Decellularized Skeletal Muscles: Model Systems to Study Changes in Extracellular Matrix and their Effect on Stem Cell Differentiation

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Statement of Purpose: Naturally derived scaffolds are ideal for stem cell growth and differentiation because they contain growth factors and chemical cues from native tissues. Many protocols used to decellularize skeletal muscle for this purpose use enzymes and detergents to remove cellular components [1,2]. The use of these agents, even in low concentrations, may alter the biochemical composition and mechanical properties of the extracellular matrix (ECM), which have been shown to have a significant effect on stem cell differentiation [3,4]. The goal of this study was to produce whole decellularized skeletal muscles while maintaining the biochemical composition and structure of the ECM in order to understand the changes ECM undergoes with aging and disease progression and how these changes affect stem cell myogenic differentiation.

Methods: This study was performed in accordance with the guidelines established by the Institutional Animal Care and Use Committee at the University of California, San Diego. Tibialis anterior (TA) muscles were isolated from 2-month-old C57BL/6 female mice. All decellularization steps were performed at room temperature with agitation unless otherwise noted. Freshly isolated muscles were incubated in 50 nM latrunculin B for 2 hrs at 37°C. Muscles were washed, incubated in 0.6 M KCl for 2 hrs, washed again, and incubated in 1.0 M KI for 2 hrs [5]. Muscles were washed overnight and then the KCl and KI incubation steps were repeated and followed by a final incubation in DNase I (1 kU/mL) for 2 hrs. The treated tissues were washed for 2 days to remove remaining reagents. Muscles were analyzed by hematoxylin and eosin (H & E) staining, immunohistochemical staining for actin and myosin heavy chain (MHC), DNA and glycosaminoglycan (GAG) quantification, SDS-PAGE, tensile testing, and scanning electron microscopy (SEM). Statistical analysis was performed using one-way ANOVA.

Results: H & E staining showed the absence of nuclear components in decellularized muscles, which was confirmed by the absence of DAPI staining (data not shown). DNA content in decellularized muscles was significantly reduced to 3.0% of normal DNA content. GAG content was reduced in decellularized muscles to 78% of untreated muscle. Bands for actin and MHC from decellularized muscle were absent on a SDS-PAGE gel and no actin immunostaining was observed. However, MHC was observed as trace staining in decellularized muscle (data not shown). Tensile testing of a decellularized bundle yielded a modulus of 1.2-1.7 GPa (Fig. 1). SEM analysis revealed the expected honeycomb-like structure of decellularized muscle (Fig. 2).

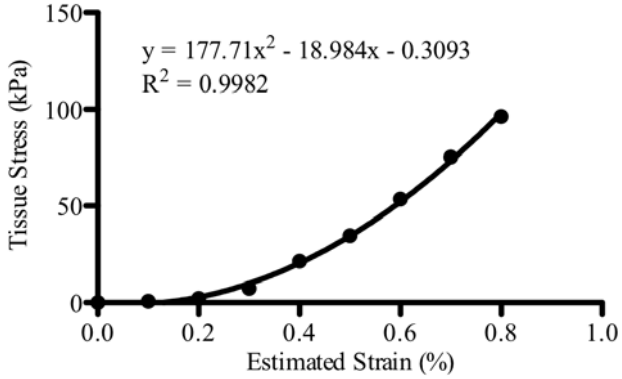

Figure 1. Stress vs. strain curve for decellularized muscles.

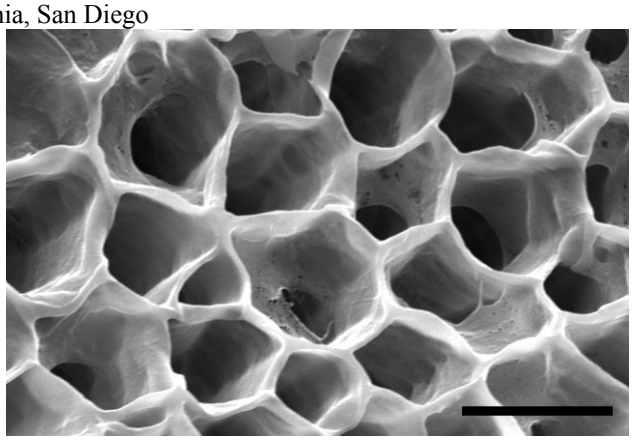

Figure 2. SEM image of decellularized TA muscle. 6000X magnification, scale bar represents 5 μm.

Conclusions: Skeletal muscles were successfully decellularized while maintaining the architectural integrity and majority of ECM components. The reduced GAG content in the decellularized muscles may be due to the loss of GAGs associated with proteoglycans on the cell membrane and does not necessarily indicate the loss of GAGs associated with the ECM. Although decellularized muscles showed positive staining for MHC, it is likely that myosin was depolymerized and remained in the muscle ECM since a myosin band was not visible on a SDS-PAGE gel. When viewed under high magnification, decellularized muscle fibers showed almost no striations, which supports the hypothesis that sarcomeres, and their associated myosin filaments, were disassembled. The modulus value obtained is consistent with isolated tendon literature and the stress-strain curve provides evidence that muscle ECM is intrinsically nonlinear. The honeycomb-like structure of the decellularized muscles may be ideal for myogenic differentiation of stem cells, allowing the cells to align themselves with native muscle ECM. Decellularized whole muscles are a promising model to study the changes that occur in muscle ECM in myopathies and muscle wasting diseases.

References:

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