THE UNIVERSITY OF ARIZONA
COLLEGE OF MEDICINE PHOENIX

# Prophylactic Dosing of Myofascial Release in a Human Fibroblast Model of Wound Closure

Travis Joseph Powell, 2012

## INTRODUCTION

Benefits of prophylactic stretch intervention on injury reduction have been well documented in the literature [1, 2, 3, 4]. Studies have shown that stretching before strenuous exercise can reduce muscle strain and injury in various applications such as military training, soccer, and high school football. Myofascial release (MFR) is a technique applied by clinicians to directly stretch and palpate soft tissue restrictions and to improve tissue elasticity, maximizing range of motion [5, 6]. However, despite a great deal of research focusing on the use of MFR after repetitive motion strain (RMS) and wound closure there is no known application of prophylactic MFR treatment and its effect on wound closure rates. By utilizing our previously reported in vitro strain models we will investigate the potential roles of prophylactic MFR in regulating fibroblast wound healing. We hypothesize that such MFR treatments will have greater efficacy when used prior to the injury and repetitive motion strain, increasing the rate of wound healing.

## METHODS

Human fibroblast (HF) were seeded at a concentration of 1x10$^5$ cells per well onto Bioflex plates arranged in 6-well format with flexible collagen coated elastomeric well bottom. Once the cultures reached 70-80% confluence, growth media (DMEM supplemented with 2% FBS) was replaced with a reduced serum (0.2% FBS) medium to induce quiescence. After twenty-four hours a modeled scratch wound was induced via a sterile 1000 ul pipet tip. The result was a "wound" strike of approximately 2 mm by 25 mm or a 50 mm$^2$ area completely devoid of cells. Debris and medium was removed with a sterile phosphate buffer saline wash. Fresh growth medium was reintroduced to each well and the plates were imaged as a time point of 0 hours and then subjected to one of the four strain paradigms (Fig 1, 2A, 2B). They groups were then reimaged at 24, 36, and 48 hours.

1. *Control (No-strain):* cells which did not receive any strain during the duration of the experiment. Representing immobilized tissue after injury
2. *RMS (Repetitive Motion Strain):* cells subjected to RMS strain profile, which consisted of a cyclic strain at 0.625 hertz (cycles/second) through a 10% strain. The strain's duration lasted 8 hours. Representing those who ignore treatment after injury and complete a RMS activity
3. *RMS+MFR (RMS plus Myofascial Release):* cells subjected to the 8 hour RMS strain profile followed three hours later by MFR stain profile for 60 seconds. The MFR strain consisted of a slow loading strain increased at a 3% a second for two seconds, held constant at roughly 6.6% strain for 54 seconds and then decreased by -1.5% per second for 4 seconds. Representing those who ignore treatment after injury and complete a RMS activity, but seek treatment following in the form of MFR therapy.
4. *MFR+RMS:* cells subjected to the 60 second MFR profile followed three hours later by RMS profile. Representing those who seek treatment after wound injury and complete a RMS activity following.

Strain profiles were conducted utilizing the Flexercell FX-4000 Tension Plus System. It encompasses a computer controlled vacuum system, which directs controlled vacuum pressures to a baseplate. 25mm platens placed beneath the flexible membrane allow for a equibiaxial strain across the membrane where by this deformation of the collagen similarly stretches the attached fibroblasts. Programming the strain magnitude, duration, direction and frequency of the negative pressure created the desired strain profile.

