Modeled Repetitive Motion Strain and Indirect Osteopathic Manipulative Techniques in Regulation of Human Fibroblast Proliferation and Interleukin Secretion

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Context: Clinical studies have supported the efficacy of a variety of osteopathic manipulative techniques. However, an evidence base for the cellular mechanisms underlying these clinical findings is lacking.

Objective: To investigate human fibroblast proliferation and interleukin secretory profiles in response to modeled repetitive motion strain (RMS) and modeled indirect osteopathic manipulative techniques (IOMT). The authors hypothesized that the RMS model would increase fibroblast proliferation and proinflammatory interleukin secretion, while the IOMT model would reverse these effects.

Methods: Human fibroblasts were exposed in vitro to one of three conditions: (1) an 8-hour RMS; (2) a 60-second IOMT; or (3) an 8-hour RMS followed by a 60-second IOMT. Data on fibroblast proliferation and interleukins present in conditioned media were obtained immediately after RMS, at 24 hours after RMS (24RMS), at 24 hours after IOMT (24IOMT), and at 24 hours after RMS and IOMT (24RMS+IOMT). Cytokine protein array and enzyme-linked immunosorbent assay were used in data analysis. Fibroblast proliferation was also measured colorimetrically with a cell proliferation assay.

Results: Fibroblasts that underwent RMS secreted several proinflammatory interleukins 24 hours after strain cessation, with substantially increased secretion of IL-1α, IL-1β, IL-2, IL-3, IL-6, and IL-16. At 24 hours after strain cessation, fibroblasts subjected to RMS also secreted increased amounts of the anti-inflammatory IL-1ra, and they displayed 15% less

proliferation, compared with baseline cells ($P<.05$). Fibroblasts that underwent IOMT, when analyzed at 24 hours after IOMT, did not display increased interleukin secretion or proliferation. However, they did display a 44% reduction in proinflammatory IL-3 secretion when compared with baseline cells ($P<.05$). The use of 24RMS+IOMT did not induce interleukin secretion in fibroblasts that were analyzed 24 hours after the combined exposure. However, cells in the 24RMS+IOMT group did display a 46% reduction in proinflammatory IL-6 secretion compared with RMS alone (24RMS; $P<.05$), as well as a 51% increase in proliferation compared with the 24RMS group ($P<.05$).

Conclusion: An in vitro strain model that simulates RMS has different effects on fibroblast proliferation and interleukin secretion than does an in vitro model that simulates IOMT. Modeled RMS appears to cause a reduction in fibroblast proliferation and a delayed inflammatory response. Modeled IOMT not only fails to induce this response, it also reverses inflammatory effects in cells that have been strained repetitively. Data from the present study suggest that fibroblast proliferation and expression/secretion of proinflammatory and anti-inflammatory interleukins may contribute to the clinical efficacy of indirect osteopathic manipulative techniques.

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Osteopathic manipulative treatment (OMT) is used to target somatic dysfunction, increasing patients' range of motion, alleviating pain, and promoting the body's natural self-healing ability. Although clinical studies[1-4] have supported the efficacy of various osteopathic manipulative techniques, an evidence base for the cellular mechanisms underlying these observed outcomes is lacking.

We previously reported on the development of an in vitro model useful for studies of strain-induced soft-tissue injury and OMT.[5,6] These studies[5,6] demonstrated that heterobiaxially strained (ie, strained unequally in two axes) in vitro human fibroblasts responded with increased secretion of proinflammatory interleukin 6 (IL-6) and nitric oxide, increased proliferation, and distinct changes in cellular structure and intracellular actin activity.

In the present study, we expand on these findings by modeling repetitive motion strain (RMS) and an indirect osteopathic

manipulative technique (IOMT), using human fibroblasts cultured on collagen matrices. Because fibroblasts are rich sources of many cytokines, including interleukins,[5-16] we expanded our search of likely cellular mediators of OMT to include a total of 13 proinflammatory and anti-inflammatory interleukins.

We hypothesized that the modeled RMS would induce increased proliferation of fibroblasts and increased secretion of proinflammatory interleukins, both of which may be responsible for the decreased range of motion, pain, and edema characteristic of repetitive strain injuries. We also hypothesized that the modeled IOMT would reverse this proliferative response and lead to attenuation of proinflammatory interleukin secretion, induction of anti-inflammatory interleukin secretion, or both.

When combined with the results of previous studies,[5,6] the results of the present study expand the cellular evidence base for the efficacy of indirect osteopathic manipulative techniques as used by osteopathic physicians to treat their patients.

Methods

In the present study, human fibroblasts were cultured on flexible-bottomed, collagen I-coated Bioflex plates (Flexcell International Corp, Hillsborough, NC) and then exposed in vitro to one of the clinically relevant modeled strain profiles (ie, strain-counterstrain or myofascial release). Fibroblast proliferation and the concentration of 13 interleukins in the conditioned media were analyzed as described below.

