The Development of Biomaterials from Porcine Skeletal Muscle Extracellular Matrix for use in Tissue Engineering and Regenerative Medicine

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Statement of Purpose: Naturally derived biomaterials hold great appeal for use in tissue engineering and regenerative medicine (TE/RM). There is currently great interest in the development of biomaterials for use in the engineering and/or regeneration of skeletal muscle tissue. Thus, the development of skeletal muscle-derived biomaterials holds great potential as such materials may provide unique cues that enhance the formation of functional muscle. Here, we describe the development of an acellular scaffolding system from porcine skeletal muscle extracellular matrix (ECM). We also demonstrate that decellularized skeletal muscle tissue can be processed into bioactive powder or gel forms that promote the growth of myogenic cells. These unique biomaterials are among the first to be generated from skeletal muscle tissue and represent an attractive alternative to both natural materials derived from non-muscle tissue and synthetic materials for use in TE/RM applications.

Methods: Porcine skeletal muscle tissue was obtained from the loin muscles of ~40 kg animals. Tissue was cut into 3-4 inch thick segments and thinly sliced using a deli slicer. Slices were decellularized by exposure to water, trypsin, and detergent. Acellular slices were lyophilized and sterilized. For use as scaffolds, slices were seeded with C_2C_{12} myoblasts. To prepare muscle ECM powder, acellular slices were processed in a cryogrinder under liquid nitrogen cooling. To form ECM gels, powder was pepsin-digested in acidic solution, which was then neutralized to allow gelation.

Results:

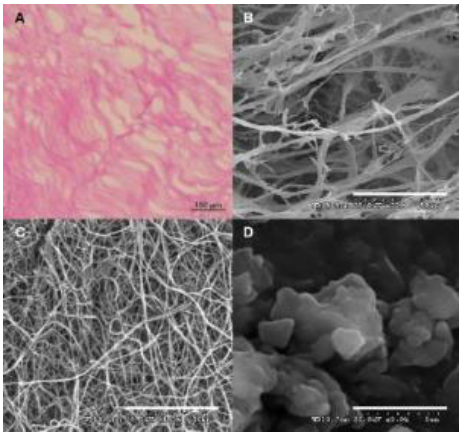

Figure 1: Biomaterials derived from porcine skeletal muscle ECM. A) H&E staining of decellularized porcine muscle. **B-D)** SEM images of **B)** a lyophilized acellular scaffold, **C)** critical point dried muscle ECM gel, and **D)** muscle ECM powder. White scale bars = 50µm, 10µm, and 5µm for B, C, and D, respectively.

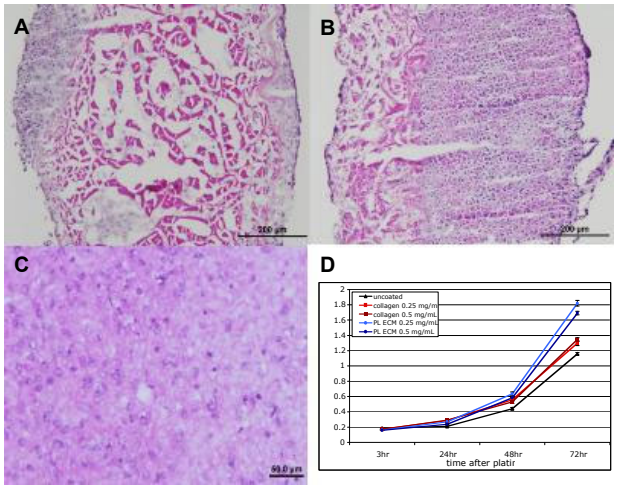

Figure 2: Utility of biomaterials derived from porcine skeletal muscle. A, B) Acellular skeletal muscle scaffolds allow the inward migration of myogenic cells. Images of 7- and 14-day timepoints, respectively. **C)** Skeletal muscle ECM gel seeded with C_2C_{12} myoblasts during gelation and cultured for 7 days. **D)** C_2C_{12} myoblasts seeded on ECM-coated surfaces experience significantly greater ($p < 0.05$) levels of proliferation over 72h relative to collagen-coated and uncoated surfaces. Proliferation was measured via MTS assays (A490 readings); n=12.

Histological analysis confirmed that the decellularization procedure removed all cells. Lyophilized acellular scaffolds and ECM gels exhibited a microstructure containing a complex network of different-sized fibers. C_2C_{12} myoblasts seeded onto acellular scaffolds penetrated all of the way into the scaffolds' centers. ECM gels also supported myoblast growth and were remodeled within 7 days. Furthermore, myoblast proliferation was enhanced on surfaces coated with ECM powder.

Conclusions: The use of biomaterials derived from porcine skeletal muscle for the engineering and regeneration of skeletal muscle tissue represents an attractive alternative to synthetic materials and natural materials obtained outside of the musculoskeletal system. The work described here demonstrates the feasibility of using bioactive materials comprised of porcine skeletal muscle ECM molecules to produce different classes of biomaterials. Acellular scaffolds, gels, and surface coatings were all created from the same starting material. Myogenic cells grew better on muscle ECM coatings compared to collagen. Muscle ECM gels self-assembled into fibrous networks that supported robust cell growth and remodeling of the gels. Most importantly, myogenic cells seeded onto acellular scaffolds exhibited the ability to fully penetrate and recellularize the scaffolds.