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FASCIA SCIENCE AND CLINICAL APPLICATIONS: NARRATIVE REVIEW

Biological effects of direct and indirect manipulation of the fascial system. Narrative review

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ABSTRACT

Introduction: Osteopathic Manipulative Treatment (OMT) is effective in improving function, movement and restoring pain conditions. Despite clinical results, the mechanisms of how OMT achieves its' effects remain unclear. The fascial system is described as a tensional network that envelops the human body. Direct or indirect manipulations of the fascial system are a distinctive part of OMT.
Objective: This review describes the biological effects of direct and indirect manipulation of the fascial system.
Material and methods: Literature search was performed in February 2016 in the electronic databases: Cochrane, Medline, Scopus, Ostmed, Pedro and authors' publications relative to Fascia Research Congress Website.
Results: Manipulation of the fascial system seems to interfere with some cellular processes providing various pro-inflammatory and anti-inflammatory cells and molecules.
Discussion: Despite growing research in the osteopathic field, biological effects of direct or indirect manipulation of the fascial system are not conclusive.
Conclusion: To elevate manual medicine as a primary intervention in clinical settings, it's necessary to clarify how OMT modalities work in order to underpin their clinical efficacies.

1. Introduction

Osteopathy has been challenged over the past decade to provide clinically relevant research relative to mechanisms and efficacy. The conduct of evidence-based osteopathic research is imperative not only for scientific, economic, and professional reasons, but also to drive health care policy and clinical practice guidelines (Licciardone, 2007). Lucas & Moran (2006), raised critical questions regarding the relevancy of contemporary osteopathy research in the evolving healthcare environment. Their ultimate challenge to the osteopathic profession was to provide the “numbers” supporting the clinical effectiveness of osteopathy (Lucas and Moran, 2006).

These assertions originated the questions that inspired the aim of this review:

- Why is osteopathic manipulative treatment effective?
- What are the biological mechanisms responsible for better outcomes in patients?

Osteopathy is a broad manual medicine that involves different body systems and fascia is a key component of osteopathic manipulative techniques.

Fascial tissue is equally distributed throughout the entire body, enveloping, interacting with and permeating blood vessels, nerves, viscera, meninges, bones, and muscles, creating various layers at different depths, and forming a tridimensional metabolic and mechanical matrix (Findley and Shalwala, 2012). Osteopathy treats the fascial system through two modalities: indirect manipulation that requires the exaggeration of the pattern of dysfunctional tissue motion, bringing the restricted fascial tissue into its position of ‘ease’ (balanced tension), maintaining it until tensional forces relax (Ward, 2003) or induce a beneficial change. The second approach is direct manipulation, involving the application of forces against a resistance barrier to achieve a change in tissue behaviour (Greenman and Destefano, 2012).

The objective of this review is to describe, through biomedical literature, the biological effects resulting from direct or indirect manipulation of the fascial system.

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2. Material and methods

Literature search was performed in February 2016 in the electronic databases: Medline, Scopus, Cochrane, Pedro, Ostmed Dr., and authors' publications (full articles only) mentioned on Fascia Research Congress Website, international world congress on fascia. The following search terms were then used to identify relevant studies in the databases from the past 10 years: "fascia", "fascial system", "fibroblasts", "myofascial release", "myofascial techniques", "manual therapy", "manual medicine", "osteopathic manipulative treatment", "indirect osteopathic treatment", "strain and counterstrain".

To be included in this review studies had to meet the following inclusion criteria: investigation into the biological effects resulting from any form of manual fascial treatment techniques, written in the English language only. Studies were excluded if they were not relevant to fascial techniques or if they added other manipulative forms. We excluded articles not related to the biological effect of fascial techniques. The various authors followed the selection process independently; validity and quality assessment were not performed in order to not impose any form of restriction to the review. The study selection process comprised 2 "phases". During the first phase, we reviewed the abstracts of the studies. Those that failed to meet the eligibility criteria on the basis of the content of their abstracts were excluded during this stage. During the second phase of the selection process, the eligibility criteria were applied to the full-text versions of the studies using the same screening method used for the abstracts. The literature search yielded 95 studies and 24 articles that met the inclusion criteria. 21 studies analyzed the biological effects of direct manipulation (3 subcategories: stretching, myofascial release, other manipulative forms) and 3 studies analyzed the biological effects of indirect manipulation (Strain and Counterstrain).

