Autologous Minced Muscle Treatment of Volumetric Muscle Loss Improves Neuromuscular Strength

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Background/Purpose: The volumetric loss of skeletal muscle has no definitive therapy and the resultant functional deficits are often considered part of the sequelae of severe musculoskeletal trauma. Volumetric muscle loss (VML) can occur directly or indirectly after various injury mechanisms (blast, crush, degloving) or surgical procedures (debridement, tumor removal, and muscle tissue resection). For example, VML occurs with type III open fractures and often results in suboptimal function of salvaged limbs. The pathophysiology of VML is not well understood. Moreover, therapeutic strategies to regenerate de novo functional muscle tissue are not established. The purpose of this study was twofold: (1) to develop a porcine model of VML to evaluate the pathophysiology of VML and (2) to investigate the capacity of autologous minced muscle grafts (1 mm3 pieces of muscle) as a therapeutic to restore muscle strength. Minced muscle grafts were chosen for this study because they are an effective therapy in rat VML studies and do not require FDA (US Food and Drug Administration) approval for clinical use.

Methods: A bilateral VML model was established in the anterior compartment of the leg in female Yorkshire Cross pigs. In five pigs, approximately 5 g of tissue was excised from the middle third of the peroneous tertius muscle (~3 cm x 3 cm x 1.5 cm volume) in each leg. In each pig, the defect in one leg was treated with autologous minced muscle (~1 mm3 pieces, derived from contralateral defect) and the contralateral defect was untreated. The fascia and skin were closed similarly in all conditions. The animals were allowed to recover for 12 weeks. Prior to injury, and every 2 weeks postinjury, in vivo muscle function was performed to assess the strength of dorsiflexor muscles (tibialis, digitorum, peroneous tertias) using a large animal force transducer (890A, Aurora Scientific). While under anesthesia, the foot of the nonrepaired leg was attached to a foot-plate of the force transducer with the foot plantar flexed and the knee at a right angle. Maximal isometric tetanic torque was elicited by stimulating (100 Hz, 0.1 ms pulse, 800 ms train) the peroneal nerve with percutaneous needle electrodes. On the final day of testing, muscles were harvested, weighed, and prepared for standard histology. This study was conducted in compliance with the Animal Welfare Act, the implementing Animal Welfare Regulations, and the principles of the Guide for the Care and User of Laboratory Animals.

Results: Over the course of the 12-week study, pigs significantly gained body weight (preinjury vs 12 weeks; 39.9 ± 1.5 vs 69.2 ± 1.9, $P < 0.001$). Therefore, isometric muscle strength was normalized to body weight to control for growth. Group strength deficits to VML injury are presented in Figure 1A. Ultimately, VML injury resulted in a ~40% loss of neural-evoked strength 12 weeks postinjury in nonrepaired legs. VML repair with autologous minced muscle grafts resulted in significantly improved strength at this time (~20% strength deficit, $P < 0.001$). Histologic analysis indicates that nonrepaired VML-injured muscles develop significant fibrosis and present no evidence of muscle fiber regeneration within the defect area (Figure 1B). In comparison, autologous minced graft treated muscles presented evidence

of de novo muscle fiber regeneration within the defect area, although collagenous tissue deposition was also present.

Conclusion: Volumetric muscle loss presents persistent strength deficits marked by severe fibrosis. Autologous minced muscle grafts restored approximately 50% of the strength deficit observed in nonrepaired muscle and presented histologic evidence of muscle tissue regeneration within the defect area. Although many challenges remain in maximizing functional recovery and muscle tissue regeneration after VML, these results encourage the clinical application of autologous minced muscle graft-based repair strategies for severe soft-tissue trauma.

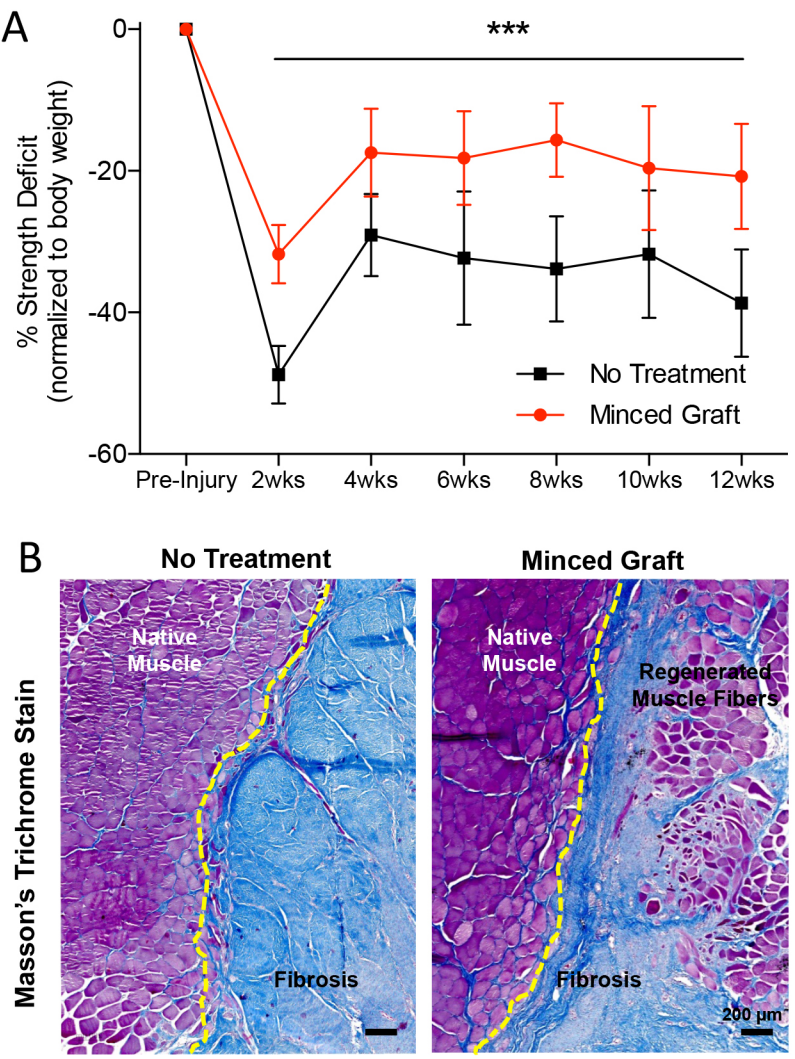

Figure 1. Autologous minced muscle graft-repair of VML in a porcine model. VML injuries were surgically created in the anterior crural muscle compartment of each leg (n = 5 pigs). One VML defect received no treatment, while the contralateral leg was treated with autologous minced muscle grafts (1 mm3 pieces of muscle). A) In vivo isometric muscle strength of the affected musculature was assessed every two weeks in each leg. At all time points post-injury, the minced muscle graft group was significantly stronger than non-repaired muscles. Values are mean ± sem; *** Minced Graft > No Treatment, $p < 0.001$. B) Representative histological images of the interface the remaining muscle mass and VML defect region 12 weeks post-injury (denoted by dashed line).

See pages 47 - 108 for financial disclosure information.