Porcine Acellular Muscle Matrix Scaffolds Enhance Restoration of Contractility in a Murine Model of Muscle Injury

Matthew M. Stern[1], Amber R. Stern[3,5], Elliott E. Goff[3], Christopher R. Bergman[4], Olivia L. Latham[4], Benjamin T. Corona[2]
[1]Winthrop University, Rock Hill, SC, [2]United States Army Institute of Surgical Research, Fort Sam Houston, TX, [3]University of Missouri-Kansas City, Kansas City, MO, [4]Wake Forest Institute for Regenerative Medicine, Winston-Salem, NC, [5]Engineering Systems Inc., Charlotte, NC

Statement of Purpose: There is great interest in the development of biomaterials that facilitate the ability to engineer and/or regenerate skeletal muscle. The use of naturally-derived biomaterials generated from the extracellular matrix (ECM) of the same tissue being targeted for repair/regeneration is a common strategy in biomaterial development. Here, we describe the development, and use of scaffolds composed of porcine acellular muscle matrix (PAMM) in the repair of a murine model of volumetric muscle loss (VML). We show that the orientation of ECM elements and the mechanical properties of PAMM scaffolds can be controled during the decellularization procedure. Furthermore, we show that PAMM scaffolds can be recellularized *in vitro* and that both recellularized and acellular scaffolds support the partial restoration of contractility in surgically repaired skeletal muscle.

Methods: Porcine longissimus dorsi (loin) was obtained from ~40 kg animals and thinly sliced. Slices were decellularized via exposure to a series of buffered, detergent solutions. Acellular slices were either lyophilized or maintained in "wet" conditions and then sterilized via gamma irradiation prior to being sized for use as scaffolds or mechanical testing. Muscle-derived cells (MDC) were obtained following collagenase digestion of tibialis anterior and soleus muscles of Lewis rats and propogated under standard culture conditions. A volumetric muscle loss (VML) injury was surgically created as a critical size defect of a latissiums dorsi (LD) muscle of nude mice. The defect was repaired with either cell-seeded or unseeded PAMM scaffolds that had been lyophilized and rehydrated. Functional assesment of whole repaired LD muscles was performed using a DMT organ bath system.

Results:

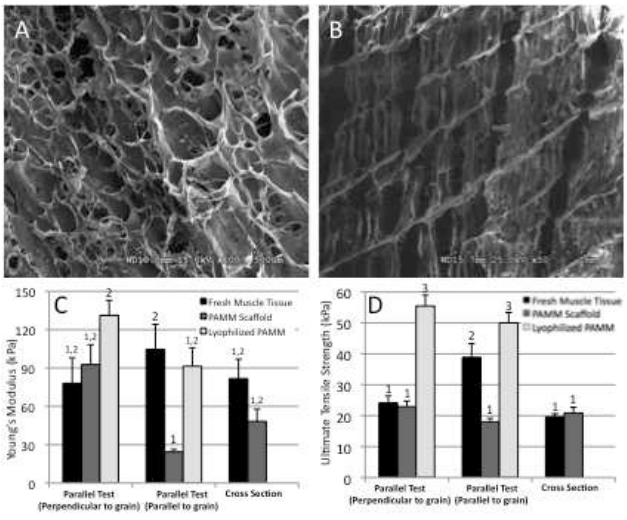

Figure 1: Altering the orientation of ECM elements impacts the mechanical properties of PAMM scaffolds. A,B) Scanning electron micrographs of the surface of PAMM scaffolds illustrate how the orientation of muscle tissue during processing can be used to control the alignment of major ECM elements relative to the long axis of the sheet-like PAMM scaffold. A = "cross section" orientation and B = "paralell to the grain" orientation **C,D)** The orientation of ECM elements and scaffold lyophilization impact the Young's modulus and ultimate tensile strength of PAMM scaffolds. Data are +/– SE; numbers indicate statistically independent groups by ANOVA; $p < 0.05$.

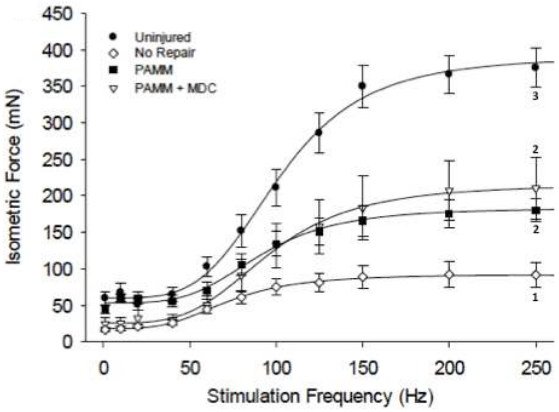

Figure 2: Acellular and recellularized PAMM scaffolds promote partial recovery of LD contractility one-month after VML repair. The scaffolds used were lyophilized and rehydrated (light bars above) and from the "paralell to the grain" group from above. Data are +/– SE; numbers indicate statistically independent groups for peak isometric force by ANOVA; $p < 0.001$. MDC = muscle derived cells.

Conclusions: Our results demonstrate the ability to take advantage of the innate organization of ECM elements within a tissue to control the architecture of a naturally-derived biomaterial scaffold we have termed PAMM. We show that this level of control provides an opportunity to produce scaffolds with different material properties, which can be further tuned based on additional optional procedures such as lyophilization. Furthermore, we demonstrate that, within one-month of implantation, both PAMM scaffolds alone and PAMM scaffolds recellularized with MDCs promote restoration of around 50% of peak isometric force when used in the repair of a murine model of VML. Together, these results suggest that PAMM scaffolds have great potential for use in skeletal muscle tissue engineering. Future work will be aimed at optimizing the production of scaffolds and determining combinations of different cell types that most effectively promote regeneration of functional tissue.