

Human Fibroblast (HF) Model of Repetitive Motion Strain (RMS) and Myofascial Release (MFR): Potential Roles in Muscle Development

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Background: Guided fascia manipulation is an osteopathic technique capable of alleviating pain and accelerating muscle trauma recovery. The fundamental cell type of fascia, HF, is known to secrete numerous cytokines with roles in muscle development e.g. FGF-2, TGF- β , IGF-1, IL-6, and IL-1 α . When HF are strained in manners modeling RMS, MFR, and RMS followed by MFR, cytokine release is altered, suggesting mechanisms for clinical efficacy.

Hypothesis: Modeled RMS and MFR differentially regulate HF release of signaling molecules involved in myoblast differentiation into functional myotubes.

Methods: To test for myotube differentiation, HF were plated onto flexible collagen coated membranes and subjected to the following 4 strain paradigms: 8 hr 10% cyclic RMS; 60 second 6% MFR acyclic strain; RMS followed 3 hrs later by MFR; and no strain. At 24 hrs post strain, HF conditioned media (CM) from all groups were collected, and aliquots used to culture C₂C₁₂ myoblasts. Differentiation media containing horse serum and untreated media served as positive and negative controls, respectively. Differentiation of myoblast to myotubes was assessed by cellular elongation, fluorescent labeling of acetylcholine receptors via α -bungarotoxin, and hematoxylin eosin staining to document multinucleation. Each experiment was performed 3 times, with duplicate measures in each. In triplicate photomicrographs obtained from each well yielded N=18 at 48, 72, and 96 hours, and an N=6 at 88 hours. All data were analyzed using ANOVA and post hoc unpaired two-tailed t-tests

Results: Although CM from all groups induced differentiation to some degree, the differentiation index (DI; represented in myotubes per cm²), varied among treatment groups. In t-test analysis vs. non strain CM, MFR resulted in the earliest myotube formation at times 72 and 88 hrs (DI = 35.6 \pm 9.9, p=0.04, 71.5 \pm 28.6, p=0.05), leveling (DI = 80.9 \pm 25.4, p=0.17) at 96 hrs. RMS DI was delayed vs. non strain CM at 72 and 88 hrs (28.5 \pm 11.5, p=0.17, 14.3 \pm 9, p=0.54), however at 96 hrs RMS DI was the greatest among all groups (114.3 \pm 30.42, p=0.03). RMS+MFR DI was also delayed vs. MFR at 72 and 88 hrs (28.6 \pm 11.0 p=0.16, 28.5 \pm 14.2 p=0.21), and was attenuated vs. RMS DI at 96 hrs (47.6 \pm 14.7 p=0.05). Non-strained CM had the lowest DI (9.6 \pm 7.4, 7.167 \pm 7.167, and 38.1 \pm 16.6) at 72, 88, and 96 hrs.

Conclusion: These data suggest that HF secrete soluble mediators of myoblast differentiation, and that biomechanical forces modeling RMS and MFR differentially regulate muscle development. Ongoing research is currently investigating the involvement of candidate cytokines and mechanisms associated with differentiation. Funding: AOA