Dosed Myofascial Release in Three-Dimensional Bioengineered Tendons: Effects on Human Fibroblast Hyperplasia, Hypertrophy, and Cytokine Secretion

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Abstract

Objective: The purpose of this study was to investigate potential differences of magnitudes and durations associated with dosed myofascial release (MFR) on human fibroblast proliferation, hypertrophy, and cytokine secretions.

Methods: Bioengineered tendons (BETs) attached to nylon mesh anchors were strained uniaxially using a vacuum pressure designed to model MFR varying in magnitudes (0%, 3%, 6%, 9%, and 12% elongation) and durations (0.5 and 1-5 minutes). Conditioned media were analyzed for cytokine secretion via protein microarray (n = 2). Bioengineered tendons were weighted and fibroblasts extracted from the BET were assessed for total cell protein and proliferation via double-stranded DNA quantification (n = 5). All data were compared by a 1-way analysis of variance with post hoc Dunnett test and Student *t* test.

Results: Changing MFR magnitude and duration did not have an effect on total fibroblast cellular protein or DNA accumulation. However, we observed a stepwise increase in BET weight with higher-magnitude MFR treatments. Longer durations of MFR resulted in progressive increase in the secretions of angiogenin, interleukin (IL)-3, IL-8, growth colony–stimulating factor, and thymus activation–regulated chemokine. Alternatively, increasing strain magnitude induced secretions of IL-1β, monocyte chemoattractant cytokine, and regulated and normal T cell expressed and secreted chemotactic cytokine.

Conclusion: Cellular proliferation and hypertrophy were not significantly changed by any treatment. However, the change in total BET dry weight suggests that production of extracellular matrix protein may be up-regulated. Different MFR parameters induce secretions of a unique subset of cytokines and growth factors that can be further enhanced by increasing the magnitude and duration of treatment. If clinically translatable, these results suggest that variations to manual therapy biomechanical parameters may differentially affect physiological responses in vivo. (J Manipulative Physiol Ther 2013;36:513-521)

Key Indexing Terms: *Musculoskeletal Manipulations; Fascia; Tendons; Fibroblasts*

Myofascial release (MFR) is a specialized form of manual manipulative therapy designed to treat a variety of conditions such as musculoskeletal injuries, somatic pain, fibromyalgia, and chronic lymphedema.[1-4] In some cases, MFR has been shown to improve mobility, reduce pain, and reduce inflammation.[5-7] Treatment typically consists of a slow-loading stretch applied to the long and transverse axis of restricted connective tissue for 90 to 120 seconds, with palpation pressure varying based on anatomy, injury type, and tissue response.[8] During manipulation, the clinician feels changes in tissue texture, thus becoming more palpable. It is believed that manual pressure can alter the plasticity of the connective tissue by modifying its viscoelastic and piezoelectric properties.[8] Despite these reports, there are few studies that provide mechanistic evidence to support these observations. In addition, clinical studies investigating the efficacy of MFR have been inconsistent, with some

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studies reporting positive clinical outcomes and others showing no difference compared with conventional standard of care.[9-11] Many of these clinical studies focus primarily on a specific manual therapy technique rather than describing the biomechanical parameters used in these maneuvers. This makes it difficult to compare clinical relevance from different studies. For example, one study investigating the effects of dosed cervical mobilization reported that higher frequencies of the same maneuver enhanced sympathetic efferent activity and increased cutaneous blood flow in the upper limb.[12] This suggests that there may be a potential physiological effect of dosed manual manipulative therapy.

Fibroblasts are the primary cell type of connective tissue, that is, fascia, tendons, and ligaments, and provide biomechanical support to the body. Their primary role is to maintain extracellular matrix homeostasis through secretion, degradation, and reorganization of collagens, proteoglycans, fibronectins, tenascins, and laminins.[13] Recent findings suggest that fibroblasts are essential to facilitating the adaptive immune response and wound healing through the secretion of specific cytokine and growth factors such as type 1 interleukins (ILs) and IL-1β, which are involved in leukocyte preservation.[14,15] The multifunctional ability of fibroblasts to respond uniquely to biomechanical strain and their relative abundance within the connective tissue nominate them as a likely candidate of mechanotransduction during manual manipulative treatments.