3. Results

3.1. Description of the studies analyzing the biological effects of direct manipulation

3.1.1. Stretching

Different biological characteristics such as cell shape, nuclear remodeling, inflammation processes, etc. consequent to static stretching of the connective tissue were analyzed in nine laboratory studies (see Table 1).

Langevin et al. (2005) hypothesized stretching fascia affects the cellular shape of fibroblasts. Using an in-vivo and ex-vivo model, the authors stretched mouse subcutaneous tissue (average 25% tissue elongation for from 10 min to 2 h) determining a significant time-dependent increase in fibroblast cell body perimeter and cross-sectional area. (Langevin et al., 2005).

Subsequently, the same authors examined the effects of tissue stretch on the distribution of α- and β-actin in subcutaneous tissue fibroblasts ex vivo (Langevin et al., 2006). The results showed that both actin isoforms react in response to tissue stretch, but in a different matter. α-actin moved centripetally organizing around the nucleus in a star-shaped pattern, β-actin extended out into newly formed lamellipodia in response to the same mechanical stimulus. The action of α- and β-actin thus may be part of a complex mechanism allowing fibroblasts to actively participate in the regulation of connective tissue tension (Brown et al., 1996).

The behavior of α- and β-actin, resulting from static stretch tissue, influenced the study of Langevin et al. (2010) towards the observation of nuclear remodeling of cell elongation. Connective tissue stretched for 30 min caused a change in the shape of fibroblast nuclei, which were wider, flatter and smoother (less concave) than those within non-stretched tissue. The prospective consequences of nuclear remodeling induced by stretch and loss of nuclear concavity are far reaching, since mounting evidence suggests that cell and nuclear shape can influence cell differentiation, chromatin structure, and histone acetylation (Chen et al., 1997; Dalby, 2005; Kim et al., 2005).

On the basis of this physiological experiment, Langevin et al. (2011) continued the observation of fibroblast function, exploring their ability to interact with the viscoelastic properties of fascial tissue. The hypothesis that fibroblasts, through cytoskeletal remodeling, induced by static stretching, actively contribute to the viscoelastic behavior of the whole tissue, has also been tested. The results show that by changing shape, fibroblasts dynamically modulate the viscoelastic behavior of areolar connective tissue through Rho-dependent cytoskeletal mechanisms. Cytoskeleton remodeling of fibroblasts, in response to static stretching, increases the cell body cross-sectional area, thereby relaxing the tissue to a lower state of resting tension. (Langevin et al., 2005, 2011a).

Abbott et al. (2013) evaluated cytoskeletal remodeling of fibroblasts in and dissociated from mouse areolar and dense connective tissue in response to 2 h of static stretch in both native tissue and collagen gels of varying crosslinking. Areolar connective tissue had a lower dynamic modulus and was more viscous than the dense connective tissue. In response to stretch, cells within the more compliant areolar connective tissue adopted a large "sheet-like" morphology that was in contrast to the smaller dendritic morphology in the dense connective tissue. By adjusting the in-vitro collagen crosslinking, and the resulting dynamic modulus, it was demonstrated that cells dissociated from dense connective tissue are capable of responding when seeded into a compliant matrix, while cells dissociated from areolar connective tissue can lose their ability to respond when their matrix becomes stiffer. This set of experiments indicated stretch-induced fibroblast expansion was dependent on the distinct matrix material properties of areolar connective tissues as opposed to the cell's tissue of origin (Abbott et al., 2013).

Schleip et al. (2012) examined a potential cellular basis for a particular characteristic of the fascial tissues, an increase in stiffness induced by stretch and subsequent rest: strain hardening of fascial tissues. The results showed an increase in stiffness in the majority of samples when fascia was isometrically stretched for 15 min followed by 30 min at rest. With sufficient resting time both matrix hydration and tissue stiffness increased. Applying these findings in-vivo clinical application of routines, for injury prevention, merits investigation (Schleip et al., 2012; Cook et al., 2006; Dolan et al., 1994; Hides et al., 2008).

Fibroblasts in whole areolar connective tissue respond to static stretching of the tissue by expanding and remodeling their cytoskeleton within minutes, both ex-vivo and in-vivo (Langevin et al., 2006, 2005, 2011). Langevin et al. (2013a,b) tested the hypothesis that the mechanism of fibroblast expansion in response to tissue stretch involves extracellular ATP signaling. In response to tissue stretch ex-vivo ATP levels increased significantly. These results support the mechanism by which ATP is involved in fibroblasts remodeling in response to static stretched tissue (Langevin et al., 2013a,b).

