Mechanisms of ischemic skeletal muscle regeneration mediated by mechanically constrained human allogeneic mesenchymal stromal cells

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INTRODUCTION

- Critical limb threatening ischemia (CLTI) occurs when there is blockage in a major blood vessel of the leg preventing complete blood perfusion to the lower limb and foot, resulting in rest pain, tissue death, and a high incidence of amputation.
- No effective pharmacological treatment is available for CLTI, and some patients, especially diabetics, are not candidates for surgical procedures.
- Injection of bone marrow-derived mononuclear cells into leg muscles of CLTI patients has been shown to reduce the need for amputation; however, mesenchymal stromal cells (MSCs), especially in 3D form (encapsulated) may be a more effective treatment for diabetics.
- Studies have shown that encapsulating stem cells in an alginate-based hydrogel has resulted in shielding from the host's defenses and longer dwell time for the cells. It may also result in an alternate phenotype of the cells that could benefit muscle regeneration.
- Mice may be used to model CLTI by ligation and excision of the femoral artery, which creates a blood perfusion deficit in the leg, leading to muscle damage and dysfunction.
- We have adopted a polygenic mouse model of type II diabetes (TALLYHO) to include modeling of CLTI in order to determine the ability of MSCs & encapsulated mesenchymal stromal cells (eMSC) to ameliorate the tissue perfusion deficit and muscle damage in the context of diabetes.

BACKGROUND

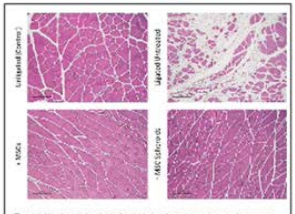


Figure 1. Muscle pathology in the ligated, ischemic gastrocnemius muscle assessed by H&E staining showed significant loss of muscle fibers. Administration of MSCs or spheroids appeared to reverse the ischemia-induced muscle fiber loss (mag=10X).

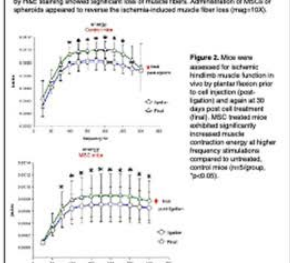


Figure 2. Mice were assessed for ischemic hindlimb muscle function in vivo by plantar flexion prior to cell injection (post-ligation) and again at 30 days post cell treatment (final). MSC treated mice exhibited significantly increased muscle contraction energy at higher frequency stimulations compared to untreated, control mice (n=5/group, *p<0.05).

OBJECTIVES

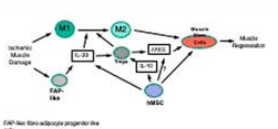
- Determine if encapsulation process caused phenotypic changes in the production of IL-10 and IL-33
- Determine if eMSCs stimulate Tregulatory cells to enhance muscle regeneration
- Determine if eMSCs stimulate muscle progenitor cells to differentiate

MATERIALS and METHODS

Animals and experimental timeline. All procedures were approved by the Indiana University School of Medicine IACUC.

- Tissue cryosections were used for immunohistochemistry (IHC) in order to assess muscle fiber regeneration and the presence of Tregulatory cells.
- Cells were encapsulated in 2% alginate using a centrifugation method. Cells were suspended in PBS, mixed with alginate, and then centrifuged through a needle into calcium chloride. This acted as a cross-linker, which formed a protective layer around the cells.
- Conditioned media from these cells along were analyzed for IL-10 and IL-33 using ELISA.

