

# Modeled Osteopathic Manipulative Treatments: A Review of Their in Vitro Effects on Fibroblast Tissue Preparations

Manal Zein-Hammoud, PhD  
Paul R. Standley, PhD

From the Department of  
Basic Medical Sciences  
at the University of  
Arizona College of  
Medicine in Phoenix.

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Address correspondence to  
Paul R. Standley, PhD,  
Department of Basic  
Medical Sciences,  
University of Arizona,  
College of Medicine,  
B-558 HSEB, 435 N 5th St,  
Phoenix, AZ 85004-2157.

E-mail:  
standley@email.arizona.edu

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**A key osteopathic tenet involves the body's ability to self-heal. Osteopathic manipulative treatment (OMT) has been evolved to improve this healing capacity. The authors' in vitro work has focused on modeling 2 common OMT modalities: myofascial release (MFR) and counterstrain. Their studies have evaluated the effects of these modalities on wound healing, cytokine secretion, and muscle repair. The key components of the host response to mechanical forces are fibroblasts, which are the main fascial cells that respond to different types of strain by secreting anti-inflammatory chemicals and growth factors, thus improving wound healing and muscle repair processes. The purpose of this review is to discuss the cellular and molecular mechanisms by which MFR and other OMT modalities work, in particular, the role of strained fibroblasts in inflammation, wound healing, and muscle repair and regeneration. Changing MFR parameters, such as magnitude, duration, direction, and frequency of strain, might uniquely affect the physiologic response of fibroblasts, muscle contraction, and wound healing. If such results are clinically translatable, the mechanisms underlying the clinical outcomes of OMT modalities will be better understood, and these treatments will be more widely accepted as evidence-based, first-line therapies.**

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**D**espite the medical training behind osteopathic manipulative treatment (OMT), some outside the osteopathic medical profession may still consider OMT modalities to be complementary or alternative therapies. This misunderstanding of OMT may result from the fact that the mechanisms behind OMT and how it affects a patient's physiologic structure and function are poorly understood.

## Fundamental Factors in OMT

### The Role of Biomechanics in Repetitive Motion Strain and OMT

Repetitive and forceful movements, awkward postures, and sustained forces often lead to repetitive motion strain (RMS), a common condition generally correlated to occupation-related factors such as physical and psychological distress and monotonous work, but which is also correlated to non-work related factors, such as sports and other recreational activities.<sup>1</sup> Symptoms may include back pain, tendonitis, headache, behavioral problems, and dental problems.<sup>2</sup> These symptoms may lead to a variety of musculoskeletal disorders, peripheral nerve entrapment, and vascular syndromes affecting tendons, muscles, joints, blood vessels, as well as the back, neck, and upper limbs.<sup>1,3</sup> In general, such disorders have, at their root, biomechanical strain-induced dysfunctions.

Known to relieve pain associated with somatic dysfunction, OMT improves the circulation and function of the body.<sup>2</sup> However, the precise cellular and molecular mechanisms of action of these treatments remain poorly understood. A common aspect of all OMT techniques is that they extracorporally impart various biomechanical stimuli to surface and deep tissues, such as the fasciae and muscle layers. Like the RMSs, these biomechanical stimuli may take many forms, nearly all of which can be attributed to tissue and cellular stretch, compression, torque, and shear. Consequently, 1 thing common to both deleterious repetitive strain injuries and curative OMT is biomechanical stimulation. This connection catalyzed our laboratory to investigate how various biomechanical strains modeling both RMSs and various OMT modalities affect cellular physiology and gene activation and suppression. More specifically, we have sought to explain the mechanisms by which those OMT modalities can clinically eliminate pain, restore motion, and improve body function. By modeling strain techniques *in vitro* using bioengineered tissues, our goal has been to show proof of concept that different strain factors might uniquely affect critical processes, such as wound healing, muscle repair and regeneration, and anti-inflammatory cytokine expression and secretion. If clinically translatable, biomechanical strain-induced effects observed *in vitro* may help explain patients' responses to OMT and the basics of the cellular and molecular mechanisms of such treatments.

### **The Role of Fibroblasts in OMT**

A soft tissue component of the connective tissue, the fasciae comprise parallel longitudinal collagen bundles and rudimentary elastic laminae and covers connecting muscles (myofasciae), tendons, bones, vessels, organs, and nerves.<sup>4</sup> The primary symptom of RMS is myofascial pain resulting from tightness along the muscle fibers caused by activation of trigger points (sensitive points) within the injured muscles.<sup>5</sup> The fibroblast, the principle cell type of the fasciae, synthesizes, organizes, and remodels collagen.<sup>6</sup> During

development, differentiation, and tissue repair, fibroblasts often differentiate into myofibroblasts in response to mechanical stress.<sup>7</sup> Fibroblasts are targets for normal and abnormal biomechanical stimuli. We have reported that fibroblasts *in vitro* respond to strain by secreting proinflammatory and anti-inflammatory cytokines, undergoing hyperplasia as well as altering cell shape and alignment.<sup>8-10</sup>

Fibroblasts respond to injury by rapid proliferation at the site of injury, forming granulation tissues and providing structural integrity to the wound.<sup>11</sup> Events that constitute the wound healing process include cellular proliferation, migration, extracellular matrix deposition, and remodeling.<sup>12</sup> By manipulating the fasciae, OMT targets fibroblasts. For instance, fascial unwinding was designed to stimulate postulated mechanoreceptors in the fasciae by applying gentle touch and stretching that induces relaxation and, presumably, activates the central nervous system until a state of ease is reached.<sup>13</sup> Our laboratory has shed light on several types of *in vitro* strains that modulate fibroblast functions. Although our work has been carried out exclusively *in vitro*, these biomechanical strains are predicted to play a role in pain relief and in improving the physiologic functions that have been disrupted during injury. The role of fibroblast strain in inflammation, wound healing, and muscle repair and regeneration needs to be investigated further through clinical translational studies designed to unravel the cellular and genetic mechanisms of OMT.