Human Fibroblast Cultures and In Vitro Strain Apparatus

Normal dermal fibroblasts (Cambrex Corp, East Rutherford, NJ) from human subjects were used for all experiments in the present study. Cells were cultured in Fibroblast Basal Media (Cambrex Corp, East Rutherford, NJ) at 37.0°C (98.6°F), 5% carbon dioxide, and 100% humidity. Cells were fed every other day with fresh media. Every 7 to 14 days, as the cells reached maximum density throughout the culture dishes, they were split into new dishes.

We used the Flexercell FX-4000 Tension Plus System (Flexcell International Corp, Hillsborough, NC) for the in vitro strain procedures. The Flexercell FX-4000 is a computer-based system that uses a vacuum to strain cells adhered to flexible collagen I-coated membranes (Bioflex plates) arranged in a format with six wells per plate. The deformation of the collagen on the plates causes the attached cells also to deform. Programming of the magnitude, duration, and frequency of the negative pressure in the Flexercell apparatus creates desired strain profiles.[17] Cells were strained equibiaxially (ie, equal strain across both axes).

Strain Profiles

Fibroblasts were seeded (120,000 cells/well) onto the collagen I-coated Bioflex plates in the Flexercell apparatus, using six wells per treatment group (ie, per strain profile). After the
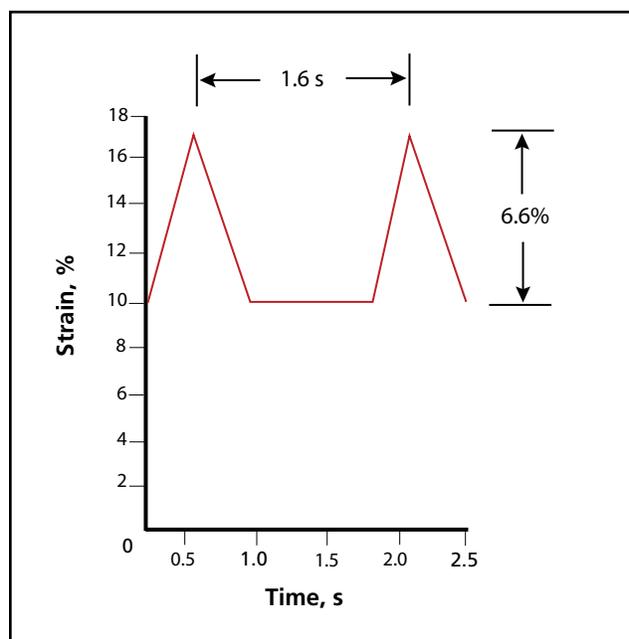

Figure 1. Profile of a 1.6-second cycle during an 8-hour cyclic strain regimen designed to simulate repetitive motion strain. This cycle is continually repeated, with the strain increasing at a rate of 22% per second every 1.6 seconds before decreasing again to the baseline level of 10% strain.

cells were approximately 50% to 60% confluent—which usually happened by 24-hours postseeding—the growth medium (2% fetal bovine serum) was replaced with a reduced-serum medium (0.2% fetal bovine serum) to induce cell quiescence.

Twenty-four hours after the induction of quiescence, the fibroblasts were subjected to the strain profiles. Briefly, strain profiles were designed to simulate 8 hours of RMS (*Figure 1*) and 60 seconds of IOMT (*Figure 2*):

- **BCS (baseline cell secretion)**—Cells were grown on membranes prestrained to 10% beyond resting length, but they were not subjected to RMS or IOMT.
- **RMS (repetitive motion strain)**—Cells were seeded onto membranes prestrained to 10% beyond resting length, subjected to the RMS profile for 8 hours, and then sampled immediately upon cessation of RMS.
- **24RMS (24 hours post-RMS)**—Cells were seeded onto membranes prestrained to 10% beyond resting length, subjected to the RMS profile for 8 hours, and then sampled 24 hours later.
- **24IOMT (24 hours post-IOMT)**—Cells were seeded onto membranes prestrained to 10% beyond resting length, subjected to the IOMT profile for 60 seconds, and then sampled 24 hours later.
- **24RMS+IOMT (24 hours post-RMS and -IOMT)**—Cells were seeded onto membranes prestrained to 10% beyond
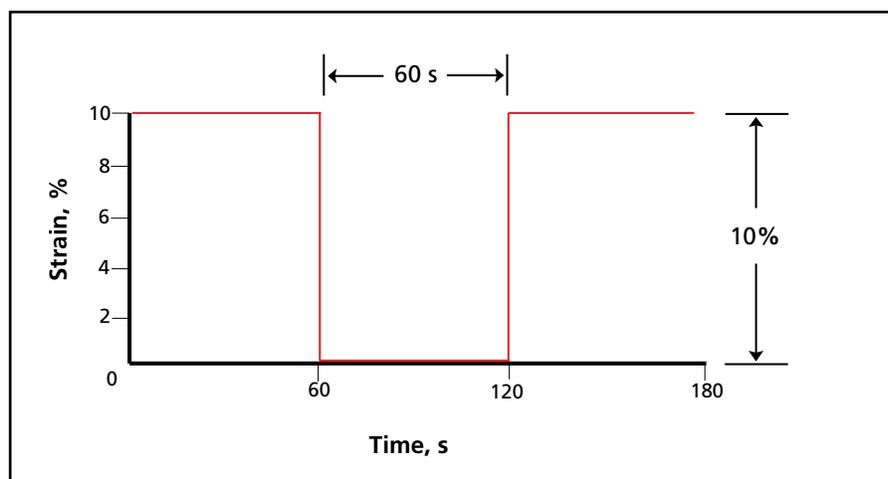
shades of the film were corrected with the AlphaEaseFC software in the final volume calculations of each duplicate cytokine spot.

