Chronic Inflammation Negatively Alters the Cellular Components of the Myofascia
Chanté Richardson, PhD, MBA and Christina Kennedy, PhD
Alabama College of Osteopathic Medicine, Dothan, AL, 36303 USA

Introduction
One of the effective noninvasive therapies for myofascial pain (MP) is the osteopathic manipulative treatment (OMT) technique, myofascial release. Studies have shown that this technique improves range of motion and pain; however, the effect of inflammatory mediators on the myofascial tissue and the cellular response to OMT needs to be elucidated. (1) We hypothesize that chronic inflammation negatively alters the myofascial tissue structure and function. These negative myofascial tissue changes, we predict are reversed by OMT and will be examined in the future.

Background
• Fibroblasts, the predominant cell type in fascia, respond to strain tension and contribute to cellular changes. (2)
• In vitro modeling of OMT has been successful in identifying important cellular changes in fibroblasts. These cells along with myogenic precursor cells regenerate muscle through cell-cell cross talk. (3)
• Proinflammatory cytokine, tumor necrosis factor-alpha (TNF-alpha), has catabolic effects on tissue function and is directly involved in loss of skeletal muscle mass and function. (4)

Methods
Human dermal fibroblasts and human skeletal muscle cells (hSkM) were exposed to various concentrations of recombinant human TNF-alpha for five days in cell culture. Cell quantification, cell viability, and light microscopy were used to evaluate the cellular growth and morphologic changes. Human transforming growth factor beta (TGF beta) ELISA kit was used to quantify the amount produced by the hSkM cells. All results were analyzed by performing unpaired two-tailed Student’s t-tests or one-way ANOVA with Tukey post-hoc tests. P-values less than or equal to 0.05 was considered significant.

Results
Figure 1. Light microscopy showing human fibroblast (A-C) and hSkM cell (D-F) organization and cell-cell interaction in the presence and absence of TNF-alpha. Cells were grown in a T-75 flask in the absence or presence of TNF-alpha in a dose-dependent manner for five days. Pictures were taken with a 10x objective in four quadrant sections of the T-75 on day 5.

Figure 2. Cell growth in fibroblast and hSkM cells was measured individually using the Neocolor colorimeter on day 5 in the presence and absence of TNF-alpha. Cells were grown in a T-75 flask in the absence or presence of TNF-alpha in a dose-dependent manner for five days, n = 3.

Figure 3. Cell growth comparison of fibroblast and hSkM cells grown in a T-75 flask in the absence or presence of TNF-alpha in a dose-dependent manner for five days.

Figure 4. TGF-beta expression in hSkM cell culture supernatant in the absence or presence of TNF-alpha in a dose-dependent manner for five days.

HSkM cells and human fibroblasts respond to TNF-alpha in a similar fashion as they do to muscle injury. We found that exposure to TNF-alpha causes HSkM cells and human fibroblasts to exhibit opposite responses to inflammation. That is, HSkM cells experienced a decrease in cell growth, while fibroblasts experienced an increase in cell growth when exposed to increasing TNF-alpha concentrations. We also show that the inflammatory stimuli, TNF-alpha, visually changes cell-cell interaction and cell growth of HSkM cells and fibroblasts. Next, we assessed the function of these two cell types looking at TGF-beta production and Collagen Ia. In skeletal muscle, TGF-beta regulates extracellular matrix remodeling and stimulates fibrosis. Muscle biopsies from patients with chronic inflammatory disease show increased TGF-beta expression in the extracellular matrix and in fibrotic tissue.

Conclusions
Our data reveals that when we exposed the HSkM cells to TNF-alpha there is a statistically significant increase in TGF-beta production suggesting that exposure to inflammation leads to the formation of fibrotic tissue. We are currently assessing the Pro-Collagen Ia production in the fibroblasts exposed to TNF-alpha.

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Results (cont.)
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Conclusions
• Supports hypothesis that the structure and function of the myofascial tissue changes under chronic inflammatory conditions.
• Understanding how the major cellular components of the myofascia respond to inflammation in isolation provides the foundation for understanding how they interact in their normal microenvironment.
• Development of a direct contact co-culture in vitro model of the myofascial tissue to mimic the microenvironment to assay the interaction and paracrine signaling.
• Elucidate the molecular mechanism underlying the inflammatory component of myofascial pain and potentially clarify the signaling changes that occur when using OMT technique, myofascial release.

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Results

**Fibroblast vs. Skeletal Muscle Cell Growth**

<table>
<thead>
<tr>
<th>Cell Growth (Fold Increase)</th>
<th>Fibroblasts</th>
<th>Skeletal Muscle Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00 ug/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.025 ug/ml</td>
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<tr>
<td>0.050 ug/ml</td>
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<td>0.10 ug/ml</td>
<td></td>
<td></td>
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<tr>
<td>0.20 ug/ml</td>
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</tr>
</tbody>
</table>

**Figure 3.** Cell growth comparison of fibroblast and hSKM cells grown in a T-75 flask in the absence or presence of TNF-alpha in a dose-dependent manner for five days.

HSKM cells and human fibroblasts respond to TNF-alpha in a similar fashion as they do to muscle injury. We found that exposure to TNF-alpha causes HSKM cells and human fibroblasts to exhibit opposite responses to inflammation. That is, HSKM cells experienced a decrease in cell growth, while fibroblasts experienced an increase in cell growth when exposed to increasing TNF-alpha concentrations. We also show that the inflammatory stimuli, TNF-alpha, visually changes cell-cell interaction and cell growth of HSKM cells and fibroblasts. Next, we assessed the function of these two cell types looking at TGF-beta production and Pro-Collagen Iα. In skeletal muscle, TGF-beta regulates extracellular matrix remodeling and stimulates fibrosis. Muscle biopsies from patients with chronic inflammatory disease show increased TGF-beta expression in the extracellular matrix and in fibrotic tissue.

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References

Graphs / Charts

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<table>
<thead>
<tr>
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<th>Absorbance at 450</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00 μg/mL</td>
<td>* 1.5</td>
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<tr>
<td>0.25 μg/mL</td>
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<td>0.50 μg/mL</td>
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<tr>
<td>1.0 μg/mL</td>
<td>0.0</td>
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