Two studies have examined the effects of stretching relative to inflammatory processes. Corey et al. (2012) developed a carrageenan-induced inflammation model in the low back connective tissue of a rodent and observed the effects of in vivo static stretch for 10 min twice a day for 12 days. Authors found that carrageenan-induced inflammation of the non-specialized connective tissues of the low back, in the rat, caused altered gait, increased local mechanical sensitivity and macrophage infiltration of connective tissues. All of these effects were ameliorated by tissue

Table 1
Stretching.

Articles	Study type	Sample (n)	Subject characteristics	Outcome	Intervention	Significance
Langevin et al., 2005	In vitro and in vivo physiological experiment	Ex vivo: 23 experimental group + 6 control group; Vivo: 6 without control group;	Mice killed by decapitation including a tissue flap containing dermis, subcutaneous muscle, and subcutaneous tissue;	cell body cross-sectional area, cell body perimeter, cell field perimeter, cell body-to-field ratio, and cell body thickness	Ex vivo: The tissue was elongated at a rate of 1 mm/s until a load of 0.02 N was registered and then maintained at that length for the duration of the incubation. Incubation time for both stretched and unstretched tissue flaps was 10, 60, or 120 min. At the end of incubation, the tissue was immersion fixed in 95% ethanol for 1 h; Vivo: 30 min in stretching position then tissue analysis;	ANOVA, $P < 0.01$ for cell body perimeter and cell body cross-sectional area;
Langevin et al., 2006	In vitro physiological experiment	n: not indicated	Subcutaneous abdominal CT containing dermis, subcutaneous muscle and subcutaneous tissue harvested after death and prepared then explanted with cells cultivated	1) Detection of smooth muscle cells (SMCs) via different methods including immunofluorescence and immunoperoxidase using different antibodies; 2) Localisation of antigenic sites for α-SMA via immunoelectron microscopy;	Stretch group = tissue stretched by placing it between stainless steel grips and elongated at a rate of 1 mm/s by advancing a micrometer connected to the distal tissue grip until a load of 0.02N was registered and then maintained at that length for 30 min Control group = tissue without load placed in grips and incubated for 30 min without tissue elongation	No p values indicated;
Langevin et al., 2010	In vitro physiological experiment	n: 28 mice randomized in two groups;	Subcutaneous abdominal CT containing dermis, subcutaneous muscle and subcutaneous tissue harvested after death and prepared then explanted with cells cultivated	Nuclear remodeling measurable as a change in the degree of nuclear concavity;	Stretch group: tissue stretch with a load of 0.02N for 30 min; Control group: tissue without load incubated for 30 min;	Fibroblast nuclei had a larger cross sectional area ($p < 0.001$), smaller thickness ($p < 0.03$) in the plane of the tissue; smaller relative concavity ($p < 0.005$);
Langevin et al., 2011	In vitro physiological experiment;	n: 28 mice;	Mice killed by decapitation harvesting connective tissue from the back;	Cytoskeletal remodeling influence viscoelasticity of the tissue;	Tissue sample were elongated until a target peak force of 4.4 mN and maintained for 60 min. Different pharmacological inhibitors interfere with cell function;	Viscoelastic parameters showed statistical significance ANOVA, $P < 0.001$;
Abbott et al., 2013	In vitro physiological experiment;	Not indicated	Mice killed by decapitation harvesting connective tissue from the back;	Cellular morphology and rheometric tests;	Cytoskeletal remodeling of fibroblasts in and dissociated from areolar and dense connective tissue in response to 2 h of static stretch in both native tissue and collagen gels of varying crosslinking;	Cell body cross-sectional area was significantly greater in areolar compared with dense connective tissue $P < 0.001$; Dense connective tissue had a higher dynamic modulus than areolar connective tissue demonstrated by both the strain sweep and frequency sweep curves $P < 0.001$;
Schleip et al., 2012	In vitro physiological experiment;	n: 9 mice; n: 4 porcine;	Fascial tissue harvested from mice and porcine;	Changes in cellular matrix hydration;	Fascial tissue stretch for 15 min and then 30 min of rest;	Matrix hydration $P < 0.05$;
Langevin et al., 2013a	In vitro physiological experiment;	n: 14 mice first experiment; n: 10 mice second experiment;	Mice killed by decapitation harvesting connective tissue from the back;	Extracellular release of ATP;	First: Stretching of fascial tissue for 50′ then analysis of ATP; Second: Examining pharmacological agents (ATP inhibitors) on fibroblast spreading in response to stretching	First: increase in ATP concentration $P < 0.01$; Second: The stretch-evoked ATP release was not observed when the tissues was treated with the Rho kinase inhibitor $P < 0.947$;
Corey et al., 2012	In vivo physiological experiment;	n: 36 mice randomize per treatment;	Adults mice subjected to a carrageenan-induced inflammation model in the low back;	Mechanical sensivity testing with Von Frey Filaments; Gait analysis; Ultrasound imaging and analysis;	Injection of carrageenan-induced inflammation or vehicle (saline) solution; 5 experimental group: - Vehicle/no treatment; - Vehicle/stretch; - Carrageenan/no	Gait analysis $P < 0.001$; Mechanical sensivity $P < 0.001$; ICCs for measurements of connective tissue thickness $r = 0.85$ and $r = 0.90$ respectively (high reliable

