Engineering 3D Cellular Scaffolds from Human Muscle Progenitors and Injured Mouse Rotator Cuff Derived Extracellular Matrix for Disease Modeling and Treatment

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Introduction:

Rotator cuff injuries present a tremendous burden because of a combination of high incidence rate and limited quality of life, diminished functionality of the shoulder joint, and the economic burden secondary to the costs of surgery and rehabilitation. Chronic injury of the rotator cuff muscle is associated with progressive fibrotic scarring and fat accumulation, originating from degenerative fibro-adipogenic differentiation of $PDGFR\beta^{+}PDGFR\alpha^{+}$, fibro-adipogenic progenitor cells (FAP). Such muscle degeneration results in pathologic alterations of the extracellular matrix (ECM). While cellular fate and the regulators of cell fate in chronically injured rotator cuff muscle have been studied in detail, the role of extracellular regulators remains less understood. Therefore, refined experimental systems are needed in which pathologic states can be recapitulated and cellular treatments as well as anti-fibrotic therapies evaluated in vitro. We therefore developed an innovative cultured artificial rotator cuff muscle from a decellularized injured murine muscle ECM colonized with human muscle FAP.

Methods:

This study was approved by the institutional IACUC and IRB. Massive rotator cuff injuries were induced in murine models through surgical tenotomy and denervation (8-10-week-old mice, n= at least 3 mice per group). At 5 days, 2- and 6-weeks postop, injured supraspinatus and infraspinatus muscles were harvested and decellularized for removal of myofibers, cells and DNA. Primary cultures of FAP were derived from human muscle biopsies and fibro-adipogenic differentiation of FAP was confirmed in fibrogenic and adipogenic cultures. Non-injured and injured 3D ECM scaffolds were seeded with fluorescently labeled FAP via dynamic seeding on an orbital shaker and cultured for 1, 3 and 6 days. Unseeded and seeded scaffolds were fixed and sectioned for histological analysis. DAPI staining was used for detection of nuclear DNA before and after cell seeding. Cell survival and growth were evaluated at 1-, 3- and 6-days post seeding through fluorescent microscopy or XTT cell viability assay. Picrosirius red staining was used for detection of collagens I and III in sections of decellularized rotator cuff scaffolds.

Results:

Histological analysis confirmed efficient muscle decellularization: H&E staining and morphological analysis before and after decellularization demonstrated elimination of muscle myofibers, picrosirius red showed the preservation of collagens I and III deposition and the removal of cellular material was further validated by lack of muscle DNA staining by DAPI (Figure 1). Fibrogenic and adipogenic differentiation ability of human muscle FAP were validated in 2D cultures prior to cell seeding into 3D scaffolds. Seeded CM-DiI labeled FAP adhered to rotator cuff 3D non-injured and injured scaffolds and were evenly distributed within the scaffolds over a period of 6 days in culture (Figure 2). Additionally, XTT cell viability assay confirmed cell viability within the 3D scaffold immediately post seeding and up to 6 days in culture. Microscopic analysis of serial sections of FAP-seeded rotator cuff 3D scaffolds revealed that FAP uniformly infiltrated the entire scaffold within 3 days post seeding and remained evenly distributed at 6 days post seeding.

Discussion:

Our data demonstrate the feasibility of the decellularized rotator cuff matrix to support 3D FAP cultures that will be further used for evaluation of cell differentiation. We will further use this platform for comprehensive analysis of cell distribution and growth in 3D scaffolds over time and for evaluation of cell degenerative differentiation in diseased scaffolds in the presence or absence of fibrosis inhibiting compounds.

Clinical Significance:

Rotator cuff derived scaffolds show great potential in clinical translational research owing to their properties to represent a near physiological model for healthy and diseased tissues. Rotator cuff-derived, structurally preserved ECM can be used as an advanced novel research tool for characterization of pathological deposition of ECM. Reconstruction of human muscle in decellularized healthy and diseased mouse rotator better recapitulates disease modeling for drug discovery and pharmaceutical applications.

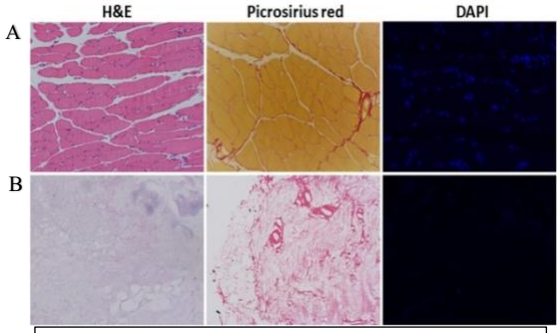

Figure 1. H&E, picrosirius red, and DAPI staining (blue) of sections of non-injured murine rotator cuff muscle before (A) and after (B) decellularization.

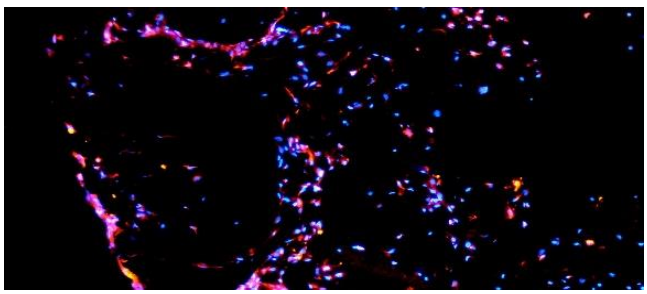

Figure 2. Uniform distribution of CM-DiI labeled (red) human muscle FAP. Seeded FAP are seen adhered to non-injured decellularized rotator cuff scaffold at 3 days post seeding. Nuclei staining with DAPI (blue).