### **The Role of Biomechanical Strain in OMT**

Symptoms of somatic dysfunction are alleviated by OMT, which aims to align the structures of the body, thereby improving circulation and function.<sup>2</sup> The 3 main categories of OMT are direct, indirect, and direct-indirect.<sup>14</sup> High-velocity, low-amplitude thrust; muscle energy techniques; and soft tissue manipulation are direct techniques, in which pressure is applied to relax muscles and improve circulation and function. Counterstrain

(CS) is an indirect technique that uses passive body positioning of spastic muscles and dysfunctional joints toward positions of comfort that compress or shorten the malfunctioning structure, hence relaxing the aberrant reflexes and alleviating pain. Myofascial release (MFR) is a direct-indirect technique involving balancing the structure in 3 planes of motion and making positional corrections that are thought to lead to tissue relaxation.<sup>2,14</sup> A key commonality of all OMT techniques is that they were designed to impart biomechanical stimuli to affected or, at least, associated tissues to bring about change in cellular function.<sup>2,15</sup> The techniques are shown to be effective in treating patients with neck pain,<sup>16</sup> shoulder pain,<sup>17</sup> tendonitis,<sup>18</sup> fibromyalgia,<sup>19</sup> tension headache,<sup>20</sup> back pain,<sup>21</sup> osteoarthritis,<sup>22</sup> and other conditions.

Besides stretching and compression, torque treatment using an acupuncture needle rotation induces rapid morphologic changes in fibroblasts.<sup>23</sup> In addition, exposing fibroblasts to shear fluid flow and equibiaxial stretch (equal strain across both axes) resulted in structural rearrangement of fibronectin, an extracellular matrix protein secreted by the fibroblasts, suggesting a role for both fibroblast and fibronectin in torque-associated pain relief.<sup>24</sup> Although we have opted not to focus on torque, we have focused on stretching fibroblasts as well as compression techniques to begin an analysis of potential cell and tissue effects that may explain patients' improvements after OMT.

## Modeling of OMT

### In Vitro Modeling of OMT Modalities in Fibroblast Tissue Preparations

Factors such as magnitude and duration of cell and tissue stretching play essential roles in fibroblast remodeling of the extracellular matrix.<sup>25</sup> In our laboratory, we are studying not only the magnitude and duration of strain, but also the direction and frequency of all strain techniques. We have used different in vitro

models to avoid influences of confounding variables such as sex, age, and body mass index, and specifically focus on 1 variable at a time: magnitude, duration, direction, or frequency. We acknowledge that our in vitro models are not ideal, because they are limited to fibroblasts and are devoid of blood vessels, nerves, and organ systems. How in vitro strain magnitudes reflect in vivo magnitudes is difficult to determine. Nevertheless, our objectives remain to demonstrate that the different strain modalities used in our in vitro models are likely to affect cell function in manners that may help explain the clinical efficacy of OMT.

We developed a 2-dimensional fibroblast matrix in which different strain paradigms were modeled in vitro. Human fibroblasts were seeded in flexible collagen-coated wells, allowing them to adhere to the well surfaces for 24 hours. Cells were then subjected to various types of strains using selected magnitudes and durations.<sup>8,9,26,27</sup> We used these engineered tissue matrices to investigate the effects of strain on fibroblast wound healing using an in vitro scratch wound strain model.<sup>28</sup> It is known that fibroblasts exhibit unique features when they interact with 3-dimensional collagen matrices,<sup>29</sup> an observation that prompted us to improve upon the 2-dimensional matrix by developing a 3-dimensional fibroblast matrix that we dubbed a *bioengineered tendon* (BET). We developed BETs attached to nylon mesh anchors to create a 3-dimensional model that would serve in studying the effects of strain on fibroblast function and modeled healing, overcome the limitations of the 2-dimensional matrix, and model the physical environment found in vivo.<sup>31</sup> The difference between the symmetric adhesive interactions in 3-dimensional matrices and the forced asymmetry of the 2-dimensional surfaces may result in changes in morphology.<sup>30</sup> Further, cells in the 3-dimensional matrix are capable of penetrating into the matrix as well as stabilizing matrix fibrils by remodeling them.<sup>6</sup> A third model that we developed is a coculture that allows interaction between fibroblasts and myoblasts and creates a mod-

eled myofascial junction, where muscle is surrounded by a single layer of fascia.<sup>32</sup> The aim of this model was to test the ability of strain-activated fibroblasts to enhance myoblast differentiation.<sup>32</sup>

Using the Flexercell FX-4000 Tension Plus System (Flexcell International Corp), we created strain profiles by programming the magnitude, duration, and frequency of the negative pressure to yield the desired profiles.<sup>26,31</sup> The specific parameters, such as frequency, direction, loading, and unloading rates, were determined by analyzing videomorphometric data of clinically applied modeled MFR as well as modeling reference parameters of RMSs.<sup>27</sup>