Cytokine data were further analyzed with RayBio Cytokine Antibody Array software (Human Cytokine Antibody Array System VI & 6.1; RayBiotech Inc, Norcross, Ga), which was used to average duplicate data for each individual membrane. Corrected cytokine spot volume data, which were normalized with cellular proliferation data, were then analyzed using Microsoft Excel software (Version 11.0; Microsoft Corporation, Redmond, Wash) and GraphPad Prism software (GraphPad Prism 4.03; GraphPad Software Inc, San Diego, Calif).

To determine the effects the different strain profiles had on fibroblast interleukin secretion, the fold changes ($\Delta F_{treatment}$) of the spot volumes were calculated by comparing each experimental cytokine (E) with the cytokine's baseline cell secretion (BCS) using the following equation:

$$\Delta F_{treatment} = E \div BCS$$

Therefore, $\Delta F_{treatment} < 1$ corresponded to condition media containing less interleukin than at baseline, whereas $\Delta F_{treatment} > 1$ corresponded to increased interleukin secretion compared with baseline. In keeping with literature standards,[18,19] $\Delta F_{treatment} \geq 2$ corresponded to notably increased interleukins as a result of strain.

For the conditioned media collected from each of the strain profiles, each interleukin of interest, as determined by the results of the cytokine protein array, were further analyzed using a human enzyme-linked immunosorbent assay (ELISA) kit. The interleukins tested with individual ELISAs included IL-1α, IL-1β, IL-1ra, IL-2, IL-4, and IL-7 (ELH-IL1α-001, ELH-IL1β-001, ELH-IL1ra-001, ELH-IL2-001, ELH-IL4-001, ELH-IL7-001, respectively; RayBiotech Inc, Norcross, Ga); IL-3 and IL-16 (KHC0031, KHC0161, respectively; BioSource International Inc, Camarillo, Calif); and IL-6 (EH2IL6; Pierce Biotechnology Inc, Rockford, Ill). In addition, ELISA data were normalized with cellular proliferation data and analyzed with two-tailed, two-sample *t* tests. Statistical significance was defined as *P*<.05.

resting length. They were then subjected to the RMS profile for 8 hours, followed by a 3-hour resting period. They were then subjected to the IOMT profile for 60 seconds, and, finally, they were sampled 24 hours post-IOMT.

Cell Viability, Growth Measurements, and Interleukin Analysis

Cell viability was confirmed and proliferation was measured using the CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega Corp, Madison, Wis). These data were analyzed with a one-way analysis of variance and post hoc Bonferroni multiple comparisons test. Cell viability and proliferation data were further used to normalize data derived from the interleukin data analysis. Statistical significance was defined as *P*<.05.

Conditioned media samples were obtained from six replicate wells in all experimental strain profiles. These samples were then frozen at –80°C (–112°F) until assayed. The cytokine array membranes (RayBiotech Inc, Norcross, Ga) were first incubated with blocking buffer at room temperature (22°C [72°F]) for 30 minutes and then incubated for 4 additional hours at room temperature with 2.5 mL of thawed conditioned media from each of the baseline and treatment groups. The membranes were next incubated overnight at 4.0°C (39.2°F) in primary biotin-conjugated antibody, followed by incubation with horseradish peroxidase-conjugated streptavidin at room temperature for 30 minutes. Finally, the membranes were briefly incubated with detection buffer, exposed to film, and developed using a Kodak X-OMAT 1000A processor (Eastman Kodak Company, Rochester, NY).