PROPOSED MECHANISM



FAP-like fibro-adipocyte progenitor like cells
Treg= Foxp3+CD+ T regulatory cells
ANEG: angiogenin
M1/M2 M1 and M2-biased macrophages
hMSC: human mesenchymal stromal cells

Figure 3. Proposed mechanism of MSC-moderated muscle regeneration after ischemic damage in tissue

RESULTS

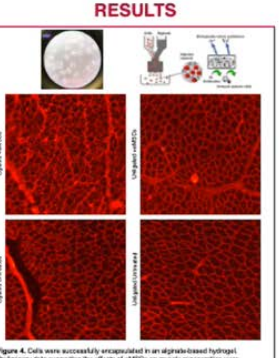


Figure 4. Cells were successfully encapsulated in an alginate-based hydrogel. Preliminary data supporting the effects of eMSCs on muscle regeneration were examined by the number of centralized nuclei in the muscle fibers. These generally suggest newer muscles, as the nuclei of mature fibers tend to drift to the perimeter. Ligated samples were expected to have more centralized nuclei than their unligated counterparts, and ligated samples treated with eMSCs appeared to have more than those that were untreated. This would suggest an increase in muscle regeneration, although further analysis needs to be completed.

RESULTS CONTINUED

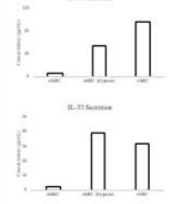


Figure 5. Preliminary ELISA results showed non-significant increases in IL-10 and IL-33 secretion by encapsulated vs. non-encapsulated MSCs. IL-10 and IL-33 secretion by encapsulated MSCs compared to that of the positive control, unencapsulated MSCs activated by hypoxia.

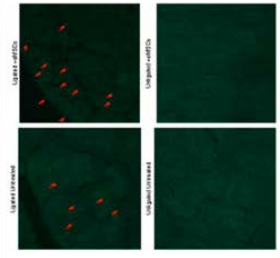


Figure 6. Immunohistochemistry was performed on 4 samples, staining for FOXP3, a marker for T-regulatory cells. Preliminary data demonstrated increased levels of FOXP3 in ligated samples treated with encapsulated MSCs. This supports our proposed mechanism involving T-regulatory cells in muscle regeneration.

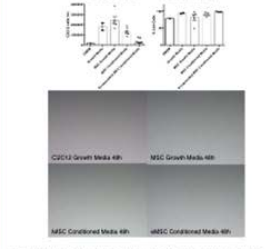


Figure 7. Myoblasts cultured with media conditioned by naked and encapsulated MSCs demonstrated decreased proliferation compared to control, but both MSC-conditioned medias caused a morphologic change in myoblasts. Cells appeared to elongate and potentially fuse. This suggests that MSCs may cause a differentiative effect on muscle progenitor cells instead of a proliferative one.

SUMMARY/CONCLUSIONS

•ELISA for IL-10 and IL-33 showed non-significant increases in IL-10 and IL-33 secretion by encapsulated vs. non-encapsulated MSCs.

•Ligated samples treated with eMSCs showed an increase in FOXP3/T-regulatory cells.

•Culturing myoblasts with media from naked MSCs and eMSCs showed a change in cell morphology along with decreased proliferation compared to the control

•This data provides initial support for encapsulated MSCs as a viable treatment option for critical limb threatening ischemia and the potential prevention of limb amputation.

•More work must be done in determining the mechanism behind skeletal muscle regeneration mediated by mechanically constrained mesenchymal stromal cells.

•Taken together, the results indicate that MSCs, and to a greater extent encapsulated MSCs, can reverse ischemic muscle damage, reduce inflammation, and increase muscle function by promoting regeneration of muscle fibers independent of tissue perfusion state.

FUTURE STUDIES

•We will repeat experiments with the Buchi Encapsulator, shown below. This machine possess the ability to mass produce beads filled with cells on a larger scale more suitable for clinical trials.

•We plan to do digital droplet PCR on RNA extracted from target tissues for small molecules that may play a role in the results demonstrated in this project. This includes amphiregulin, TSG-6, and TGF-B.

•In depth characterization of the myoblasts described in Figure 7 is necessary in order to assess the mechanistic effect this result brings and how it can be utilized clinically.

•Other cells will begin to be encapsulated, including earlier passage versions of the cells used in the experiment, vertebral body stem cells, and iPSC mesodermal cells.

•Results from figures 4 and 6 will be analyzed using a scanning microscope in order fully quantify the magnitude of those results.

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