### **Effects of Strain Direction on Fibroblast Morphology and Function**

Before we modeled clinical MFR, our laboratory investigated potential changes that acyclic in vitro biophysical strain has on the cellular shape, proliferation of normal human dermal fibroblasts, nitric oxide (NO) production, and interleukin 1 (IL-1) and IL-6 secretion.<sup>8</sup> Acyclic strain was applied in a heterobiaxial direction (unequal strains in both axes) in magnitudes of 10% to 30% beyond resting length and durations of 12 to 72 hours. Our results indicated that strain magnitude at 10% for 48 and 72 hours induced mild cellular rounding and pseudopodia truncation compared with the spindle-shaped appearance and well-defined pseudopodia of the control nonstrained fibroblasts. However, increasing strain magnitude to 30% caused reduced cell viability, cell membrane decomposition, and pseudopodia loss. Interleukin 1 secretion was not different, whereas IL-6 secretion and NO levels were increased in strained cells at 10% for 48 and 72 hours compared with nonstrained cells (*Table 1*).<sup>8</sup> These data conclusively showed that biomechanical strain had profound and, perhaps, clinically relevant effects on several cellular processes, such as proliferation, apoptosis, and cytokine production. Additionally, these results were the first to suggest a potential use of dose-dependent OMT in patient care.

After recording and analyzing videos of osteopathic physicians performing various OMT techniques in multiple axes, we developed modeled OMT strain profiles and subjected our 2- and 3-dimensional tissue preparations to them (*Figure 1*). In addition, we investigated the possible role of strain direction in mediating changes in cell function. We reported that differences in strain direction resulted in differential effects on cell growth, morphology, and IL-6 secretion.<sup>26</sup> Our results showed that human fibroblast morphology and cellular proliferation are affected by strain direction. For instance, heterobiaxial but not equibiaxial strain affects fibroblast morphology. This difference in response to different strain direction is likely correlated to actin, which mediates strain-induced cellular Ca<sup>++</sup> release. Our study suggested that the increase in cell number and changes in cytokine production might be influenced by strain direction (*Table 2*).<sup>26</sup> Therefore, we suspect that if our in vitro results are clinically translatable, strain direction may also confer unique responses to OMT applied by physicians.

### **Modeled CS and MFR Reverse the Inflammatory Effects in RMS-Treated Fibroblast Preparations**

For the following discussions that reference our work in this area, 2 different terms for each technique were used, and the choice of the term depended on the journals' requirements. *Repetitive motion strain* and *cyclic short duration strain* refer to the same technique; *MFR* and *acyclic long duration strain* refer to the same technique; and *CS* and *indirect OMT* refer to the same technique. The effect of CS is due to muscle shortening, which is important for its own protection, and tender points develop in those shortened muscles. Therefore, this position of comfort obtained by shortening tissues is central in CS (*Figure 2B*).<sup>33</sup>

We have used modeled RMS in an attempt to model a typical tissue injury profile and observe the accompanying changes in cell physiology. Fibroblasts

**Table 1.**  
**Main Effects of RMS, CS, Acyclic Strain, and MFR on Morphology, Proliferation, Apoptosis, and Cytokine Production of Normal Human Dermal Fibroblasts**

Measures RMS+MFR <sup>27</sup>	RMS <sup>9,27</sup>	CS <sup>9</sup>	RMS+CS <sup>9</sup>	Acyclic Strain <sup>9</sup>	MFR <sup>27</sup>	
Magnitude/time	22%/s every 1.6 s	10%	RMS: 22%/s every 1.6 s; CS: 10%	10% for 48-72 h	6% for 60 s	RMS: 22%/s every 1.6 s; MFR: 6% for 60 s
Morphology	Elongation of lamellipodia; cell perimeter ↑; cellular decentralization; intercellular distances ↑; cell-cell contact ↓	NA	NA	Cell rounding; loss of pseudopodia; no changes in cell viability	Cell perimeter ↓	NA
Proliferation	↓ by 15%	No change	↑	↑	NA	NA
Apoptosis	Apoptosis rate ↑; Phospho-DAPK ↑; Phospho-FAK ↑	No change	NA	↑ Nitric oxide	No change	No change
Cytokines	IL-1α, IL-1β, IL-2, IL-3, IL-6, IL-16, and IL-1RA ↑	IL-3 ↓	IL-6 ↓	IL-6 ↑	NA	GRO ↑

**Abbreviations:** CS, counterstrain; DAPK, death-associated protein kinase; FAK, focal adhesion kinase; GRO growth-related cytokine; IL, interleukin; MFR, myofascial release; NA, not available; RA, receptor antagonist; RMS, repetitive motion strain.