(*continued on next page*)

Table 1 (*continued*)

Articles	Study type	Sample (n)	Subject characteristics	Outcome	Intervention	Significance
				Histology and immunofluorescence;	treatment; - Carrageenan/sham; - Carrageenan/stretch;	for the rodent inflammation model); The carrageenan/no treatment group had increased thickness relative to the vehicle/no treatment group ($P < 0.001$); Tissue stretch resulted in decreased tissue thickness measurements in the carrageenan/stretch group compared to the carrageenan/no treatment group ($P < 0.0.01$) but not in comparison to the carrageenan/sham group ($P = 0.12$).
Bouffard et al., 2008	In vitro physiological experiment;	n: 44 mice;	Mice killed by decapitation harvesting connective tissue from the back;	Measure of TGF-β1; Measure of LDH (indicator of apoptosis);	Two experiments: 1) Time course (n = 8) with cell cultures in pairs. - Stretch (n = 4) - No-stretch (n = 4) 2) Assayed (n = 36) paired for 1. - Stretch (n = 18) - No-stretch (n = 18)	TGF-β1 $P < 0.004$ for the first experiment; $P < 0.001$ for the second experiment; LDH $P < 0.05$ for the first experiment; $P < 0.81$ for the second experiment (not significative);

stretch. These findings suggest that connective tissues of the low back could be an important therapeutic target because inflammation can cause pain and impair function and the response of these tissues to a static stretch intervention could improve these outcomes.

Bouffard et al. (2008), tested the hypothesis that short (10 min) static tissue stretch attenuates Transforming Growth Factor β1 (TGF- β1) in response to injury. TGF-β1 is one of the key cytokines regulating the response of fibroblasts to injury, as well as the pathological production of fibrosis (Barnard et al., 1990; Sporn and Roberts, 1990; Leask and Abraham, 2004). The results showed a decrease of TGF- β1 in both in-vivo and ex-vivo samples. Decrease TGF-β1-mediated new collagen formation may be important for limitation of scarring and fibrosis, post-injury (Bouffard et al., 2008).

Overall static stretching is thought to decrease tension, inflammation and stiffening of connective tissue. Although these results highlight the relevance of connective tissue stretching, in vivo studies are necessary to confirm and translate these findings in a clinical scenario.

3.1.2 Myofascial release (MFR)

Eight studies analyzed the biological effects of MFR on fascia (see Table 2). MFR is a widely employed direct manual technique treatment, which utilizes specifically guided mechanical forces to manipulate and reduce myofascial restrictions of various somatic dysfunctions. MFR when used in conjunction with conventional treatment has been shown to be effective in providing immediate relief of pain and reduction of tissue tenderness (Hou et al., 2002; Fernandez de las Penas et al., 2005).

In a preliminary in vitro study, Dodd et al. (2006) investigated the effects of acyclic biophysical strain on normal human dermal fibroblasts and observed potential changes in cellular shape, proliferation, production of nitric oxide, interleukin (IL) 1β, and IL-6. The results showed that low strain magnitudes (10%) induced mild cellular rounding and pseudopodia truncation. High strain magnitudes (30%) decreased overall cell viability and induced cell membrane decomposition and pseudopodia loss. IL-6 concentrations increased two-fold, while nitric oxide levels increased three-fold, in cells strained at 10% magnitude for 72 h.

