

# In Vitro Human Fibroblast (HF) Injury Repair in Response to Modeled Repetitive Motion Strain (RMS) and Myofascial Release (MFR)

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**Background:** Despite clinical efficacy, the cellular basis for osteopathic manipulative therapies (OMT) is not well understood. By utilizing various in vitro strain profiles, we investigated commercially available HF cellular responses to modeled RMS and MFR. We focused here on wound closure rate of HF in response to mechanical strain.

**Hypothesis:** We hypothesize that (1) RMS will delay HF wound closure and (2) these effects are mediated by signal transduction dependent on HF secretions in response to strain and that they are reversed by MFR treatment and/or inhibition of nitric oxide synthase (NOS).

**Methods:** A modeled scratch-wound approximately 2 mm wide was applied to a sub-confluent monolayer of cultured HF plated on flexible bottom collagen coated plates. And then subjected to one 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. Each treatment was conducted in the presence and absence of the NOS inhibitor L-NMMA. Additionally, unstrained HF were inoculated with condition media (CM) from these strain groups and assessed for wound closure rates microscopically at times 0, 24 and 48 hrs post-injury. All data were analyzed using unpaired two-tailed t-tests.

**Results:** At 48 hrs post-injury, RMS treated HF responded with a 79.5% ( $p < 0.0001$ ,  $n=9$ ) reduction in wound closure when compared to non-strained control HF. CM derived from RMS treated HF also impaired wound closure by 30.5% ( $p < 0.05$ ,  $n=4$ ) when compared to non-strained CM. This phenomenon was *not* observed in HF treated with CM from MFR nor in combined RMS+MFR strained groups. MFR treatment alone improved the rate of wound closure by 138.5% ( $p < 0.01$ ,  $n=6$ ) in HF subjected to RMS. L-NMMA treatment had no significant effect on wound closure in non-strained HF. However, equivalent NOS inhibition resulted in wound closure improvement in RMS and RMS+MFR treated HF as compared to RMS treatment alone (191.0%  $p=0.004$ ,  $n=6$ ; 194.8%  $p=0.009$ ,  $n=3$  respectively).

**Conclusion:** These data suggest that injurious strain results in significant impairment of wound closure and that CM from strained fibroblasts is sufficient to mimic this impairment. These results support the stated hypothesis; that impairment of wound closure is induced by both biomechanical strain and soluble mediators secreted by HF in response to strain. The reduced wound closure rate caused by RMS can be normalized by treatment with modeled MFR and/or inhibition of NOS. Taken together, these in vitro studies suggest additional cellular evidence that may explain the clinical efficacy of OMTs post-injury.

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