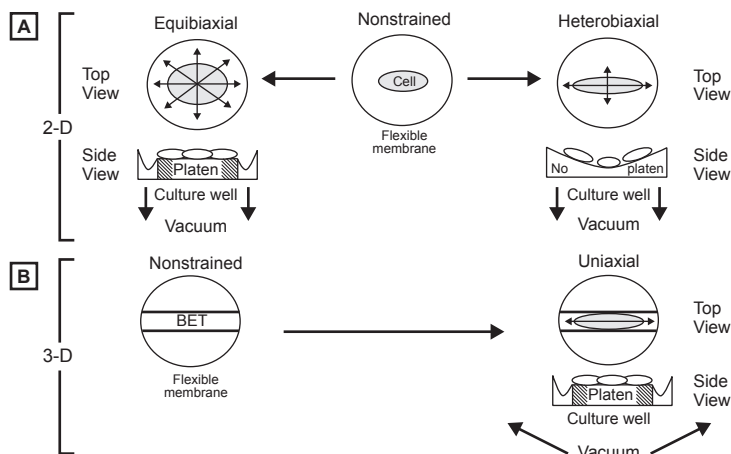
were seeded onto membranes prestrained to 10% beyond resting length,<sup>9</sup> subjected to the RMS profile, and then either sampled immediately on cessation of RMS or 24 hours later<sup>27,28</sup> (*Figure 2A*). In related experiments, we combined both modeled RMS and modeled CS to investigate potential changes in human fibroblast proliferation and interleukin secretion.<sup>9</sup> After videotaping clinicians performing MFR on patients, we arrived at basic modeled MFR profiles defined by appropriate loading and unloading rates and strain magnitude and duration (*Figure 2C*). We aimed to investigate possible cellular and molecular mechanisms that would explain the immediate clinical outcomes associated with combining RMS and MFR.<sup>27</sup> These

studies were designed to be a surrogate to a clinical scenario in which a patient with a repetitive motion injury was treated with OMT. Our data revealed that human fibroblasts respond to various types of strains differently by changing cellular morphology, proliferation, and cytokine and NO secretions (*Figure 3* and *Table 1*). Although modeled RMS produced a delayed inflammatory response and reduction in cellular proliferation, both modeled CS and MFR reversed those effects.<sup>9,27</sup> In addition, using different strain magnitudes enabled us to establish minimum and maximum thresholds, which affect physiologic change and cellular viability, respectively. The results of these studies suggested that cellular shape is a product of both strain

duration and magnitude.<sup>8</sup> Herein, we have shown proof of concept that both clinical CS and clinical MFR may equivalently reverse RMS injury in patients in manners that affect cytokine and NO signaling as well as cellular proliferation. If these data are clinically translatable, a cellular mechanism of understanding may be revealed to explain the efficacy of CS and MFR treatments.

### Varying MFR Strain Magnitudes and Durations Produce Differential Effects on Fibroblast Tissue Preparations

To our knowledge, large-scale clinical studies looking at variables such as magnitude and duration of MFR treatment, or any other specific OMT techniques, have not been published. After watching video recordings of clinically applied MFR and measuring the strain direction, frequency, duration, and magnitudes applied during this technique,<sup>27</sup> we started investigating the possibility that changing MFR magnitude or duration induces unique fibroblast responses in BETs.<sup>31</sup> These studies were meant to simulate pharmacologic studies in terms of assessing the optimal dose, dose form, and duration of treatment. In addition to MFR dosing, we sought to determine whether modeled MFR affects tissue function in the absence of RMS. Such a finding would suggest a potential for prophylactic use of MFR and other OMT modalities. In these studies, fibroblast hyperplasia, hypertrophy, and secretion of cytokines and growth factors were examined with different MFR magnitudes (3%, 6%, 9%, and 12%) and durations (0.5, 1, 2, 3, 4, and 5 minutes). Neither BET cellular protein nor DNA accumulation were affected by changing MFR magnitude and duration, suggesting that proliferation is only affected by strain direction.<sup>26,31</sup> However, BET weight was increased with greater-magnitude (12%) treatment, suggesting that production of extracellular matrix proteins such as collagen may be up-regulated. Greater MFR magnitude also led to a statistically significant increase in IL-1 $\beta$ , monocyte chemoattractant cytokine, and regulated and normal T-cell expressed and



**Figure 1.**

Profiles of various directions of strain modeled in the authors' laboratory. A, Applied equibiaxial (with platen) and heterobiaxial (without platen) strain vectors to the culture plate membrane and attached cells in 2-dimensional (2-D) matrices. B, Applied uniaxial (with platen) strain vector to 3-dimensional (3-D) bioengineered tendons (BETs).

secreted chemotactic cytokine compared with non-strained fibroblasts. Alternatively, holding the strain magnitude constant at 6% and varying the duration of MFR increased secretions of angiogenin, IL-3, IL-8, growth colony-stimulating factor, and thymus activation-regulated chemokine only in the 5-minute MFR group. Therefore, changing magnitude and duration of MFR appears to enhance the secretion of a unique subset of cytokines and growth factors, possibly affecting physiologic responses in vivo. Further, these findings suggest that dose-dependent and prophylactic MFR may potentially regulate inflammation and wound healing responses in patients.<sup>31</sup> If clinically translatable, changing doses and maneuvers of OMT would produce different effects on patients in manners that may be mediated by differential cytokine production. The potential prophylactic effect of MFR (and perhaps other OMT modalities) may prevent injury in persons with risk factors for musculoskeletal injury.

**Table 2.**  
**Main Effects of Equibiaxial and Heterobiaxial Strain on Morphology, Proliferation, and Cytokines in Normal Human Dermal Fibroblasts<sup>26</sup>**

Measures	Equibiaxial	Heterobiaxial
Magnitude/time	10% over the initial resting length for 48 h	10% over the initial resting length for 48 h
Morphology	No change	Lamellipodia truncation; cytoplasm condensation; cell membrane destruction
Proliferation (compared with heterobiaxial)	NA	↓ by 22%
dsDNA	↑	↑↑
IL-6 (compared with nonstrained and heterobiaxial)	↓	No change
IL-7	Not significant ↓ (compared with nonstrained and heterobiaxial)	NA
MDC/chemokine	↓ (compared with nonstrained and heterobiaxial)	Not significant ↑ (compared with nonstrained)

**Abbreviations:** dsDNA, double-stranded DNA; IL, interleukin; MDC, macrophage-derived chemoattractant; NA, not available.

