Immuno-regulatory Roles of Cyclic Loading that Promotes Skeletal Muscle Regeneration

Bo Ri Seo[1], Christopher Payne[1], Brian Kwee[1], Conor Walsh[1], and David Mooney[1]
[1]Harvard University, Cambridge, MA

Introduction: 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.

Materials and Methods: To generate severe injury on the skeletal muscle of mouse hind limb, we challenged mouse with a combination of intramuscular injection of myotoxin and hind limb ischemia surgery. We then applied mechanical intervention on the severely injured tibialis anterior muscle of mice by utilizing a soft robotic device, which can externally apply cyclic loading on the tissue. After 3, 7, and 14 days of MS, the muscle was harvested to assess its functional performance by measuring the contraction force. Also, we screened the broad range of cytokines via cytokine array, and immune cell population by flow cytometry. In addition, we utilized *in vivo* imaging technique to assess any possible roles of MS on the mass transport, and intraviatal microscopy to examine the changes in lymphatic vessel contraction in response to MS.

Results and Discussion: First, our analysis on the functional performance of muscle tissue indicates that the injured muscle exhibited significant functional improvements after 14-day MS as compared to a control group without MS; MS for the first 7 days out of 14 days did not show the same functional outcome as a full 14 days of MS. For the group that received MS for 14 days, the total number of immune cells and specific immune cell populations (neutrophils, macrophages, monocytes, dendritic cells, and T cells) in injured muscle decreased relative to the control while the group harvested after 3 day stimulation did not show a decreasing trend except neutrophils. Interestingly, cytokine array analysis indicated that MS decreased the majority of cytokines, although to varying degrees; The group received MS for 3 days showed more pronounced changes. Significantly reduced cytokines were associated with inflammation, specifically immune cell chemotaxis, and this finding aligns with the reduced immune cell populations. The reduced levels of cytokines might be due to cyclic MS-driven mass transport in the injured tissue, as the transport of intramuscularly injected dextrans was significantly increased following stimulation. Lastly, intravital microscopy revealed popliteal lymphatic vessel diameter changed by MS, which could impact lymphatic drainage.

Fig. 1

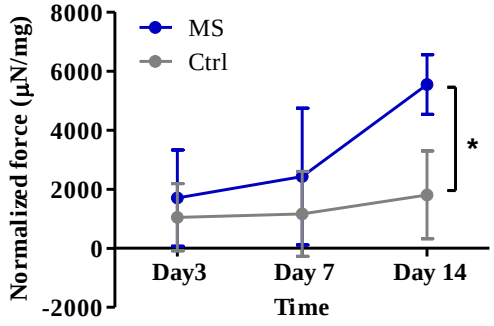

Fig. 2

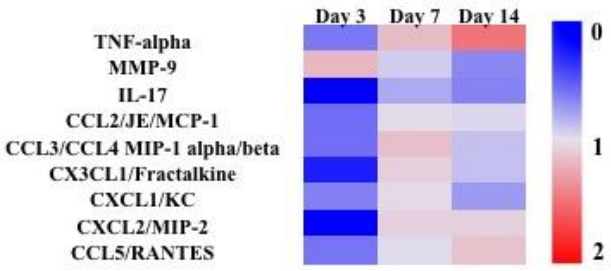

Figure 1. The bar graph shows the change in muscle contraction force over time in response to MS.
Figure 2. Color-coded pattern depicts the change in the selective cytokines of MS group relative to control group.

Conclusions: In summary, cyclic loading modulates local transport, 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.