Engineered Collagen-Glycosaminoglycan Scaffold Arrays for Understanding Regulators of MSC Fate

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Statement of Purpose: Biomaterial strategies for regulating stem cell fate are necessary for numerous regenerative medicine applications. For example, the repair of tendon injuries is limited by the inability to produce large numbers of tenocytes. Here we discuss the use of a regulatory compliant three-dimensional collagen-glycosaminoglycan (CG) scaffold system to analyze the combined effects of architectural (pore size and shape), compositional (biomolecule presentation), and mechanical cues on mesenchymal stem cell (MSC) signal transduction and fate. We are focused in particular on orthopedic tissue interfaces such as tendon-bone junctions (TBJs). TBJs, including the rotator cuff, are oft-injured tissues with a history of poor clinical outcomes. Therefore, the goal of this work was to identify sets of biomaterial instructive cues to regulate MSC signaling and differentiation down tenogenic, chondrogenic, and osteogenic lineages. In order to more efficiently accomplish this goal, we have developed arrays of porous CG scaffolds, enabling analysis of wider ranges of biochemical and biophysical regulators of MSC behavior.

Methods: CG scaffolds were fabricated by freeze-drying a suspension of type I collagen and chondroitin sulfate in acetic acid. Scaffold arrays consisted of a polysulfone chip (2 mm thick) with circular holes (6.5 mm diameter) mounted on a removable base. The geometry of the nodes was designed to be identical to the dimensions of a 96-well plate. Human bone marrow-derived MSCs (Lonza) were seeded on the scaffolds and cultured for 1-3 weeks. Gene expression was evaluated by real-time PCR. Small molecule inhibitors such as blebbistatin were used to isolate effects of scaffold microstructure and mechanics on MSC phenotype. SMAD8 activity was selectively silenced using a commercially-available siRNA kit (Santa Cruz Biotechnology).

Results: We first examined the dual roles of microstructural (pore alignment) and biomolecular cues on tenogenic MSC differentiation. The influence of scaffold pore anisotropy alone was shown to promote up-regulation of the tenogenic marker scleraxis (n = 3). When blebbistatin was added to the culture media, the effects of scaffold pore anisotropy on tenogenic MSC differentiation were abrogated (*Figure 1a*). TGF-β superfamily growth factors, including BMP-12 and BMP-14, were shown to promote up-regulation of scleraxis (n = 3). SMAD8 has been implicated specifically in BMP signal transduction during tendon development. Treatment of MSCs with SMAD8 siRNA resulted in significantly reduced cell coherency (elongation) and down-regulation of tenogenic genes including COMP, SIX1, and TNC (*Figure 1b*).

We next evaluated the influence of incorporating a mineral phase within the CG scaffold on MSC osteogenesis. MSC-seeded mineralized scaffolds displayed significant up-regulation of bone markers osteocalcin and bone sialoprotein as well as depressed expression of chondrogenic markers compared to non-mineralized CG scaffolds (n = 3). BMP-2 supplementation was shown to further influence cell bioactivity, eliciting significant increases in alkaline phosphatase expression (n = 3).

In order to more efficiently screen combinations of scaffold microstructural and biochemical cues we developed CG scaffold arrays (*Figure 1c*). Scaffold arrays with two distinct microstructural regions were fabricated by controlling local heat transfer during the freeze-drying process. The removable base contained an aluminum section and a polysulfone section ($k_{aluminum}/k_{polysulfone} \sim 900$); this disparity in thermal conductivity was intended to control ice crystal growth kinetics during freezing. The mean pore sizes for scaffolds were 88 ± 28 μm within the aluminum section and 157 ± 47 μm within the polysulfone section of the array for a freezing temperature of -40ºC.

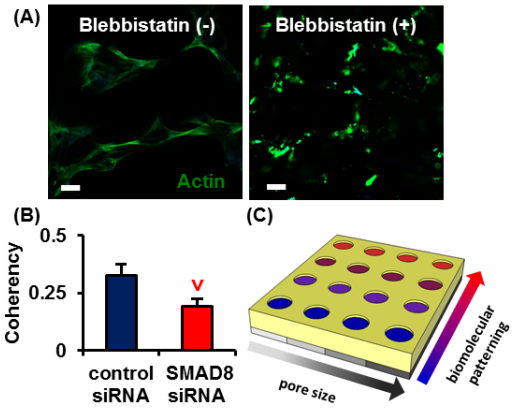

Figure 1. (A) Blebbistatin reduced cell elongation and promoted down-regulation of tenogenic genes. Scale bar: 50 μm. (B) SMAD8 silencing leads to significantly reduced cell coherency (elongation) and down-regulation of tenogenic genes. (C) Schematic of CG scaffold array.

Cells seeded in the scaffold array and stained with calcein showed no significant differences in fluorescence compared with cells seeded in a standard 96-well plate. Within the array we could also pattern near-linear ($R^2 = 0.91$) step-wise gradients of a model protein (biotinylated concanavalin A) using carbodiimide chemistry. Using this array we showed that the combination of a scaffold with large pores (> 150 μm) and patterned BMP-12/14 induced tenogenic MSC differentiation as shown by significant up-regulation of collagen I.

Conclusions: We have shown that the combination of scaffold (cell) anisotropy and SMAD8-mediated BMP signaling influences tenogenic MSC differentiation. We have also developed a scaffold array tool to efficiently screen combinations of biochemical and biophysical regulators of MSC behavior. Ongoing work with these systems is continuing to define optimal biochemical and biophysical cues for guiding MSC differentiation and understanding MSC signal transduction.