### Effects of Modeled OMT on Muscle Differentiation

Fibroblast expression and secretion of collagen into the extracellular matrix is essential for the development and migration of new blood vessels in injured areas during repair.<sup>34</sup> We investigated the ability of fibroblasts to induce muscle differentiation through IL-6 in response to modeled MFR following modeled RMS, hypothesizing that this effect might improve muscle repair in vivo.<sup>35</sup> Using fibroblast-conditioned media in a uniculture of fibroblasts and fibroblast-myoblast cocultures, we hypothesized that RMS would reduce muscle repair by inhibiting muscle differentiation, whereas MFR would reverse this effect. Indeed, our data revealed that RMS followed by MFR produced a statistically significant increase in muscle differentiation and myoblast fusion efficiency into myotubes compared with RMS alone and with nonstrained groups.<sup>35</sup> If

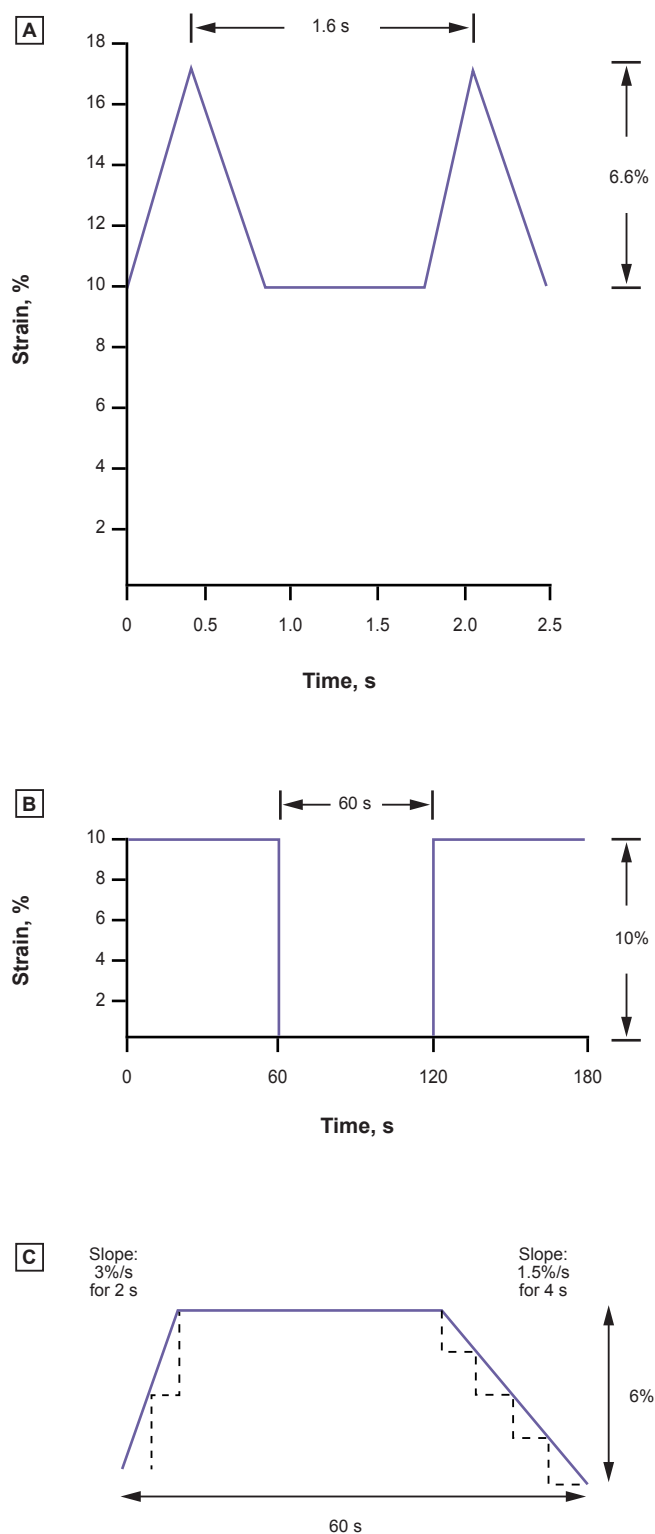
clinically translatable, our results suggest that although RMS would clinically reduce the ability to regenerate and repair muscles, MFR would enhance these effects.

On discovering MFR's ability to enhance muscle differentiation in vitro, we investigated whether the formed muscle myotubes were functional and, if so, whether MFR-treated fibroblasts conferred unique myotube functional differences compared with those not treated with modeled MFR.<sup>32</sup> Modeled RMS followed by modeled MFR, but not MFR alone, caused increased nicotinic acetylcholine receptor (nAChR) expression in coculture vs uniculture, suggesting the possibility of MFR-induced alterations in contractile sensitivity. After all, the nAChR mediates neuromuscular transmission in vitro. Additionally, RMS disrupted nAChR clusters and hypersensitized muscle contraction, and MFR aggregated nAChR clusters. These nAChR clustering findings are important given that in vivo, AchR is well documented to cluster

specifically at motor endplates, thus allowing coordinated electrochemical transmission and consequent muscle contraction. Our results showed that fibroblasts activated by MFR can enhance myoblast differentiation and that differentiated muscles formed nAChR clusters. Thus, fibroblasts strained by MFR are suggested to mediate muscle differentiation and nAChR organization. If clinically translatable, MFR may modify the sensitivity of muscle to the contractile actions of acetylcholine *in vivo*, as well as play a direct role in new muscle growth.<sup>32</sup>

