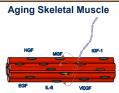


Stem Cell Preconditioning and Transplantation as a Therapeutic Strategy to Revitalize Growth and Function in Aged Skeletal Muscle

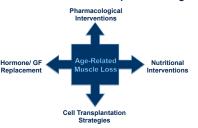


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Aging is associated with decreased regenerative potential as well as greater susceptibility to damage and reduced anabolic response to factors that stimulate skeletal muscle maintenance and growth. With numerous mechanisms involved such as decreased growth factor levels, denervation and declining vascular supply, developing effective therapeutic strategies for the age-related loss of muscle mass and function has proven to be a considerable challenge

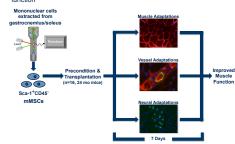
Limitations of Current Therapeutic Strategies

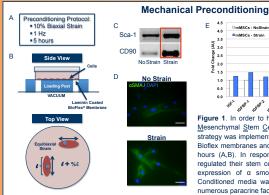


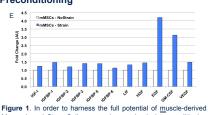
To date, efforts to repair and maintain skeletal muscle mass and function in aged individuals have been single target focused leading to limited clinical utility. This can be explained, at least in part, by the complexity of the local microenvironment as well as the numerous factors contributing to pathogenesis. Although a number of treatments are currently under investigation the complex etiology of age-related muscle loss likely warrants a multifactorial multisystem approach

Experimental Design and Purpose

- 1. Develop preconditioning methods to improve stem cell phenotype and potency following transplantation into aged skeletal muscle
- 2. Assess key markers of skeletal muscle adaptation
- 3. Assess vascular and neural adaptations within skeletal muscle
- 4. Measure the cumulative effects of tissue level adaptation on muscle function





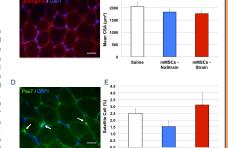


Mesenchymal Stem Cells an ex vivo mechanical preconditioning strategy was implemented. mMSCS were plated on laminin coated Bioflex membranes and exposed to biaxial mechanical strain for 5 hours (A.B). In response to strain mMSCs transcriptionally upregulated their stem cell markers (C), and down-regulate protein expression of α smooth muscle actin; scale bar 10μm (D). Conditioned media was collected post stretch and analyzed for numerous paracrine factors important for growth and repair.

Skeletal Muscle Adaptation



Figure 2. Following preconditioning ex vivo, mMSCs were transplanted directly into the gastrocnemius and soleus muscles of 24 month old C57BL6 mice. Saline only and no strain controls were performed. Seven days following transplantation mice were euthanized and tissues were collected (A). To assess skeletal muscle specific adaptation changes in myofiber cross-sectional area (CSA) and satellite cell activation were assessed. Measuring an average of 250 fibers, no differences in CSA were observed (B,C). Using Pax7 as a marker for satellite cells, total numbers of satellite cells were assess and expressed as a percentage of total myonuclei (D.E). Although no statistically significant differences were detected, a trend for an increase in the preconditioned cell injected group was observed. Scale hars 10um.



mMSCs Strain

mMSCs -NoStrain

Saline

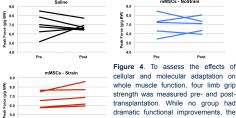
Vascular and Neural Adaptations

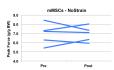
mMSCs -Strain Figure 3. Due to the heterogeneous nature of muscle tissue and age-related effects on other systems, vascular and neural adaptations within skeletal muscle were evaluated. While no significant increases were observed in the microvasculature (A), a trend toward an increase was observed in vessels larger than 5um; *p= 0.067, Strain vs. Saline (B). Finally, while denervation is a hallmark of aging, muscle injected with strained mMSCs was protected from this phenomena, #p < 0.05. Strain vs. Saline (C). Scale bars 20um.

mMSCs -NoStrain

Saline

Skeletal Muscle Function

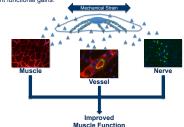




cellular and molecular adaptation on whole muscle function, four limb grip strength was measured pre- and posttransplantation. While no group had dramatic functional improvements, the mMSCs - Strain group consistently showed minor functional gains.

Conclusion

This study provides strong evidence that mechanical strain increases the therapeutic potential of mMSCs through increased release of several paracrine factors. This enhanced paracrine function has the potential to coordinate the adaptation of several tissue types within skeletal muscle. Combined, these minor adaptations in the muscle, cardiovascular, and nervous systems lead to important functional gains



Future Directions PDMS Flastomer Gels



To better understand how the mechanical microenvironment regulates behavior, preconditioned mMSCs will be plated on PDMS elastomer gels with stiffnesses within the physiologically relevant range of young, healthy to a much stiffer aged skeletal muscle microenvironment. Proliferation and growth factor release kinetics will be assessed

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