After the films were scanned (ScanJet 6200C; Hewlett Packard Company, Palo Alto, Calif), the volume (ie, color density × spot area) of each representative cytokine spot was quantified using AlphaEaseFC software (Version 4.0.0; Alpha Innotech Corporation, San Leandro, Calif). Each cytokine spot was assayed in duplicate for each membrane probed. Intraspot variability for any specific interleukin that was assayed in this manner typically averaged less than 20%. Varying background

Results

Three observations from the present study indicate that all of the strain profiles conserved fibroblast viability. These observations are as follows:

- the cells' continued ability to bioreduce tetrazolium/formazan[20]
- lack of cell detachment from the collagen elastomere membranes
- lack of cell rounding as determined by phase-contrast microscopy[5,6]

The 24RMS profile resulted in significantly attenuated cellular proliferation relative to baseline, while the 24RMS+IOMT profile resulted in significantly increased cellular proliferation versus baseline (*Figure 3*) ($P<.05$). Two of the profiles, RMS and 24IOMT, resulted in no significant change in proliferative response versus baseline.

Cytokine Protein Array

All interleukins assayed in the present study are documented to have proinflammatory, anti-inflammatory, or dual-action characteristics. In addition, all interleukins in this study, except for IL-4 and IL-5, are secreted by human fibroblasts (*Figure 4*).

Figure 5 presents observed changes in interleukin secretion, as determined by cytokine protein array, in response to RMS, 24RMS, 24IOMT, and 24RMS+IOMT, compared with baseline secretion. The RMS profile resulted in significant induction in IL-1α ($P<.05$), but all other interleukins displayed little change from baseline. Cells subjected to the 24RMS profile displayed additional induction of IL-1α, as well as notable induction of four other proinflammatory interleukins (IL-1β, IL-2, IL-3, and IL-6), the anti-inflammatory IL-1ra, and the dual-action IL-16 ($P<.05$). Cells exposed to the 24IOMT profile alone or to the 24RMS+IOMT profile showed no induction of interleukins (*Figure 5*).

Enzyme-Linked Immunosorbent Assay

Of the nine interleukins analyzed via ELISA (*Table*), IL-3 displayed a significant decrease in secretion when comparing baseline levels with 24IOMT ($P<.05$). Levels of IL-1α and IL-6 were significantly elevated 24 hours after RMS, compared with immediately after RMS ($P<.05$). Secretion of IL-6 was significantly decreased in 24RMS+IOMT, compared with 24RMS alone ($P<.05$).

No significant relationships involving IL-7 were observed with the ELISAs. Moreover, the ELISAs were unable to detect IL-1β, IL-1ra, IL-2, IL-4, or IL-16.

Comment

Strain profiles that mimic repetitive motion and indirect osteopathic manipulative techniques each elicit different types and quantities of interleukin secretion and fibroblast proliferation. Repetitive motion strain appears to cause a delayed inflam-
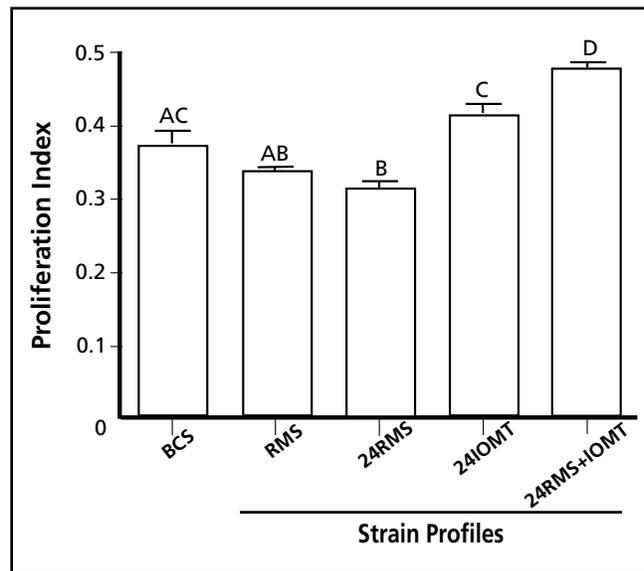

Figure 3. *Proliferation indices of fibroblasts that underwent various strain profiles involving repetitive motion strain (RMS) and indirect osteopathic manipulative techniques (IOMTs). The letters at the top of the error bars indicate the presence or absence of statistically significant differences among profile groups, as determined by the post hoc Bonferroni test (*$P<.05$*). If two bars have a letter in common, there is no significant difference between those bars. If two bars do not have a letter in common, there is a significant difference between them. **Abbreviations**: BCS, baseline cell secretion; 24IOMT, 24 hours post-IOMT; 24RMS, 24 hours post-RMS.*

matory response and a reduction in fibroblast proliferation. The IOMT profile not only failed to induce an inflammatory response or decrease in proliferation, it actually reversed these effects in cells subject to repetitive strain.

These results suggest that fibroblast proliferation and expression/secretion of both proinflammatory and anti-inflammatory interleukins may contribute to the clinical efficacy of indirect osteopathic manipulative techniques.