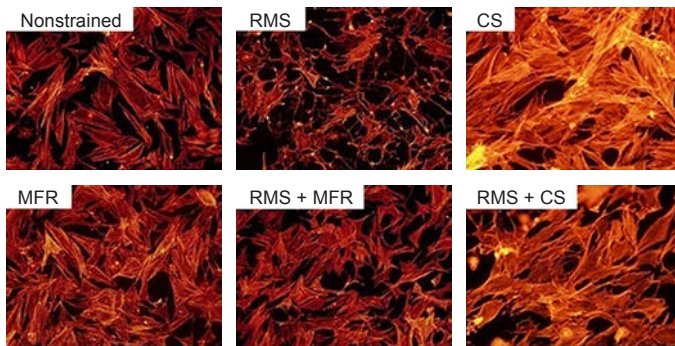
### Effects of Modeled MFR on Wound Healing in Fibroblast Tissue Preparations

Activation of fibroblasts is a key step in the wound healing process, involving secretions of necessary proinflammatory cytokines and extracellular matrix proteins that enhance proliferation, migration, and angiogenesis.<sup>34</sup> Although no published studies have focused specifically on OMT mediation of wound healing *per se*, strain-directed therapy such as vacuum compression therapy (VCT) is clinically effective, especially in the treatment of foot ulcers, to speed up the wound healing process. Despite this finding, VCT's mechanism of action is not very well understood.<sup>36</sup> Hence, we speculated that by applying external VCT to the wound and then releasing this pressure repetitively, the biomechanics used in the process would improve and facilitate wound healing by promoting cytokine production and cellular differentiation. Therefore, we assessed the effects of various biomechanical strain patterns—including our well-studied modeled MFR treatment—on fibroblast scratch wound closure.<sup>28</sup> Our results indicated that RMS alone caused reduced wound closure rates compared with the control group, which was not seen when RMS was followed by 6% MFR.<sup>28</sup> If clinically translatable, our data show the importance of a single MFR dose in correcting the RMS-induced impaired wound healing in patients.



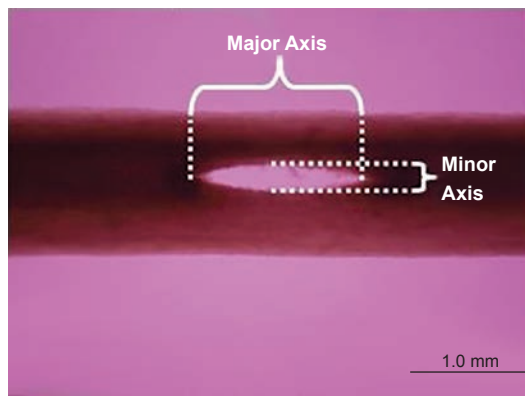
**Figure 2.** Profiles of various types of strain modeled in the authors' laboratory. A, 1.6-second cycle of repetitive motion strain. This cycle is continually repeated, with the strain increasing at a rate of 22%/s every 1.6 second before decreasing again to the baseline level. B, Single static 60-second release of strain designed to simulate counterstrain. C, Complete 60-second cycle of myofascial release. Reprinted with permission of Elsevier.<sup>9,27</sup>





**Figure 3.**

The effect of different osteopathic manipulative treatment modalities on fibroblast morphology and physiology. Representative photomicrographs of human fibroblast construct morphology, growth patterns, and actin architecture of the 6 treatment groups: nonstrained, repetitive motion strain (RMS), counterstrain (CS), myofascial release (MFR), RMS followed by MFR (RMS+MFR), and RMS followed by CS (RMS+CS). Reprinted with permission of Elsevier.<sup>27,39</sup>



**Figure 4.**

Image of nonstrained bioengineered tendon immediately after wounding. White brackets indicate the direction of the major and minor axes of the wound. Reprinted from *The Journal of the American Osteopathic Association*.<sup>37</sup>

To further these wound healing studies in 3-dimensional preparations as well as to test our dosed MFR treatments described previously, wound healing was assessed in MFR-strained BETs.<sup>37</sup> The main objective was to unveil potential effects of specific modeled MFR parameters that may have been masked by the use of a single strain magnitude and duration setting. Myofascial release was applied at 6% beyond resting length, held for 0, 1, 2, 3, 4, or 5 minutes, and then released to baseline. Similarly, magnitudes were set at 0%, 3%, 6%, 9%, and 12% beyond resting length, held for 90 seconds, and then released to baseline. The BETs' width, wound area, wound shape, and changes in the major and minor axes of the wound were measured and quantified (*Figure 4* and *Table 3*, *Table 4*, and *Table 5*). The results showed that greater magnitudes led to statistically significant reductions in the BET's width compared with other groups. Greater magnitude (12%) also showed an immediate and continuous increase in the major axis (long axis) of the wound. However, short magnitude (6% for 90 seconds) showed reduced minor axis (short axis) 18 hours after strain (*Table 3*). In light of this finding, 12% strain resulted in a larger wound area than nonstrained BETs at all time points. In contrast, a statistically significant reduction in wound area was observed with 3% strain by 24 hours and 48 hours after strain compared with no strain, 6% strain, and 9% strain (*Table 4*). Fixing the magnitude at 6% for 5 minutes resulted in a statistically significant decrease in wound area compared with no strain (*Table 5*). Thus, lower magnitude (3%-6%) and longer duration ( $\geq 5$  minutes) of MFR was shown to improve wound healing in vitro. This mechanism could be attributed to changes in the extracellular matrix (eg, collagen synthesis, secretion, and architecture) and gene activation that might result from MFR applied for longer than 2 minutes. The above effects did not occur in fibroblast-free BETs (BETs formed entirely of Purecol collagen [Advanced BioMatrix Inc]), suggesting that MFR-mediated changes in wound healing require the

