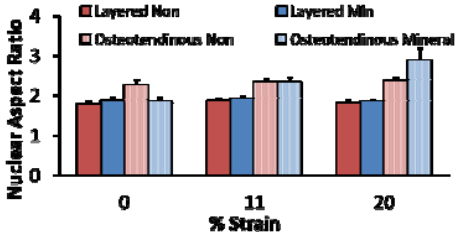

Figure 3: Nuclear aspect ratio in layered and osteotendinous scaffolds under strain

Conclusions: We have shown that an interdigitated scaffold interface can increase the mechanical competence a CG scaffold under development for TBJ repair. Nuclear aspect ratio and orientation increased with strain when the cellular microenvironment was aligned, suggesting that MSCs respond to multiple cues. Ongoing work is using mechanical strain and spatially patterned biomolecular cues to influence cellular response and differentiation to regenerate the TBJ.

References: [1] Genin, et al. Biophys J. 2009;97:976-985. [2] Song, et al. J Mech Mat. 2011;4:699-712 [3] Caliari & Harley. Biomaterials 2011;23:5330-5340