Clinical Relevance of Strain Profiles

The injury strain profiles (RMS, 24RMS) used in the present study were based on the timing and theorized intensity profiles of such common repetitive motion strains as are often the result of exercise (eg, running, swimming) and manual labor (eg, hammering nails, assembly line shift). The strain frequency and magnitude of these profiles reflect fibroblast strains exhibited in vivo.[12,21-23] The 24IOMT profile was intended to simulate an indirect osteopathic manipulative technique, in which the tissue being treated is positioned away from the restrictive barrier to a point of ease.[24,25]

In the present study, the restrictive barrier was located in the direction of the repetitive strain injury. Thus, to position the tissue away from this barrier, the prestrained (10% beyond

Interleukin	Role in Inflammation, With References	References for Human Fibroblast Expression
■ **Proinflammatory**		
□ IL-1α	Induces IL-1α and IL-1β. Mediator of inflammatory reactions.[7]	7,8
□ IL-1β	Similar to IL-1α.[7,11]	7-9
□ IL-2	Immune response regulator. Causes allergen-induced inflammation and rheumatoid arthritis.[10,48,50]	10
□ IL-3	Stimulates IL-1 and IL-6 secretion.[52]	11
□ IL-5	Stimulates proliferation of eosinophils. Causes pulmonary inflammation.[59]	None reported
□ IL-6	Mediates acute phase reaction.[7,35]	5,7,8,12
□ IL-7	Enhances IL-2, IL-3, and IL-6 secretion.[13,60,61]	13
□ IL-15	Similar to IL-2. Causes chronic inflammatory disease.[62]	10,13
■ **Anti-inflammatory**		
□ IL-1ra	Competitively blocks the binding of IL-1α, IL-1β, and IL-2. Reduces severity of inflammatory disease.[48]	14
□ IL-4	Suppresses proinflammatory cytokines. Induces IL-1ra.[41]	None reported
□ IL-10	Inhibits the synthesis of many proinflammatory cytokines, including IL-2.[41]	8
■ **Dual Action**		
□ IL-13	Reduces production of proinflammatory cytokines (IL-1, IL-6). Causes pulmonary inflammation.[41,63,64]	15
□ IL-16	Causes chronic intestinal inflammation. Pro- or anti-inflammatory role in rheumatoid arthritis still debated.[16,53]	16

Figure 4. *Some inflammatory properties of the interleukins investigated in the present study.*

resting length) collagen-coated membrane was released to a strain-free state for 60 seconds. A typical clinical treatment length for an indirect osteopathic manipulative technique varies, depending on the acuity of the injury and the skill of the osteopathic physician. In the simulation of RMS, as performed in the present study, a treatment length of 60 seconds is deemed clinically relevant (W.H. Devine, DO, oral communication, March 2006). We observed notable changes in interleukin secretion from such a 60-second IOMT, supporting the idea that this period of time is sufficient to induce a clinically relevant response.

Human fibroblasts are ideal for modeling repetitive strain injuries and indirect osteopathic manipulative techniques because they are the principle cell type of the myofascial junction. In the myofascial junction, fibroblasts secrete extracellular matrix (ECM) proteins (eg, collagen, fibronectin[26]) and cytokines (eg, growth factors, interferons, interleukins[7-16]), which are deliverable both locally and systemically. Another primary role of the myofascial junction is to endow cells and tissues with their specific mechanical and physiochemical properties. As such, the ECM represents a three-dimensional scaffold that supports cellular adhesion and contributes to tissue-specific function. Since imposed mechanical loads affect the fascia, the myofascial junction plays important simultaneous roles in sensing and transmitting force input generated by muscle contractions and by such external forces as physical injury and OMT.

