Immmuno-regulatory roles of cyclic loading that promotes skeletal muscle regeneration

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Physical manipulation aids skeletal muscle recovery after injury, and muscle regeneration is clearly governed by inflammation. However, the link between mechanical stimulation (MS) and inflammation in muscle regeneration is unknown. Here, we investigated the impact of cyclic loading on interstitial inflammation, and the functional consequences on muscle regeneration following severe injury. For this study, we developed a robotic actuator, which can deliver various mechanical parameters to mouse hind limb skeletal muscle in a controlled manner. To generate severe injury in the skeletal muscle of the mouse hind limb, mice were subjected to a combination of intramuscular injection of myotoxin and induction of hind limb ischemia. Mechanical intervention was then applied to the severely injured tibialis anterior muscle by utilizing our robotic robotic device. First, injured muscle was found to exhibit significant improvements in contraction force and histological features (i.e. reduction in fibrosis and calcification) after 14 and 21 day-MS, respectively as compared to a group without MS. Interestingly, the group treated with MS showed a significant reduction in pro-inflammatory and phagocytic immune cells relative to its control counterpart after 3 days. Cytokine array analysis indicated that the majority of cytokines were reduced with MS, and significantly decreased cytokines were specifically associated with pro-inflammatory cytokines. Lastly, in vitro studies indicated that muscle progenitor cells cultured with pro-inflammatory factors, which were found to be significantly reduced showed increased proliferation, but reduced myogenic differentiation. This data implies that prolonged pro-inflammatory responses may adversely affect the differentiation of muscle progenitor cells, which are key players in the regenerative process. In summary, cyclic loading modulates the inflammatory cytokine profile, and the immune cell populations in injured tissue, suggesting these may mediate MS effects on muscle regeneration. These findings may be broadly applicable to other MS-driven regenerative processes.

Figure 1. i) Schematic of experimental time frame to summarize the induction of muscle injury and MS treatment in vivo. ii) The muscle force measured after 14 days of MS. iii) & iv) Representative H & E and Massons’s Trichrome images of the muscle with and without MS on 21 days. * or *** indicate p-value < 0.05 or < 0.0001 respectively.