Neurotization of Decellularized Muscle Matrix Improves Functional Recovery and Promotes Unique mRNA Profiles in a Volumetric Muscle Loss Model

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Statement of Purpose: Current surgical interventions for volumetric muscle loss injuries use autograft muscle flap transfer. While these grafts provide a source of muscle to fill large voids, they typically result in development of fibrosis and fatty tissue infiltrate. To overcome this limitation, we developed a decellularized muscle matrix (DMM) that preserves extracellular matrix (ECM) components and architecture of native muscle ECM. Previously, we implanted this into a volumetric muscle loss model and demonstrated reduced fibrosis and increased immature neuromuscular formation compared to collagen and autograft controls[1]. However, these de novo neuromuscular junctions in the DMM lack re-innervation. Better strategies are needed to promote functional muscle recovery, otherwise the grafted tissues will continue to become fibrotic. The purpose of this study was to determine the effects of surgical innervation (neurotization) into DMM and autologous grafts implanted into the gastrocnemius muscle using a peroneal and tibial nerve neurorrhaphy. We hypothesized that neurotized DMM grafts would increase functional muscle recovery.

Methods: A volumetric muscle loss injury model was produced in left leg of male Sprague Dawley rats (n=8 rats/group) with a 1.5x1 cm defect in the lateral gastrocnemius muscle belly while under 4% isoflurane/400 mL/min O_2. DMM or an autologous graft was implanted to the defect site followed by graft neurotization either by a peroneal swing over or tibial nerve connection using a peroneal nerve transfer. Peroneal nerve was cut and implanted into the cut wall of the empty defect, while peroneal nerve was cut and implanted 1 cm proximal to the defect in graft alone controls. Functional muscle recovery was assessed at 2, 4, 8, and 9 weeks with muscle force testing using the 1300A Whole Animal System (Aurora Scientific). After 63 days, the animals were euthanized and muscle biopsies from the implant site were taken for RNA isolation. The isolated RNA was sent to Nanostring Technologies (Seattle, WA) for a homologous rat neuropathology and muscle panel. These Nanostring data were analyzed using the nSolver™ software. The muscle force data are presented as mean ±SEM analyzed using GraphPad Prism 6.0 (GraphPad, La Jolla, CA).

Results: The DMM treatment group neurotized using the peroneal technique showed increased max twitch and tetanic force over time with a max output by week 8 compared to autologous graft which showed only increased twitch force by week 8. Tibial neurotization demonstrated no change compared to controls, and empty defects showed no change from control muscles (Fig. 1). Distinct mRNA profiles were produced across all groups. Further, the DMM peroneal treatment group showed unique expression similar to the randomized contralateral controls. Additionally, this group showed downregulation of mRNA associated with oxidative stress, autophagy, and apoptosis. (Fig. 2)

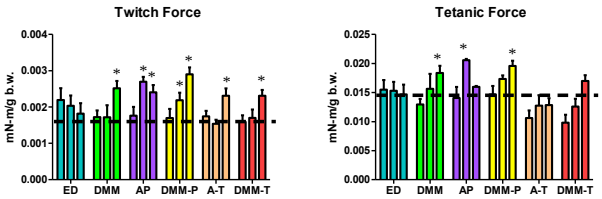

Figure 1. Time course from week 2, 4, 8 twitch and tetanic muscle force. Dashed line represents age matched control. * represents significant difference from control. ED = empty defect, DMM = graft alone, AP = autograft peroneal, DMM-P = DMM peroneal, A-T = autograft tibial, DMM-T = DMM tibial.

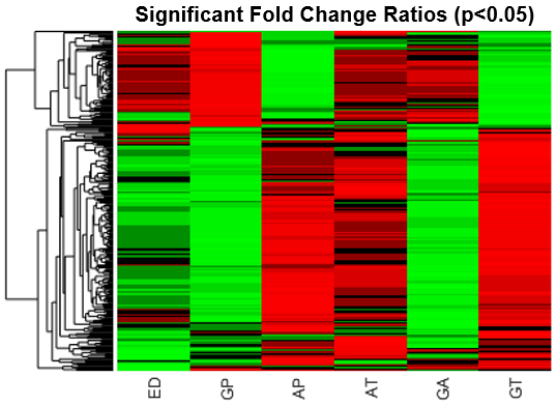

Figure 2. Heatmap of significant fold change ratios compared to randomized contralateral control legs. ED = empty defect, GP = DMM peroneal, AP = autograft peroneal, AT = autograft tibial, GA = DMM graft alone, GT = DMM tibial.

Conclusions: This study indicates the combinatory effect of neurotization and decellularized muscle matrix leading to improved functional recovery. Additionally, data demonstrated differential mRNA expression across all groups with a particular effect in the DMM treated group with a peroneal neurotization technique. Further bioinformatic big data analysis is required to better understand these differential expressions. In summary, we demonstrated that neurotized grafts enhanced functional muscle recovery while encouraging a reduced oxidative stress, autophagy, and apoptotic mRNA profile.

References: [**1**] Michael McClure, et al. Tissue Eng. Part A (2018) 24(15-16):1228-1241