Electrospun Nanofibrous Scaffolds Enhanced with Decellularized Extracellular Matrix to Promote Tissue-Specific Bioactivity for Tendon and Cartilage Repair

Benjamin Rothrauff, Guang Yang, Rocky Tuan.
University of Pittsburgh, Pittsburgh, PA, USA.

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Introduction: Musculoskeletal soft tissues, such as tendon and cartilage, are characterized by highly organized extracellular matrix (ECM) containing collagen and non-collagenous proteins, the composition of which dictates tissue function and cellular phenotype.[1] Nanofibrous scaffolds have been explored as biomaterials to enhance soft tissue healing, as they can provide topographical cues resembling collagen fibrils of the native tissue. However, nanofibers derived from synthetic polymers (e.g. poly-L-lactic acid, PLLA) lack cell binding motifs of natural proteins (e.g., collagen). While natural ECM proteins (e.g., gelatin) may be electrospun into nanofibrous scaffolds, these materials often lack sufficient mechanical integrity to support surgical repairs. Furthermore, the non-collagenous fraction of the ECM that imparts tissue-specificity is absent.[2] In this study, we have applied a recently developed protocol[3] to prepare and analyze the tissue-specific bioactivity of urea extracts of tendon and cartilage ECM on human bone marrow mesenchymal stem cells (MSCs). Additionally, the bioactive extracts were added to gelatin and co-electrospun with PLLA to investigate the feasibility of providing tissue-specific bioactive cues to mechanically robust nanofibrous scaffolds.

Methods: Bovine tendon and hyaline cartilage were minced into small pieces and pulverized using a cryomill. Tendon and cartilage powder were decellularized by 1% Triton X-100, treated with 200 U/ml DNase and 50 U/ml RNase, and then extracted in 3 M urea. The ECM extract was collected, dialyzed against H2O, lyophilized, and stored at -20 °C until use. Human MSCs were isolated from bone marrow aspirate of three donors, with Institutional Review Board approval (University of Pittsburgh) and cultured in growth medium (DMEM, 10% fetal bovine serum [FBS], 1% Anti-Anti) until passage 2. MSCs were plated at a density of 2.0 x104 cells/cm2 in 6-well plates. Following cell attachment for 24 hours, cells were serum-starved for an additional 24 hours in basal medium (DMEM, 1% ITS, 1% Anti-Anti). Cells were incubated up to 7 days in one of four conditions: (1) serum-free basal medium; (2) FBS-supplemented (10% v/v); (3) tendon ECM (tECM)-supplemented (50 μg/ml); (4) cartilage ECM (cECM)-supplemented (50 μg/ml). qRT-PCR was performed on days 1 and 7 to assess tissue-specific gene expression, including scleraxis (SCX, tenogenic), SOX9 (chondrogenic), and RUNX2 (osteogenic).

For nanofiber fabrication, tECM and cECM fractions (400 μg/ml) were combined with 20% gelatin (w/v) and dissolved in 60% acetic acid containing 0.9% (w/v) NaCl. 10% PLLA was independently dissolved in 4:1 dicholoromethane (DCM):dimethylformamide (DMF). Polymer solutions of PLLA and gelatin/ECM were ejected from independent syringe pumps positioned on opposite sides of a rotating mandrel at a flow rate of 2.0 ml/hr and 1.2 ml/hr, respectively. Voltage applied to each polymer solution was individually optimized. Scanning electron microscopy (SEM) was performed to visualize scaffold architecture.
**Results:** Tendon and cartilage ECM was sufficiently decellularized, as shown previously[3] and confirmed by the absence of nuclei on histological sections and dsDNA content falling below 50 ng/mg dry weight (data not shown).

Supplementation of basal medium with tECM or cECM promoted tissue-specific gene expression in MSCs on both days 1 and 7 (Fig. 1). tECM supplementation upregulated SCX expression on days 1 and 7 (Fig. 1A), as compared to all other conditions. Likewise, cECM supplementation most strongly upregulated SOX9 expression on days 1 and 7 (Fig. 1B), but had a negligible effect on SCX expression on day 1. By day 7, cECM supplementation slightly upregulated SCX expression, to a lesser extent than tECM. Neither tECM nor cECM supplementation drastically affected osteogenic differentiation, as confirmed by RUNX2 expression (Fig. 1C).

As shown in Fig. 2, nanofibers of PLLA (Fig. 2A) and gelatin/ECM (Fig. 2B) were successfully electrospun independently and simultaneously (Fig. 2C), with no qualitative effect on the fiber structure of each polymer.

**Discussion:** Native musculoskeletal soft tissues possess unique architecture and biochemical components that provide a particular structure and function to the tissue. While topographical cues resembling collagen fibrils have been successfully patterned by several material fabrication techniques, including electrospinning synthetic polymers or natural proteins, replication of tissue-specific biochemical motifs remains a challenge. As shown in this study, a urea-soluble fraction of tendon and cartilage ECM can promote tissue-appropriate gene expression and may be used to enhance the tissue-specific bioactivity of novel biomaterials. In particular, it is feasible to electrospun tECM and cECM as part of a gelatin nanofibrous scaffold, either independently or in combination with PLLA nanofibers.

**Significance:** Extraction of proteins from decellularized tissue ECM can promote site-appropriate cell differentiation. Therefore, these tissue-specific bioactive fractions may be combined with nanofibrous scaffolds to better replicate the structural and biochemical characteristics unique to each musculoskeletal tissue.
Fig. 2. SEM images (1000x) of electrospun nanofibers of (A) PLLA, (B) Gelatin/ECM and (C) PLLA co-spun with Gelatin/ECM.
Fig. 1. Gene expression of markers of (A) tenogenesis, (B) chondrogenesis, and (C) osteogenesis.