Regulation of Mesenchymal Stem Cell Differentiation by Controlling Moduli of Core-Shell Electrospun Fibers

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Statement of Purpose: It has been shown that environmental factors such as spatial structure of substrates, exogenous growth factors and external biomechanical stimulation influence the differentiation of stem cells. Among the factors which affect stem cell fate, substrate modulus is thought to affect differentiation. However, controlling the mechanical properties of substrates without changing either their morphology or chemical structure can be difficult to achieve. This has created challenges in pinpointing the effects of modulus on cellular response. In this study, we develop a method to generate scaffolds having two distinctive mechanical properties but with identical morphology and surface chemistry using- "core-shell" electrospinning. Subsequent chondrogenesis of stem cells on these scaffolds was investigated using histology, immunofluorescence microscopy, and real time RT-PCR.

Methods: 6.7 wt.% poly(ε-caprolactone) (PCL) dissolved in hexafluoroisopropanol (HFIP) was electrospun to produce 100 um thick layers of fiber. In addition, 100 um-thick core-shell fibers were electrospun using 8 wt.% poly(ether sulfone) (PES) (in HFIP) for the 'core' and 6.7 wt.% PCL for 'shell.' The average fiber diameter was assessed by SEM image analysis, and the mechanical properties (ultimate tensile strength, modulus and strain at fracture) measured. Fifty thousand C3H10T1/2 mesenchymal stem cells were cultured on these substrates for 2 days. Subsequently, their morphology and glycosaminoglycan (GAG) synthesis were observed by immunofluorescence microscopy and Alcian blue staining, respectively. Furthermore, chondrogenic/chondrocytic gene expression in each substrate was compared to that in the cells cultured on tissue culture polystyrene (TCPS) dishes using RT-PCR with custom made primers.

Results: The average fiber diameter of PES-PCL core-shell and PCL nanofibers were 867±257 and 750±490 nm, respectively. Even with similar fiber size and structure, they exhibited different mechanical characteristics having elastic moduli of 30 and 7 MPa for PES-PCL and PCL nanofibers, respectively, while having similar ultimate tensile strengths of ~ 2.8 MPa. The nanofibrous structure induced chondrogenesis of the mesenchymal stem cells in the absence of any exogenous factors. However, the variation in stiffness led to differences in chondrogenesis. Softer PCL nanofibers induced higher upregulation in chondrogenic (Wnt5a and Sox9) and chondrocytic (Aggrecan and Collagen type 2) gene expression (Fig. 1). Sox9, which directly regulates collagen type 2 gene expression and is heavily involved in chondrocyte maturation, was significantly upregulated only on PCL nanofibers. Furthermore, aggrecan expression was upregulated more than 300 times on PCL nanofibers compared to the stiffer PES-PCL nanofibers. This was confirmed by the histological observation of greater GAG accumulation in the PCL substrates by Alcian blue staining (Fig. 2A and 2B). It appears that the modulus of the substrate affects the cytoskeletal organization (F-actin) as well as the distribution and expression of adhesion molecule (integrin β1) (Fig. 2C and 2D). More stress fibers were observed in cells cultured on the stiffer PES-PCL fibers; softer PCL fibers induced higher expression and cytoplasmic distribution of integrin β1.

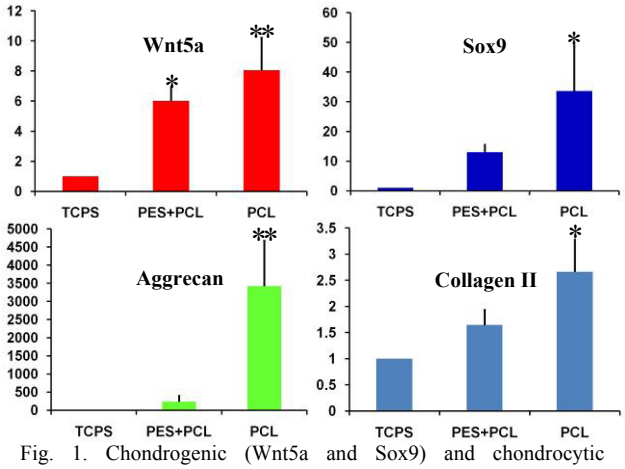

Fig. 1. Chondrogenic (Wnt5a and Sox9) and chondrocytic (Aggrecan and Collagen type II) gene expression of mesenchymal stem cells cultured on different substrates (TCPS, PCL nanofiber, PES+PCL core-shell nanofibers). N=6, *: $p < 0.05$, **: $p < 0.01$ compared to TCPS.

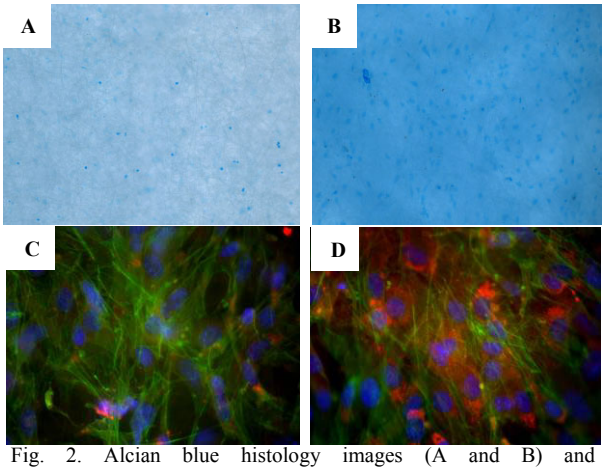

Fig. 2. Alcian blue histology images (A and B) and immunofluorescence images (C and D; blue: nuclei; green: F-actin; red: integrin β1) of C3H10T1/2 cells on PES-PCL core-shell (A and C) and PCL (B and D) nanofibers.

Conclusions: In this study, we have shown that nanofibrous structure itself induces chondrogenesis of mesenchymal stem cells. In addition, modulus alone can significantly affect chondrogenic processes in stem cells. In follow-on studies, we will analyze how the different mechanics of the substrate affect cell-cell communication by examining Cadherin proteins shown to greatly affect chondrogenesis.