Eagen et al. (2007), using the same strain-induced model sought to determine if strain direction (equibiaxial vs heterobiaxial) differentially regulates human fibroblast function. Authors found no alterations in cell morphology in equibiaxial strain despite such changes in heterobiaxial strain. The equibiaxial strain when compared with heterobiaxial strain exhibited a significant decrease in proliferation (22%), inflammatory interleukin 6 secretion (75%), and macrophage-derived chemoattractant/chemokine secretion (177%). The authors concluded asserting that effectiveness of manual medicine treatments is potential strain direction dependent.

Subsequently, tests were performed regarding the ability of fibroblasts to respond as a mechanotransducer to a modeled Repetitive Motion Strain (RMS), an injury profile that correlates with repetitive and forceful movements, awkward postures and sustained forces (Yassi, 1997), and modeled MFR (Meltzer et al., 2010). This in vitro study aimed to investigate possible cellular and molecular mechanisms that could explain the immediate clinical outcomes associated with RMS and MFR. The results showed that the modeled injury (RMS) displayed enhanced apoptosis activity and loss of intercellular integrity. Treatment with MFR following RMS resulted in normalization in apoptotic rate and cell morphology.

Muscle fascia fibroblasts may influence muscle hyperplasia and repair through paracrine cytokine activity (Cooper et al., 2004; Quinn et al., 1990). Hicks et al. (2012) investigated the mechanisms by which fibroblast manipulation could be responsible for accelerated recovery from muscle injury. Using a fibroblast-conditioned medium to construct a novel myoblast-fibroblast coculture, the authors could directly measure paracrine effects of fibroblasts on myoblast growth and differentiation. They hypothesized that fibroblasts exposed to RMS inhibit myoblast differentiation, and treatment with modeled MFR reverses the effects. Results revealed that RMS followed by MFR produced a statistically significant increase in muscle differentiation and myoblast fusion efficiency into myotubes. If clinically translatable, this study suggests that although RMS would clinically reduce the ability to regenerate and repair muscles, MFR would enhance these effects.

Studies investigating the clinical effects of MFR are focused primarily on a specific manual therapy technique rather than

Table 2
Myofascial release.

Articles	Study type	Sample (n)	Subject characteristics	Outcome	Intervention	Significance
Dodd et al., 2006	In vitro physiological experiment;	n = 40 −70,000 cells per well using 6 wells per treatment group with 50 −60% confluency;	Normal human dermal fibroblasts;	Cell shape; Proliferation; Nitric Oxide secretion; IL-1β e IL-6 secretion;	Cell exposure to in vitro strain profiles using a computer-assisted (Flexercell FX-2000) using a vacuum to strain cells: Strain = acyclical strain for 0.25 h −72 hrs at a magnitude of 10% −30% over their initial resting length; Control = no strain;	IL-6 concentration two fold higher ($P < 0.05$) with magnitude 10% at 48−72 h; NO concentration three fold higher ($P < 0.05$) with magnitude 10% at 24-48-72 h;
Eagen et al., 2007	In vitro physiological experiment;	n = 120,000 cells per well using 6 wells per treatment group with 50−60% confluency;	Normal human dermal fibroblasts;	Cell morphology; Secretion profile of 60 cytokines using ELISA;	Cell exposure to in vitro strain profiles using a computer-assisted (Flexercell FX-4000) using a vacuum to strain cells: Heterobiaxial: acyclical strain for 0.25 h−72 hrs at a magnitude of 10% −30% beyond their resting position; Equibiaxial: equal strain across both axes for 48 h at a magnitude of 10% beyond their resting position; Control: no strain;	Cytokines secretion: in EQUI strained cells, fractalkine significantly increased, macrophage-derived chemoattractant/chemokine and pulmonary and activation-regulated chemokine significantly compared with control ($P < 0.05$). The EQUI compared with HETERO exhibited a significant decrease in proliferation, inflammatory IL-6 secretion (ELISA), and macrophage-derived chemoattractant/chemokine secretion (ELISA, $P < 0.05$);
Meltzer et al., 2010	In vitro physiological experiment;	n = 120,000 cells per well using 6 wells per treatment group with 50−60% confluency;	Normal human dermal fibroblasts;	Cell morphology; Cytokines secretion; Proliferation; Cell hypertrophy;	Cell exposure to in vitro strain profiles using a computer-assisted (Flexercell FX-4000) using a vacuum to strain cells: - 8 h RMS; - 60s MFR; - 8 h of RMS + 60s MFR; - control: no strain;	RMS: elongation of lameopodia, cellular decentralization, reduction of cell to cell contact and significant decreases in cell area to perimeter ratios compared to all other experimental groups ($P < 0.0001$). Increase of apoptosis rate compared with control ($P < 0.05$); RMS + MFR: increase in GRO secretion (P not indicated);
Hicks et al., 2012	In vitro physiological experiment;	Not indicated;	Fibroblasts and myoblast colture, together and separated;	IL-6 secretion after MFR (responsible of myoblasts differentiation);	Cell exposure to in vitro strain profiles using a computer-assisted (Flexercell FX-4000) using a vacuum to strain cells: - Control: no strain; - Cyclical strain 8 h (lesional model); Acyclical strain 60s-120s (MFR); - Cyclical + Acyclical;	Increase of myoblast differentiation for all strain groups ($P < 0.05$); Cocolture: increase of myoblast differentiation compared with uniculture ($P < 0.05$); Increase of IL-6 with acyclical strain in cocolture compared with uniculture ($P < 0.05$);
Cao et al., 2013	In vitro physiological experiment;	n= (1000 cells/μL) with collagen type I;	3-dimensional bioengineered tendons (BETs) to more closely mimic the physical environment found in vivo;	Variation in modeled MFR strain magnitude or duration can induce unique fibroblast cellular response; Dose-dependent effects of modeled MFR durations and strain magnitudes on fibroblast hyperplasia, hypertrophy, and secretion of cytokines and growth factors;	Cell exposure to in vitro strain profiles using a computer-assisted (Flexercell FX-4000) using a vacuum to strain cells: - Control: no strain; - MFR 6% magnitude at 0,0.5,1,2,3,4, and 5 min;	Increasing strain magnitude increased cytokine secretion ($P < 0.05$ for 9% IL-1β and 12% I-309 compared with control); 5 min strain with 6% magnitude significant increase in angiogenin, IL-3, GCSF, TARC and IL-8 ($P < 0.05$);

