INTRODUCTION:
The efficient repair of skeletal muscle injuries is important specifically those related to war or athletic injuries. The treatment of injured skeletal muscle with muscle-derived stem cells (MDSCs) has shown increased skeletal muscle regeneration and decreased fibrosis formation in comparison to control muscles. While these histological results are promising, the outward functionality of the regenerated muscle is the most important, yet least explored aspect of MDSC transplantation. Analysis of gait kinematics has been effective for quantifying the effect of injury on gait in other models (1).

The purpose of this study was to: (1) determine whether changes in ankle kinematics could be detected following a contusion injury and (2) determine if treadmill running and/or the injection of MDSCs improved the functionality of skeletal muscle following a contusion injury.

METHODS:
Animals: Eight 7-week old B6-SCID mice were used. Animals were housed within regulation with Children’s Hospital of Pittsburgh of UPMC’s Animal Research and Care Committee (ARCC), in the Rangos Research Building animal facility.

Injury, Treadmill Running, and Cell Injections: Injuries were made by dropping a 16 gm stainless steel ball 100 cm through an impactor onto the tibialis anterior (TA) muscle of both legs of all 8 mice using a previously described protocol (2). The extent of this injury can be compared to dropping a bowling ball off an 8-story building onto the leg of an individual on the ground. 100,000 MDSCs in 7ul of PBS were then injected into each mouse’s right TA, while an equal volume of PBS was injected into the left as a control. Next, n=4 of the injured mice were subjected to a strict running protocol on a modified rodent treadmill. This exercise included running the mice for 5 weeks, 5 days a week. The mice began running at a 0° incline at 10 meters per minute (mpm) for 5 minutes and the speed increased to 15 mpm for 15 minutes for the first 3 days. Times and speeds, as well as inclination, were increased over time until 5 minutes at 10 mpm and 55 minutes at 20 mpm with a 10° incline were achieved. A total of 4 groups were then compared: MDSC+control activity (NCA), (n=4), MDSC+treadmill (TM) running (n=4), PBS+NCA (n=4) and PBS+ TM (n=4).

Measurement and analysis of gait kinematics: Gait kinematics were measured while the mice walked along a level runway. Trials were recorded with a high-speed digital video camera (100 frames/second). Motion of the hindlimb was recorded by tracking the positions of reflective markers attached to the skin overlying the iliac crest, hip, knee, ankle and metatarsal-phalangeal (MTP) joints. The skin was shaved prior to attachment of the markers with eyelash glue. Tattooing could be utilized to ensure that the reflective markers, tattooing was slightly altered for each test due to the movement of the animals’ skin over the joints and inconsistent placement of the markers by the researchers.

RESULTS:
Average ankle range of motion (ROM) angles for the swing phase of the step cycle were calculated and compared to the baseline values. Nonparametric 1-tailed independent and related t-tests were performed (SPSS).

Table 1. Ankle ROM during swing phase

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 1</th>
<th>Day 3</th>
<th>2 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS + NCA</td>
<td>56.3 +/- 15.7</td>
<td>57.3 +/- 12.2</td>
<td>77.98 +/- 14.8</td>
</tr>
<tr>
<td>MDSC+NCA</td>
<td>44.5 +/- 16.4*</td>
<td>46.0 +/- 17.5</td>
<td>81.56 +/- 13.4</td>
</tr>
<tr>
<td>PBS+TM</td>
<td>50.6 +/- 16.2*</td>
<td>52.9 +/- 11.3</td>
<td>69.34 +/- 19.1</td>
</tr>
<tr>
<td>MDSC+TM</td>
<td>51.8 +/- 14.2*</td>
<td>65.18 +/- 6.2</td>
<td>83.10 +/- 11.7</td>
</tr>
<tr>
<td>Baseline</td>
<td>69.6 +/- 9.6</td>
<td>70.3 +/- 14</td>
<td>79.56 +/- 11.4</td>
</tr>
</tbody>
</table>

*denotes statistically different from baseline controls at p<0.05

DISCUSSION:
Functional deficits in the injured tibialis anterior 1 day following a contusion injury were detected using kinematic analysis. Further, although not statistically significant, a trend towards improved ankle ROM in the MDSC+TM group was observed when compared to MDSC only and TM only treatments at 3 days. This result suggests that a combination therapy of stem cell transplantation and exercise training may aid in a faster return of muscle function following injury. In the future, this study should be repeated with a larger sample size.

Obstacles Encountered: The unwillingness of the mice to perform the walking trial following injury appeared to contribute the largest variability in the data. Their starting and stopping pattern was inconsistent with their normal gait, preventing an accurate evaluation of muscle function. Secondly, the location and placement of the reflective markers was slightly altered for each test due to the movement of the animals’ skin over the joints and inconsistent placement of the markers by the researchers.

Possible Solutions: To address some of these issues, the trials could be recorded while the mice are running on a treadmill with a clear side panel. This rig would capture the normal gait pattern while also decreasing testing time.

In order to decrease the variability stemming from the placement of the reflective markers, tattooing could be utilized to ensure that the markers are placed in consistent locations; however, tattooing is both a time consuming and expensive alternative.

Once these alterations are made, kinematic testing has the potential to enable researchers to accurately identify and evaluate functional differences between injured muscles treated with MDSCs and/or exercise protocols when compared to controls. In the future, pairing kinematic testing with in situ force testing could provide greater insight into the functional healing of MDSC-intused skeletal muscles following injury.

REFERENCES:

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