**Table 3.**  
**Measurements of the Wound Axes in Bioengineered Tendons**  
**Undergoing Myofascial Release at 6% for 90 s and 6% for 5 min<sup>37</sup>**

Variable	Wound Measurement (Mean [SEM] mm) by Follow-up				
	0 h	3 h	18 h	24 h	48 h
<b>Major Axis</b>					
Nonstrain <sup>a</sup>	1.18 (0.052)	1.17 (0.067)	1.12 (0.076)	1.11 (0.082)	1.13 (0.070)
6% magnitude <sup>b</sup>	1.18 (0.037)	1.23 (0.077)	1.18 (0.105)	1.17 (0.112)	1.12 (0.114)
5 min duration <sup>c</sup>	0.97 (0.056)	0.91 (0.079)	0.96 (0.077)	0.87 (0.093)	0.77 (0.120)
<b>Minor Axis</b>					
Nonstrain <sup>a</sup>	0.19 (0.011)	0.19 (0.014)	0.16 (0.014)	0.16 (0.014)	0.14 (0.014) <sup>d</sup>
6% magnitude <sup>b</sup>	0.21 (0.014)	0.18 (0.014)	0.15 (0.015) <sup>d</sup>	0.14 (0.016) <sup>d</sup>	0.12 (0.087) <sup>d</sup>
5 min duration <sup>c</sup>	0.15 (0.010)	0.13 (0.016)	0.11 (0.015)	0.10 (0.014) <sup>d</sup>	0.08 (0.011) <sup>d</sup>

<sup>a</sup> The nonstrain group received no treatment.

<sup>b</sup> 6% magnitude at 90 seconds.

<sup>c</sup> 5 minute duration at 6% magnitude.

<sup>d</sup> Significant decrease ( $P < .05$ ), compared with the pretreatment wound axes at time 0.

**Table 4.**  
**Percent Change in Wound Area of Bioengineered Tendons 48 h After**  
**Wounding by Myofascial Release Strain Duration and Magnitude<sup>a,37</sup>**

Magnitude, %	Percent Change in Wound Area			
	3 h	18 h	24 h	48 h
Nonstrain	109.12 (16.11)	85.89 (20.81)	82.16 (16.38)	66.02 (28.83) <sup>b</sup>
3	77.74 (25.68)	61.44 (25.10)	56.54 (23.60) <sup>c</sup>	49.46 (21.91) <sup>d</sup>
6	87.45 (17.12)	71.07 (19.46)	63.35 (19.50)	52.29 (18.49) <sup>c</sup>
9	95.62 (20.58)	81.52 (12.58)	75.87 (10.64)	64.96 (11.16) <sup>e</sup>
12	129.15 (46.89)	120.40 (42.66)	121.47 (37.80) <sup>f</sup>	108.19 (37.62) <sup>g</sup>

<sup>a</sup> Data are given as mean (SD).  $P$  values were determined via 1-way analysis of variance with post hoc Dunnett multiple comparison test.

<sup>b</sup> Statistically significant decrease compared with the pretreatment wound axes at time 0 ( $P < .05$ ).

<sup>c</sup> Statistically significant decrease compared with the pretreatment wound axes at time 0 ( $P = .01$ ).

<sup>d</sup> Statistically significant decrease compared with the pretreatment wound axes at time 0 ( $P = .003$ ).

<sup>e</sup> Statistically significant decrease compared with the pretreatment wound axes at time 0 ( $P = .002$ ).

<sup>f</sup> Statistically significant increase compared with the pretreatment wound axes at time 0 ( $P = .01$ ),  $P < .05$  vs nonstrain at the same time point.

<sup>g</sup> Statistically significant increase compared with the pretreatment wound axes at time 0 ( $P = .03$ ),  $P < .05$  vs nonstrain at the same time point.

presence of fibroblasts.<sup>37</sup> In this study, we provided an overview for the direct effects of MFR parameters in the process of wound healing. If clinically translatable, our results suggest that valuable achievements might be

acquired through the use of optimum MFR magnitude and duration to mediate wound repair in patients.

Further, our findings warrant clinical studies asking at least 2 additional questions: (1) Is there a role for

**Table 5.**  
**Percent Change in Wound Area of Bioengineered Tendons Receiving Varying Durations of Myofascial Release Strained 6% Beyond Initial Resting Length<sup>a,37</sup>**

Time, min	Percent Change in Wound Area			
	3 h	18 h	24 h	48 h
Nonstrain	99.64 (19.98)	78.24 (20.02)	75.29 (16.80)	57.50 (26.52) <sup>b</sup>
1	78.46 (16.09)	62.40 (17.87)	54.35 (19.32)	48.10 (24.79) <sup>b</sup>
3	68.50 (18.30) <sup>c</sup>	57.88 (20.15) <sup>b</sup>	52.50 (22.21) <sup>b</sup>	45.24 (24.81) <sup>b</sup>
5	59.91 (17.77) <sup>c</sup>	50.24 (19.01) <sup>b</sup>	43.12 (17.24) <sup>b</sup>	26.97 (14.47) <sup>b</sup>

<sup>a</sup> Data are given as mean (SD). Changes in wound area measured as a percent change from time 0 (100%) measured 3, 18, 24, and 48 h after myofascial release. *P* values were determined using a 1-way analysis of variance with a post hoc Dunnett multiple comparison test.

