

Age-Conscious Regenerative Engineering and Rehabilitation Following Musculoskeletal Injury

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INTRODUCTION: Volumetric muscle loss (VML) occurs when greater than 20% of muscle mass is injured through trauma or surgery, irreparably damaging the intrinsic regenerative ability of skeletal muscle, and leading to impairment of muscle function. Previous preclinical models, motivated by the current lack of effective clinical treatments, have demonstrated that VML can be significantly repaired using regenerative rehabilitation strategies such as biomaterial constructs and exercise. The addition of rehabilitative exercise not only recapitulates the clinical practice of prescribing physical therapy post-injury, but also enhances the therapeutic effects of transplanted constructs by improving muscle force production, innervation, and vascularization.^{1,2} The population of interest for VML has historically been active military service members, thus these preclinical models have primarily utilized young mice. However, given that a predicted 1 in 6 individuals will be over the age of 65 by 2050,³ a growing number of elderly patients will present with VML injuries which result from situations such as vehicular trauma, power tool incidents, or resection of soft tissue sarcomas. Age-related changes in muscle, such as reduced satellite cell function,⁴ can obstruct muscle regeneration and may also influence the healing response following VML. Regenerative strategies must be evaluated in a broader patient population to design musculoskeletal therapeutics which are effective across all ages. The objective of this study is to investigate how aging impacts the therapeutic effectiveness of engineered skeletal muscle and rehabilitative exercise following VML.

METHODS: Young (~8-week-old) and aged (~80-week-old) C57BL/6 mice, which roughly equate to 20- and 70-year-old humans respectively,⁵ underwent a 20-30% surgical VML injury (by mass) of the tibialis anterior (TA) muscle. The VML was left untreated or was treated using engineered skeletal muscle. The engineered muscle consisted of aligned collagen nanofibers which were fabricated using a shear-based extrusion method and then seeded with GFP+ primary mouse myoblasts. The myoblasts were induced to undergo myogenic fusion in low serum media prior to transplant.⁶ Mice were further subdivided into cohorts that received voluntary running exercise and those that did not. For the exercised cohort, mice were placed into cage wheels 7 days post-surgery and allowed to free run for 21 days while the non-exercised cohort received normal cage activity (**Fig. 1a**). Muscle function was then assessed using *in situ* muscle physiology which measured the maximum contractile force of the TA muscle. Additionally, histological analysis was conducted utilizing ImageJ and Cellpose⁷ to quantify metrics such as muscle size, vascularization, and myofiber area. Animal work was approved by and performed in accordance with the guidelines of the Oregon Health & Science University Institutional Animal Care and Use Committee (IACUC). One-way ANOVAs with *post hoc* Tukey's adjustment were used to compare groups unless the data was nonparametric, then a Kruskal-Wallis test with *post hoc* Dunn's adjustment was performed ($\alpha=0.05$). Outliers were removed via the Grubbs test.

RESULTS: Young mice which received the dual treatment of engineered muscle and exercise demonstrated significantly larger TA muscle mass ($N \geq 4$, $p < 0.05$) (**Fig. 1b**) and improved muscle function with a 2.45-fold increase in maximum contractile force ($N \geq 3$, $p < 0.001$) compared to young no-treatment controls 28 days post-injury (**Fig. 1c**). The combination of engineered muscle and exercise in young mice also saw a 1.9-fold increase in the density of blood vessels within the defect site compared to controls ($N \geq 3$, $p < 0.01$) (**Fig. 1d**). Aging diminished the efficacy of the dual regenerative treatment as there were no significant differences in TA muscle mass, force production, or vascularization among the aged experimental groups (**Fig. 1b-d**). Both young and aged mice saw a significant decrease in average myofiber cross-sectional area with the combined treatment of exercise and engineered muscle when compared to age-matched no-treatment controls (Young: $N \geq 4$, $p < 0.05$ Aged: $N \geq 4$, $p < 0.01$) (**Fig. 1e**).

DISCUSSION: Despite receiving treatment through exercise and engineered muscle, aged mice exhibit inadequate muscle healing after VML compared to young mice. No significant improvements in muscle mass, force, or vascularization were observed. These findings suggest that age-related differences in muscle regenerative capacity decrease the effectiveness of regenerative rehabilitation strategies following VML and highlights the importance of considering age when designing musculoskeletal therapies. Ongoing work is investigating how treatment and age influence cytokine profiles in the blood plasma and future studies will focus on adapting these therapies to optimize outcomes for older individuals. This will involve adjusting the onset of exercise and exercise type (aerobic vs resistance) as well as the composition of the engineered muscle (cells vs growth factor vs extracellular vesicle immobilization). Furthermore, future studies will explore myofibrillar protein isoforms and oxidative capacity. This will clarify the intriguing observation that despite both dual treatment age groups demonstrating a population of regenerating myofibers, as evidenced by a smaller average myofiber cross-sectional area, only the young animals exhibit improved muscle mass and function.

SIGNIFICANCE/CLINICAL RELEVANCE: This study demonstrates differences in the efficacy of regenerative rehabilitation therapies between young and aged populations. These findings implicate the need for broader evaluation of regenerative and rehabilitative strategies, tailored to age group, for treating musculoskeletal injuries and thus addressing a more inclusive potential patient demographic.

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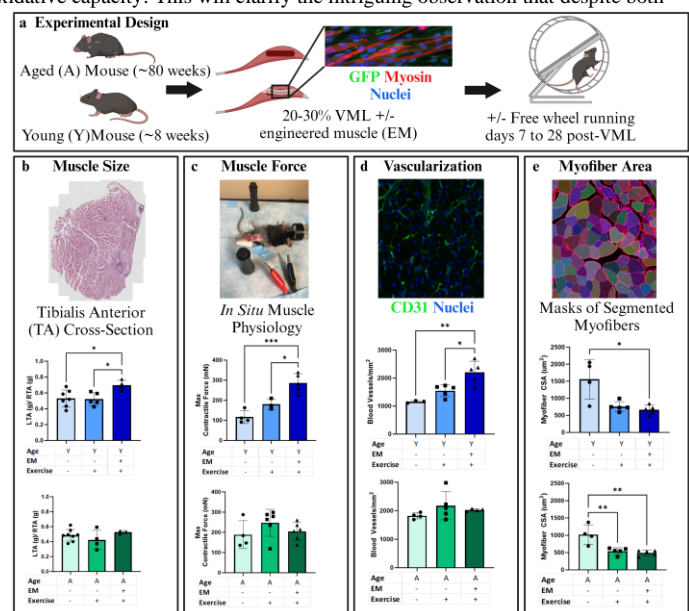


Figure 1. Age decreases the effectiveness of regenerative rehabilitation strategies for the treatment of volumetric muscle loss (VML). **a**) Experimental design. **b**) Post-mortem muscle mass in young (Y) and aged (A) mice expressed as a ratio of the injured TA muscle (LTA) to the control TA muscle (RTA). **c**) Y and A mouse muscle force was collected using *in situ* muscle physiology. **d**) Blood vessel density was quantified via immunofluorescent (IF) staining of CD31+ cells (green). **e**) Myofiber cross-sectional area (CSA) was assessed via IF staining of laminin (red) and subsequent Cellpose segmentation.