Growth Factor Presentation to MSCs within Micro-fiber/Collagen Composites for Ligament Tissue Engineering

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Statement of Purpose: Connective tissue engineering holds promise in overcoming the limitations of existing autologous and allogeneic options through the fabrication of novel scaffold materials that can support cell proliferation, differentiation, and organization into a functional tissue. Instructive biomaterial scaffolds that recapitulate the multifaceted stimuli found in native extracellular matrix – such as contact guidance, anisotropic mechanical properties, and bioactive signals – is key for the generation of a functional tissue scaffold. Therefore, to present these signals we developed model electrospun micro-fiber/collagen hydrogel composites (Figure 1a). Previously we have varied the mechanical properties of the micro-fibers to influence cell fate [1] and currently we are investigating the presentation of growth factors (GFs) in the system. Ultimately, our goal is to develop multilayered composites whose sparse distribution of bioactive fibers present a multitude of stimuli to cells to guide tissue formation and could thus be utilized in a wide variety of target tissues.

Methods: Chitosan was spincoated on glass coverslips to serve as model surfaces to analyze growth factor incorporation. Surface concentrations of incorporated GFs (fibroblastic growth factor (FGF)-2 and growth and differentiation factor (GDF)-5) were quantified via a modified ELISA technique. Then, sparse aligned PU-PCL/chitosan (core/shell) fibers (~1 μm diameter) were co-axially electrospun to form thin (~5-10 μm) aligned meshes, transferred to PDMS rings, and then functionalized with an equal mixture of (FGF)-2 and (GDF)-5 through covalent conjugation, or adsorption to heparin-coated or untreated chitosan fibers. Resultant bioactive fiber meshes (1.2 cm diameter) were embedded within 0.5 wt% collagen gels containing 5×10^4 mesenchymal stem cells (MSCs) and cultured for up to 7 days and compared to samples without GFs. Composites were analyzed for cellular distribution and morphology by confocal imaging, proliferation by total DNA quantitation, and expression of mRNA markers of ligament phenotype by quantitative real-time PCR.

Results: GF concentrations on the model chitosan surfaces after conjugation with GF solutions with concentrations from 25 ng/mL to 500 ng/mL ranged from 8 to 58 ng/cm^2. A surface concentration of 147 ng/cm^2 was measured after the adsorption of a 250 ng/mL solution onto heparin and chitosan fibers. The conjugated samples exhibited stability while the adsorbed surfaces exhibited minimal release (10%) after 7 days. PCR on micro-fiber/collagen composites revealed elevated expression of scleraxis, tenomodulin, and collagen 1α1 in the surface conjugated GFs group (FGF-2 and GDF-5 mixture) after 7 days of culture (Figure 1b). In contrast, the incorporation of GFs onto heparin-coated and chitosan fibers did not enhance expression, perhaps due denaturation of the growth during adsorption or rapid diffusion if released. We postulate that GF conjugation to the fibers enhances the expression of ligament markers due the localization of GF/growth factor receptor complexes within or near focal adhesion complexes. This co-localization may mimic the native ECM, by supporting cell adhesion, migration, and guide behavior due to integrin association with both growth factors and adhesive proteins [2].

Conclusions: Sparse micro-fiber/collagen composites that could act as a model platform were developed to study how MSC differentiation can be guided within a hydrogel network under the influence of incorporated stimuli. In this study, GF presentation modality on MSC morphology, distribution, and ligament differentiation was analyzed. However, this model system (~300 μm thick) could be utilized to analyze a myriad of stimuli combinations such as mechanical, topographical, chemical, and biological for the engineering of diverse tissue targets. Ultimately, the utilization of complementary stimuli strategies could be used to generate gradients to guide MSCs into tissues comprised of spatial ECM and phenotypical gradients.

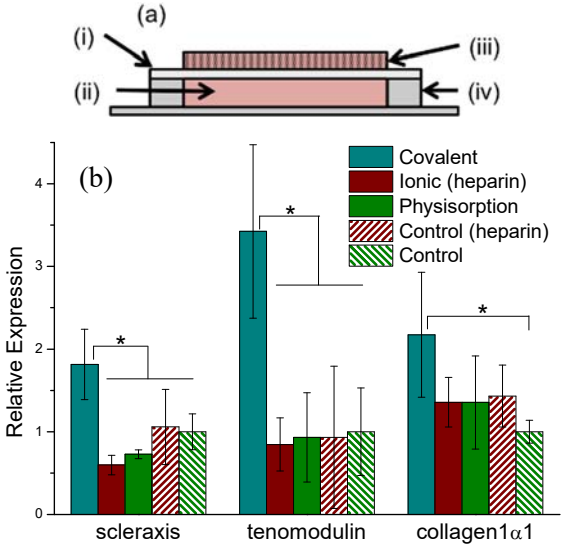

Figure 1: (a) a model composite (i) micro-fibers, (ii) acellular collagen, (iii) cellularized collagen, (iv) PDMS for support (b) Expression of collagen I, tenomodulin, and scleraxis after 7 days of culture.

References: [1] Thayer P, *et al.* J Biomed Mater Res A. 2016. [2] Knuchel S, *et al.* Oncotarget. 2015;6:14300.