<sup>b</sup> Significant decrease ( $P \leq .03$ ) in wound area compared with time 0.

<sup>c</sup> Significant decrease ( $P < .05$ ) in wound area compare with time 0.

prophylactic OMT in patients with no preexisting dysfunction or are these 2 issues mutually exclusive? and (2) Might focused dose-response studies of OMT yield better clinical outcomes for patients?

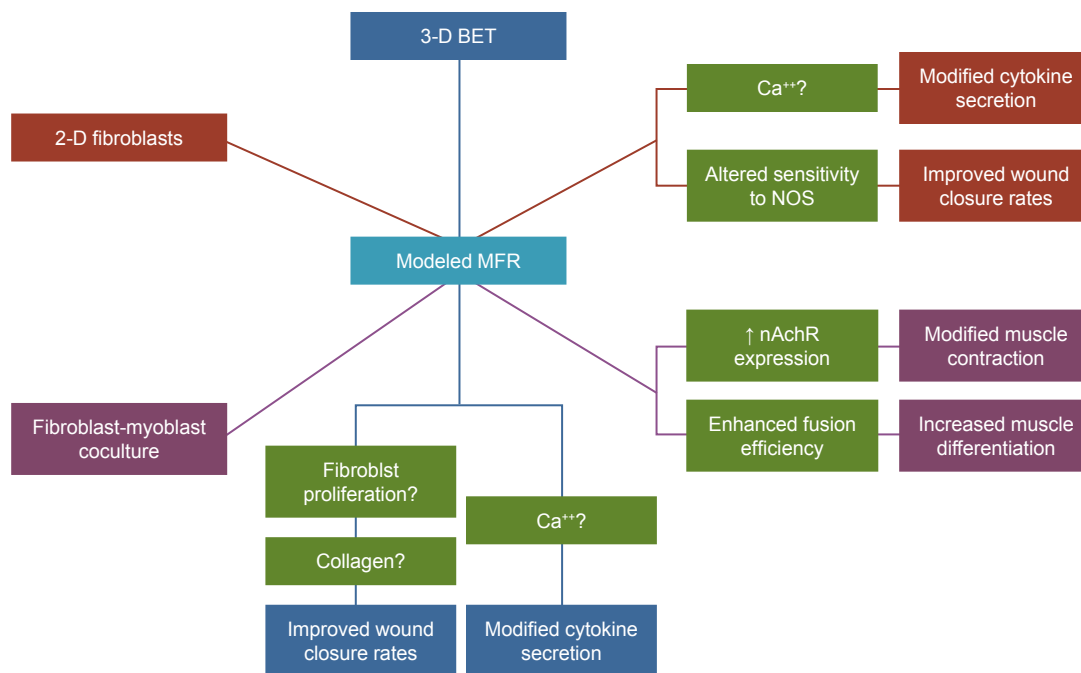
Another important issue is the combinatorial OMT therapies that are currently applied in patient studies (ie, “black box” treatments). The results of our dose-response studies as well as those showing divergent effects of strains (directed in different directions), suggest that black box treatment paradigms may result in patients remaining unresponsive or less responsive to treatment, because divergent treatments may mask each other’s cellular effects. Instead of lumping any or all OMT modalities in a “treatment group” and comparing them with a control or sham therapy group (an issue by itself that requires further scrutiny), we might be better served with focused investigations of specific OMT maneuvers and techniques and then in proscribed combinations much in the same way pharmacologic effects of medications are studied. This protocol would include data analysis among various research groups. Such a focused initiative would accurately describe every OMT maneuver in a manner that makes the process unambiguous as well as repeatable by any trained clinician.<sup>38</sup>

## Conclusion

In our laboratory during the past 10 years, we have aimed to show proof of concept that different OMT modalities might uniquely affect cell function, direct muscle contraction, and influence critical processes such as wound healing by cellular mechanisms. *Figure 5* summarizes the main points and known and postulated mechanisms involved in the effects of modeled MFR in vitro in the 3 models used in our laboratory. Understanding the molecular mechanisms by which MFR and other OMT modalities work would likely also help define the underpinnings of their clinical efficacies and perhaps propel them to the class of evidence-based, first-line therapies. Because first-line therapies are much more likely to be covered by third-party payers, such treatment methods may then be available to a much larger cohort of patients who could benefit from their uses.

## Author Contributions

All authors provided substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; all authors drafted the article or revised it critically for important intellectual content; all authors gave final approval of the version of the article to be published; and all authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.



**Figure 5.**

Main points and known or postulated mechanisms involved in the effects of modeled myofascial release (MFR) in vitro in 2-dimensional (2-D) fibroblast (red), fibroblast-myoblast coculture (purple), and 3-dimensional (3-D) bioengineered tendons (BETs) (blue). Some effects were shown to reverse repetitive motion strain (RMS) outcomes, and others were independent of RMS. The green shading indicates the known or postulated mechanism of action for each outcome.

**Abbreviations:** Ca<sup>++</sup>, calcium; nAChR, nicotinic acetylcholine receptor; NOS, nitric oxide synthase.

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