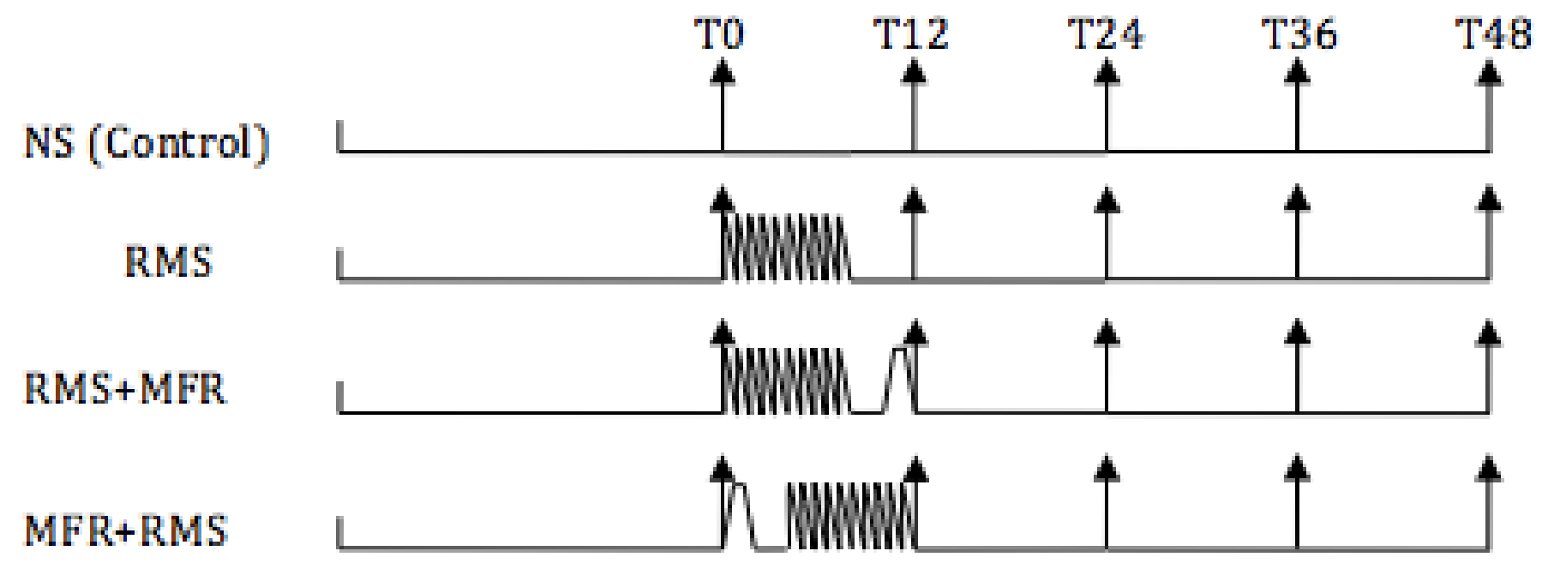

*Figure 1:* Strain time-line for the four treatment groups: non-strain (NS) control, repetitive motion strain (RMS), Repetitive motion strain followed by myofascial release (RMS+MFR) and myofascial release followed by repetitive motion strain (MFR+RMS). The cells were wounded and imaged immediately and that point acted as time zero. Time points 12, 24, 36, and 48 hours were imaged based on time zero.