Using video recordings of clinically applied MFR, we previously measured the strain direction, frequency, duration, and magnitudes applied during the technique and use these parameters to develop an in vitro model of MFR by seeding fibroblasts on Bioflex plates.[16] We have shown that modeled MFR effectively normalizes fibroblast apoptosis, proliferation indices, and actin architecture, as well as simultaneously acting to suppress secretions of various inflammatory cytokines that are induced by a modeled repetitive motion strain (RMS).[16-18]

The purpose of this study is to determine whether variation in modeled MFR strain magnitude or duration can induce unique fibroblast cellular response. This study improves on our previous 2-dimensional in vitro model by using 3-dimensional bioengineered tendons (BETs) to more closely mimic the physical environment found in vivo. In addition, we also investigated the dose-dependent effects of modeled MFR durations and strain magnitudes on fibroblast hyperplasia, hypertrophy, and secretion of cytokines and growth factors. We hypothesized that variation in MFR strain duration and or magnitude will generate unique cellular responses. The results from this study may help to explain clinically why clinicians whom use slightly different maneuvers might obtain different results and provide a proof of concept to establish a basis for the quantification and standardization of MFR and other manual manipulative techniques in the clinical setting.

Methods

Cell Culture

All experiments were conducted using commercially available normal human dermal fibroblasts obtained from Cambrex Laboratories (East Rutherford, New Jersey). The protocol was reviewed by the University of Arizona Office of Human Research Protections and is exempted from institutional review board review. Cells were cultured in Dulbecco modified Eagle medium supplemented with 2% fetal bovine serum and 1% penicillin-streptomycin at 37°C, 5% CO_2, and 100% humidity. The medium was replaced every other day with fresh prewarmed growth medium. Subconfluent cultures (acquired in 7-10 days) were passaged at a ratio of 1:3; all experiments used cell passages between 4 and 10.

Fabrication of BET

Human fibroblasts (1000 cells/μL) were added to a 70% Purecol collagen type I (Advanced Biomatrix, San Diego, CA)–30% 5 × Dulbecco modified Eagle medium mixture to create a collagen/fibroblast gel. A loading trough was created for the collagen/fibroblast gel using a linear Trough Loader placed beneath the flexible membranes of the Tissue Train Plates (Flexcell Int, Hillborough, NC). These plates are arranged in a 6-well format and consist of a flexible elastomeric well bottoms attached to a nondeformable matrix bonded by nylon mesh anchors. A vacuum of −85 kPa was then applied to create a trough between the 2 anchors into which 200 μL of the collagen/fibroblast gel was then added to the fabricated loading trough to create a tube shape structure attached at the 2 anchors. The collagen was allowed to polymerize for 2 hours in a humidified 37°C incubator. Subsequently, the vacuum was released, thereby allowing the BET to be free from attachments to the well, except at the anchor points (Fig 1A). Fresh culture medium supplemented with 2% fetal bovine serum was then added to each well, and the BETs were allowed to acclimate for 24 hours before commencing strain treatment.

Biomechanical Strain Paradigm: In Vitro Dose Response of MFR

All strain profiles were implemented using the Flexercell FX-4000 Tension Plus System (Flexcell International Corp). Vacuum pressure is applied at the elastomeric well bottom, which strains the BET uniaxially in the direction of its primary axis (Fig 1B). In this 3-dimensional model, there are many factors that contribute to overall biomechanics. The uniform structure, elasticity, and ductile nature of the tissue allow the BET to deform under tensile stress. With increasing strain, the length of the BET also increases, which causes the mass to be redistributed across the structure. The phenomenon demonstrated by this process is a Poisson effect, in which a transverse strain is generated perpendicularly to the direction of applied load.[19] This