Furthermore, numerous studies have found that fibroblast proliferation and collagen production are regulated by biophysical strain.[27-32] This finding is particularly relevant to OMT because abnormal collagen secretion may lead to fibrosis, thereby decreasing tissue compliance.[33,34] Moreover, increases
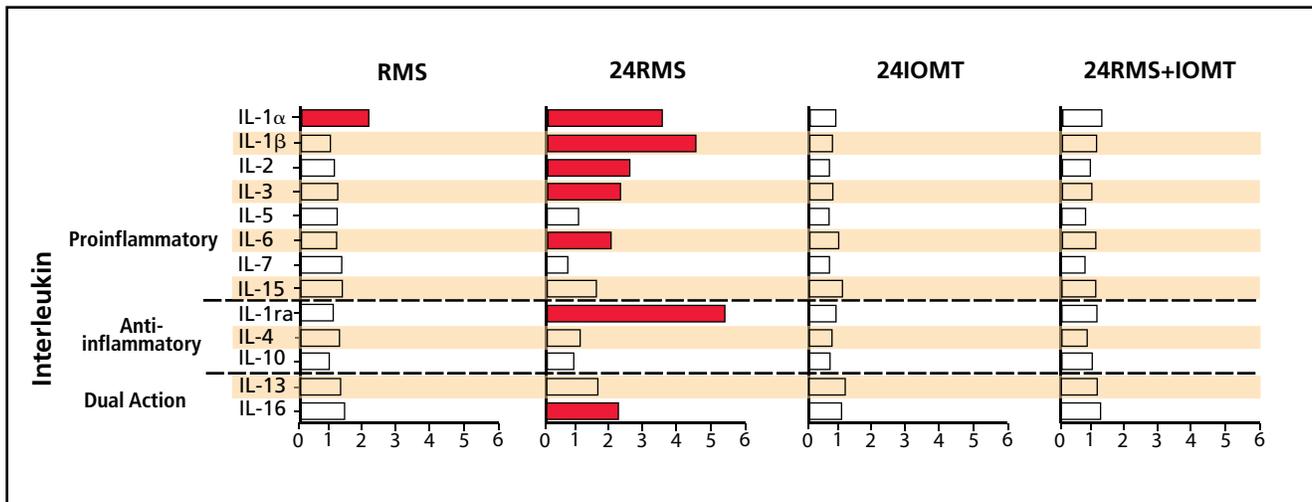
Figure 5. *The fold changes of 13 interleukins secreted by fibroblasts in conditioned media from four strain profiles, relative to baseline cell secretion. Red (darkened) bars represent a minimum twofold change in secretion compared with baseline. All other bars represent a less than twofold change in secretion compared with baseline. **Abbreviations**: BCS, baseline cell secretion; IOMT, indirect osteopathic manipulative techniques; RMS, repetitive motion strain; 24IOMT, 24 hours post-IOMT; 24RMS, 24 hours post-RMS.*

in fibroblast proliferation may exaggerate the interleukin response and, depending on the type of interleukins secreted, potentially increase or decrease pain, inflammation, and/or tissue regeneration.[7,35] Therefore, fascial fibroblasts, whose extracellular fluid is in equilibrium with lymph and vascular fluid, secrete ECM proteins and mediators of pain and inflammation (ie, interleukins). They also transduce biophysical force into cellular proliferation. As such, fibroblasts are uniquely poised to be targets of both soft tissue injuries and indirect osteopathic manipulative techniques.

Cytokine Protein Array and Enzyme-Linked Immunosorbent Assay Agreement
The protein array used in the present study can simultaneously detect multiple cytokines with high specificity, sensitivity, and reproducibility.[18,36,37] By detecting protein expression per se rather than DNA or messenger ribonucleic acid, molecules that do not always correspond well with protein secretion,[38] we were able to assess the bioactive molecules responsible for cell function.

Generally, we observed good agreement between the cytokine protein array and ELISA analyses, a finding that is consistent with other researchers' results.[36,37] Our result suggests that the cytokine protein array is a reliable initial indicator of potential patterns of interest regarding interleukins, but post hoc ELISAs are required for interleukin quantification on a molar basis. Unfortunately, some interleukins could not be detected with ELISA but could be detected with the cytokine protein array—a situation likely resulting from the fact that the detection sensitivity of the protein array is typically a magnitude greater, with a detection range 100-fold higher than ELISA.[36]

Cytokine Induction Post-Repetitive Motion Strain
Fibroblast proliferation and interleukin secretion responses to strain were not seen immediately after RMS, but rather 24 hours poststrain. This delayed proliferative response is consistent with several other human fibroblast studies.[27,39] Fibroblast proliferation was significantly decreased at 24-hours post-RMS ($P<.05$). This finding is contrary to our hypothesis that RMS would lead to increased proliferation. The contrary finding may be related to the fact that, in previous research,[5] we used acyclic strain, while in the present study, we used cyclic strain.

Other studies that have used cyclic strain[27,31] have also shown significant decreases in fibroblast proliferation. These studies suggest that cyclic strain may cause a stall at the G_0/G_1 phase of the cell cycle,[31] or that a long duration of cyclic strain may inhibit fibroblast proliferation.[27] Furthermore, IL-1β and IL-6 are known inhibitors of fibroblast proliferation.[32,35,40] We observed significant inductions of both interleukins at 24 hours post-RMS, corresponding to the timing of a significant decrease in fibroblast proliferation. The application of cyclic strain and the upregulation of IL-1β and IL-6 may have led to the decreased fibroblast proliferation observed after RMS.