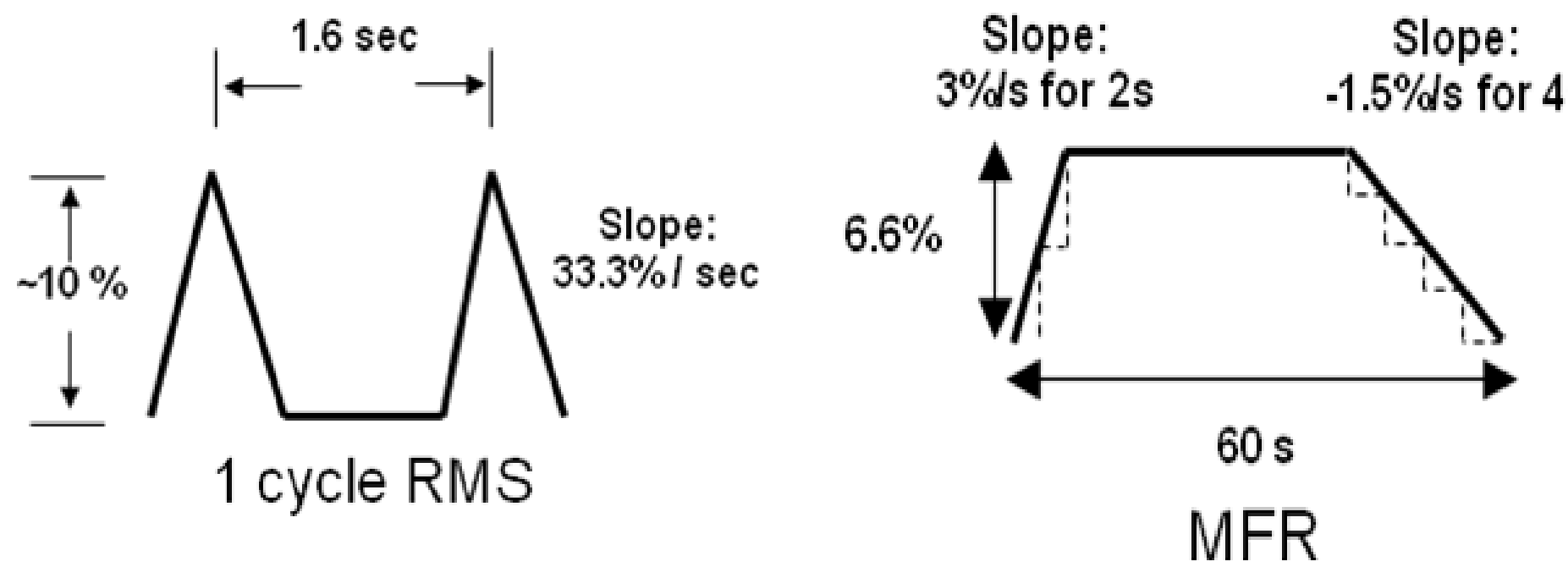

*Figure 2:* (A) Specifics of strain profile repetitive motion strain (RMS) and for (B) Myofascial Release (MFR)

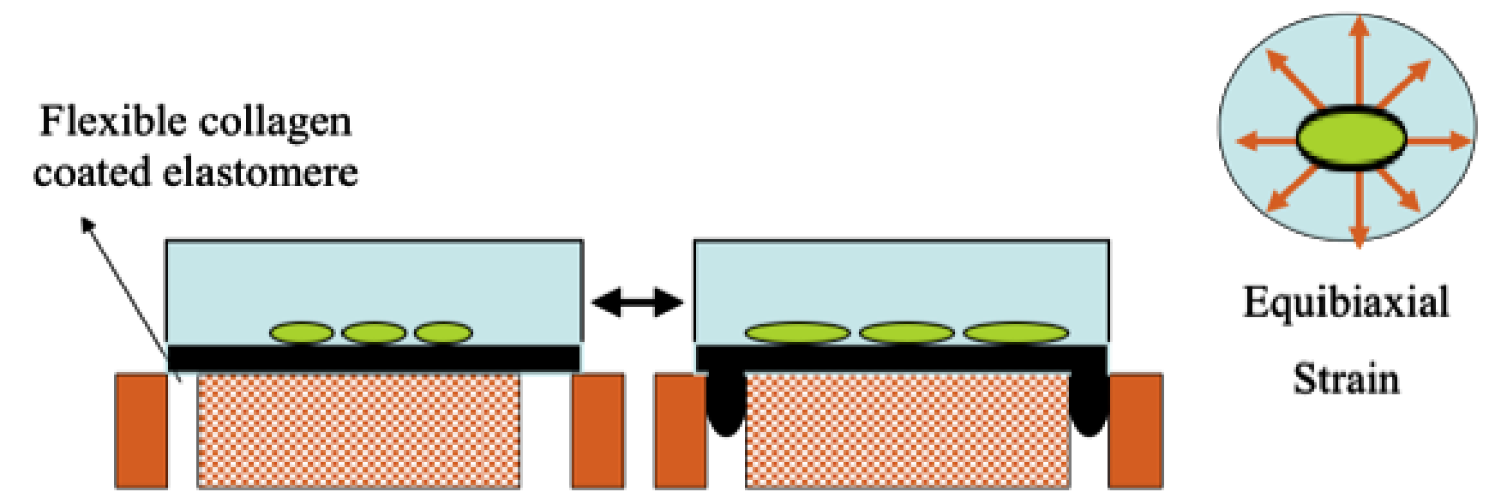

*Figure 3:* Diagram of Bioflex plate and strain apparatus. Green ovals represent fibroblasts attached to collagen well. Under each well is the 25mm platen. When vacuum is applied membrane is pulled over and down platen in the direction of black arrows as shown on right diagram.

## RESULTS

Meta-analysis was completed for the 36-hour time points with the experimental data normalized as a percent of non-strain. This was calculated by determining the non-strain average for the experimental trial and using this value to normalize the strain groups for that same trial (Fig. 5). Thirty six-hours was chosen because wound the edges had begun to approximate and close at the 48 hour time point making the measured values of closure rate unreliable. Each strain paradigm was represented by 12 wells (n=12). The combined data showed that RMS groups closed 32% faster than combined RMS+MFR treated group and 30.5% faster than non-strain control (n=12; p<005).