Interleukins are secreted in response to injury[7,41] and are one of the underlying causes of inflammation.[12,35] The data in the present study support our hypothesis that RMS would induce interleukin secretion, suggesting that fibroblasts interpreted the strain model as an injury by responding with the induction of seven interleukins, five of which were proinflammatory. Delays from hours to days in cytokine induction, inflammatory processes, and pain after a repetitive motion strain or a

Table
Enzyme-Linked Immunosorbent Assay Results for Interleukins, by Strain Profile,
Normalized by Relative Proliferation Indices

Interleukin	BCS	Strain Profile, Mean±SE*			
		RMS	24RMS	24IOMT	24RMS+IOMT
■ Proinflammatory					
□ IL-1α	3.41±0.47	2.44±1.69†	4.48±0.54	4.05±0.21	2.54±0.74
□ IL-3	8.63±0.44	6.46±1.12	5.80±0.67	3.89±0.54‡	3.42±0.85
□ IL-6	80.32±7.42	70.65±1.16†	92.47±4.36	89.07±7.94	42.73±1.28†
□ IL-7	1.63±0.78	3.94±2.23	4.86±3.58	4.63±2.40	2.15±1.60

* Mean of three samples, with standard error, for each interleukin, calculated by dividing enzyme-linked immunosorbent assay result by proliferation index result. Enzyme-linked immunosorbent assay was conducted for IL-1β, IL-2, IL-1ra, IL-4, and IL-16, but these interleukins were not detected.
† $P<.05$ vs 24RMS.
‡ $P<.05$ vs BCS.

Abbreviations: BCS, baseline cell secretion; IOMT, indirect osteopathic manipulative techniques; RMS, repetitive motion strain; 24IOMT, 24 hours post-IOMT; 24RMS, 24 hours post-RMS.

soft-tissue trauma have previously been reported.[42-44] For example, delayed-onset muscle soreness may take up to 3 days to manifest and is associated with inflammation and cytokine induction, among other possible causes.[44] In addition, cytokines are not typically stored as vesicle-bound proteins. Instead, they are secreted in a regulated manner only after cellular activation and subsequent gene transcription—processes that are often delayed and transient.[45] This pattern is consistent with the 24-hour delay in interleukin secretion we observed after RMS.

The increase we observed in IL-1β secretion is consistent with that previously reported for strained human periodontal ligament fibroblasts, in which IL-1β secretion is directly proportional to loading time (ie, static strain) and strain magnitude (8%-16% beyond resting length).[9] Yang et al[46] found that the inflammatory response induced by IL-1β secretion from strained human patellar tendon fibroblasts was caused by large-magnitude stretching (ie, 8% beyond resting length). Yang et al[46] also found that low-magnitude stretching caused an anti-inflammatory response. These findings support our former proposal[5] that fibroblasts have a normal range of function that includes low-magnitude cyclic use, and that high-magnitude use may lead to injury and inflammation.

Lee et al[47] reported that secretion of IL-1α from keratinocytes parallels cyclic strain magnitude, an example of a linkage between biomechanical strain and inflammatory interleukin secretion that might explain the cause of inflammatory skin diseases that tend to occur in areas where the dermis is subject to repetitive strain. Also consistent with the findings of the present study is the fact that IL-6 secretion—a key component of the acute phase response[35]—is regulated by fibroblast strain and displays delayed induction after strain application.[5,12]

Interleukin 1ra, an anti-inflammatory interleukin that is a competitive inhibitor of IL-1, is secreted in response to systemic inflammation.[48] The large increase in IL-1ra levels seen in the cytokine protein array is consistent with that observed in keratinocytes during cyclic strain.[47] Interleukin 1ra has been found to ameliorate local inflammation, specifically by reducing IL-1β and IL-6 production and/or secretion.[48,49] The comparatively large fivefold increase seen in IL-1ra levels for the 24RMS group, versus upregulated proinflammatory interleukins (eg, IL-1β, IL-6), is again consistent with the increase observed in keratinocytes during cyclic strain—a response that may be the cells' reaction that allows a return to homeostasis.[47]

Novel findings of interleukin strain regulation were noted in the present study with IL-2, IL-3, and IL-16 secretion. To our knowledge, these findings marked the first time that these three interleukins have been implicated in biophysical strain regulation of fibroblasts. Although IL-2 is mainly produced by T cells, functioning as a central regulator of the immune response, it is also secreted by fibroblasts[10] and has been implicated in allergen-induced inflammatory reactions[50] and such diseases as rheumatoid arthritis.[51] Interleukin 3 leads to increased secretion of both IL-1 and IL-6,[52] exemplifying the mutually supportive roles of many of these proinflammatory interleukins. Interleukin 16, secreted by fibroblasts,[16] is also secreted by a variety of other cells. Its upregulation is involved in chronic intestinal inflammation and upregulation of other proinflammatory cytokines.[53]

Modeled IOMT and Inflammatory Response to Repetitive Motion Strain

The indirect osteopathic manipulative techniques we modeled in the present study in the 24IOMT profile did not significantly alter cellular proliferation compared with baseline. However, cellular proliferation with 24IOMT was significantly higher compared with 24RMS. Furthermore, the interleukins secreted in the 24IOMT group (depending on the particular

interleukin) either did not change compared with baseline cell secretion or actually showed a reduction in concentration when compared with baseline, as observed in the cytokine protein arrays (*Figure 5*) and in the ELISA results (*Table*). These results are in stark contrast to the cytokine array profile for 24RMS, which showed a dramatic induction of multiple interleukins. These inductions were abrogated by the addition of the IOMT strain profile.

There are several ways to interpret the reduction in interleukin concentration observed in the 24IOMT group. First, it is possible that the 24IOMT profile did indeed result in decreased interleukin transcription, translation, and/or secretion, as compared with baseline secretion. This result would suggest that IOMT did not injure the cells, as exemplified in the delayed proinflammatory response seen in the RMS group. Second, the interleukin secretion rate may not have been altered in response to IOMT. Instead, the 24IOMT profile may have resulted in metabolism of the interleukins' immunogenic motif so that, on protein array hybridization, the metabolically altered interleukins would not have bound to the respective membrane-bound capture antibodies to the same degree as the nonmetabolized, intact interleukins. Third, the 24IOMT profile may have resulted in receptor-mediated cellular uptake of the interleukins,[54] thereby lowering the interleukin concentration in the conditioned media. We have designed additional experiments to evaluate these possibilities.

Fibroblasts exposed to RMS and IOMT (24RMS+IOMT) showed a substantial increase in proliferation, reversing the decrease that was observed with RMS alone. Although we incorrectly hypothesized that IOMT would decrease fibroblast proliferation caused by RMS, we correctly hypothesized that IOMT would reverse the proliferative effects of RMS. We remain uncertain as to why fibroblast proliferation responds differently to RMS than to IOMT or acyclic strain.[5,28] One possibility is that cells respond to the most recently applied strain profile. In both the 24IOMT and 24RMS+IOMT profiles, we observed increased fibroblast proliferation compared with baseline. These cells may have been responding to the acyclic strain, which was the most recently applied strain profile. By contrast, in both the RMS and 24RMS profiles, there was a decrease in fibroblast proliferation compared with baseline, a finding that is consistent with those of other researchers.[27,31,32]

Furthermore, IL-1β and IL-6 are known growth inhibitors of fibroblasts.[32,40] These interleukins were both reduced in the 24RMS+IOMT profile compared with the 24RMS profile, thereby lowering the barrier to fibroblast growth and allowing cell proliferation to occur. The application of IOMT after RMS reversed the interleukin induction seen with RMS alone, and, with several interleukins, IOMT actually led to decreased interleukin secretion compared with baseline. These data support our hypothesis that secretion of inflammatory interleukins would increase with RMS and reverse with IOMT. The increase in fibroblast proliferation we observed with IOMT may have been related to fibroblast repair processes post-RMS and driven

by the interleukin induction. Alternatively, IOMT may have led to a decrease in RMS-induced apoptosis. We are conducting additional research to investigate these possibilities.

The direction of the biophysical strain vector imparted to cells plays a role in inducing alterations in the expression of interleukins and other molecules. In the present study, opposite strain vectors were imparted to fibroblasts in the 24RMS+IOMT profile—RMS elongated the cells, while IOMT shortened them. Lee et al[29] reported on human cardiac fibroblasts that underwent 10% uniaxial tensile strain and compression strain, noting that they displayed differentially regulated expression of extracellular matrix protein. As we have previously shown,[5,6] straining cells heterobiaxially versus equibiaxially causes reciprocal regulation of IL-6 and nitric oxide, proliferative response, and cell morphologic changes. In related research,[55] rat atrial fibroblasts depolarized when compressed, but they hyperpolarized when stretched between two patch pipettes.

The variety of responses incurred by changing only the strain vector suggests that the simple addition of a counterstrain to previously strained (ie, potentially injured) cells may result in a cascade of ameliorative actions.

Conclusion

Strain profiles that simulate repetitive motion and indirect osteopathic manipulative techniques elicited different types and quantities of interleukin secretion and cellular proliferation. Repetitive motion strain resulted in a delayed inflammatory response and reduction of cellular proliferation. The IOMT profile not only failed to induce proliferative and inflammatory responses, it reversed these effects in cells that were repetitively strained. These results suggest that proliferation of fibroblasts and expression and secretion of proinflammatory and antiinflammatory interleukins may contribute to the clinical efficacy of indirect osteopathic manipulative techniques when this form of therapy is provided by osteopathic physicians to their patients. Further research is underway to help refine clinically relevant strain profiles and cellular responses to other indirect osteopathic manipulative techniques.

Several studies have reported increased plasma interleukin levels resulting from injury, disease, and OMT.[56,57] A profound limitation of measuring systemic interleukin levels, however, is that the clearance rates of interleukins and the dilution of interleukins in the large plasma volume could cause considerable underestimation of the true interleukin response. In other words, plasma interleukin levels may not be representative of local tissue levels of interleukins in cases where the cytokines' actions are most clinically relevant and important.[58] The data from the present study enable us to estimate the equivalent of local tissue levels of interleukins rather than systemic levels. Nevertheless, strain regulation of interleukin secretory profiles provides a compelling cellular mechanism to explain injury-induced inflammation and the clinical efficacy of OMT.

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