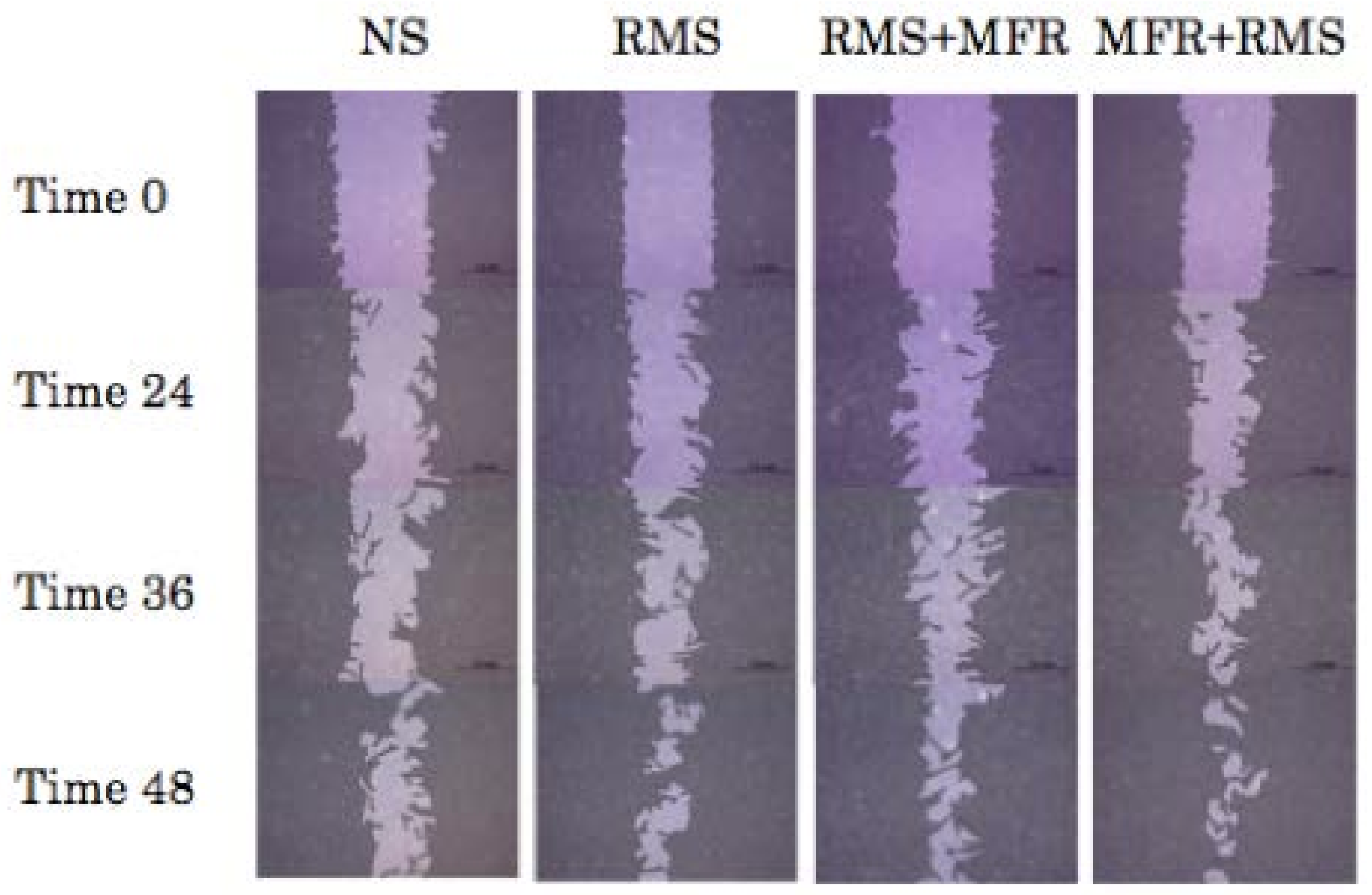

*Figure 4:* Representative photomicrographs of NS, RMS, RMS+MFR and MFR+RMS human fibroblasts immediately after wounding (time 0 hours), 24 hours, 36 hours and 48 hours. All images were captured at 40x magnification and the scale bar shown indicates 1.0mm

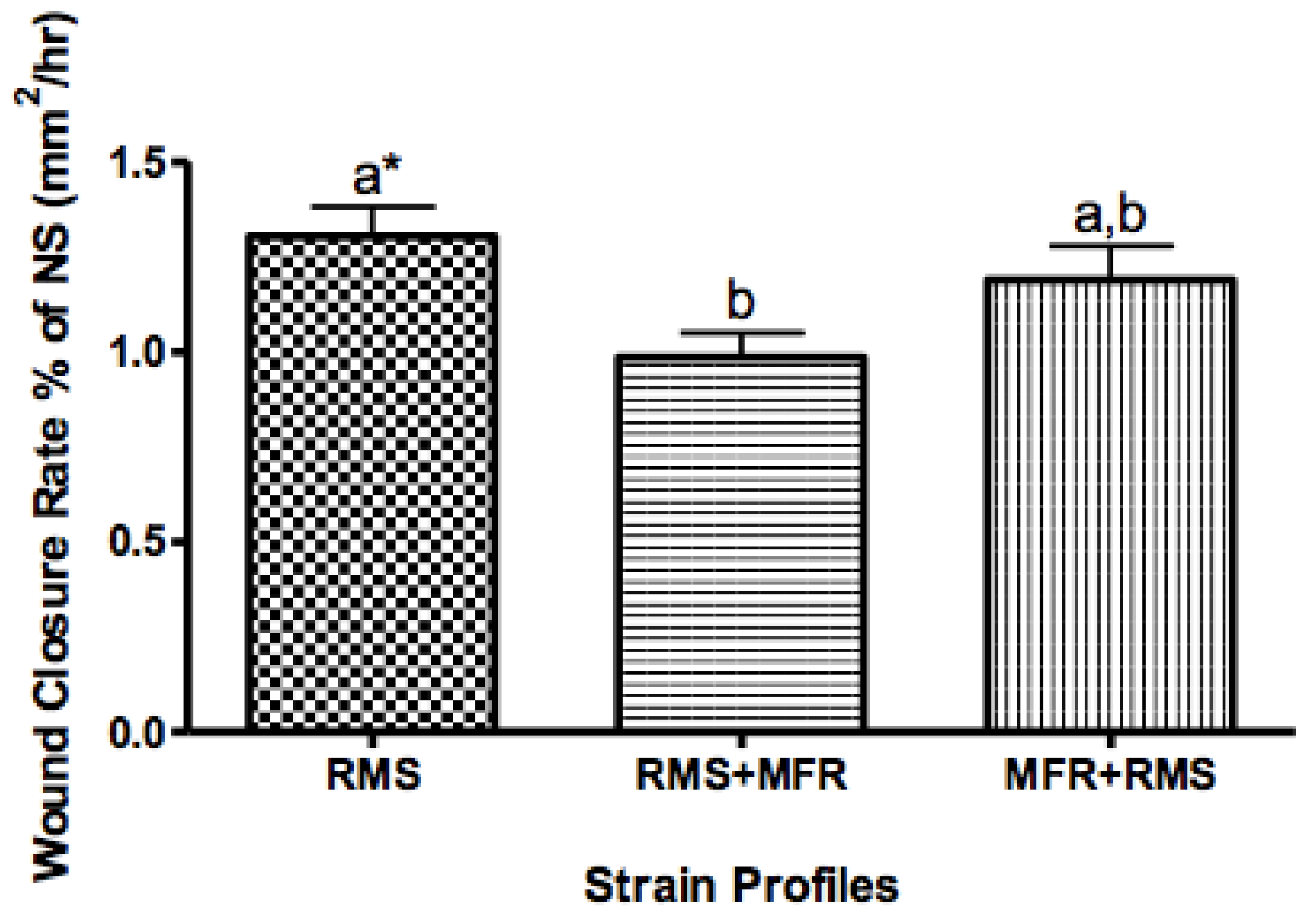

*Figure 5:* Meta-analysis of would closure rate (n=12) as a percent of non-strain. Different letters (a, b) denote significant difference between the respective groups ($p<0.05$) * Indicates a significant difference of strain profile compared to control ($p<0.05$).

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## DISCUSSION

Prophylactic application of MFR did not increase wound closure rate when normalized to a non-strain control and therefore supported the null-hypothesis. The discrepancy between our experimental modeling and previous experiments may lay in the complex nature of wound healing. Various factors in the experimental model may have influenced our findings for both the prophylactic MFR as well as the RMS+MFR groups. Errors may have been encountered with cell plating densities. There were instances that we observed complete wound closure of the wound edge during imaging. The experimental model itself may not have tested components important to the stretch response mechanism such as extracellular matrix, different cell types, transport vessels and other components which might contribute to wound closure. Despite these limitations, if these data remain consistent and translatable in vivo the results suggest that MFR applied pre- and post RMS does not affect wound closure. Research has shown wound healing to involve a mix of mechanisms including apoptosis, inflammation, secretory response, migration and replication. Although we did not observe enhanced wound closure with our modeled strains, it does not preclude the possibility that MFR may potentially reduce the risk of injury in differently modeled situations. Our model only applied a single 60-second MFR treatment where many of the other pre-stretch therapies utilized multiple segmented and repeated treatments. We believe that to fully appreciate the true effect of prophylactic MFR application additional studies are necessary which can expand on our model to include multiple dosing of treatment MFR, varied MFR strain patterns or the use of animal models focusing the emphasis on prevention of injury instead of wound healing. Through our findings it was clear that our in-vitro model did not support our hypothesis that prophylactic myofascial release wound increase the rate of wound closure.

## CONCLUSION

The reason for the unexpected outcome in wound healing seen in this experiment may have multiple layers of complexity. The fibroblasts may have been cultured in slightly different densities, the cells may have been exposed to unintended strain and the blinding process could have been faulty. We did our best to control for variation and consistency between the two final experiments and our laboratory will continue to actively research both myofascial release and repetitive motion injuries into the future. We think that it would be valuable to run additional experiments in the future to determine which specific mechanisms of wound closure may have played a role in our observations and to deduce the difference seen with this wound closure model compared to our previous models.

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