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Table 2 (*continued*)

Articles	Study type	Sample (n)	Subject characteristics	Outcome	Intervention	Significance
					- MFR magnitude 0%, 3%, 6%, 9% and 12% for 90s;	
Cao et al., 2013a	In vitro physiological experiment;	n = 1 × 10^5 cells per well using 6 wells per treatment group with 70–80% confluency;	Normal human dermal fibroblasts using an in vitro scratch wound strain model;	IL-1β and NO secretion;	Cell exposure to in vitro strain profiles using a computer-assisted (Flexercell FX-4000) using a vacuum to strain cells: - Control: no strain; - RMS 8 h; - MFR; - RMS + MFR;	- RMS reduced wound closure rates (vs nonstrain, $P < 0.05$), which are partially attenuated by a single dose of MFR; - Fibroblasts treated with combined RMS + MFR resulted in a 39% decrease ($P < 0.05$) in wound closure rate as compared with control fibroblasts; - Wound constructs treated with RMS + MFR exhibited the highest NO secretion ($P < 0.05$);
Cao et al., 2014	In vitro physiological experiment;	n= (1000 cells/μL) with collagen type I;	3-dimensional bioengineered tendons (BETs) to more closely mimic the physical environment found in vivo;	Variation in modeled MFR strain magnitude or duration on wound healing;	Cell exposure to in vitro strain profiles using a computer-assisted (Flexercell FX-4000) using a vacuum to strain cells: - Control: no train; - MFR magnitude 6% duration 0,0.5,1,2,3,4, and 5 min; - MFR magnitude 0%, 3%, 6%, 9% and 12% duration 90s;	An 11% and 12% reduction in BET width were observed in groups with a 9% ($P < 0.01$) and 12% ($P < 0.05$) strain, respectively. Reduction of the minor axis of the wound was unrelated to changes in BET width. In the 3% strain group, a statistically significant decrease (−40%; $P < 0.05$) in wound size was observed at 24 h compared with 48 h in the nonstrain, 6% strain, and 9% strain groups;
Henley et al., 2008	Repeated measure study	n = 17 healthy subjects: nine males and eight females aged 19–50 years;	Exclusion criteria chronic cardiovascular diabetes, asthma, pregnancy, smoking, premature ventricular contractions exceeding 20% of total heart beats, resting supine heart rate greater than 75 bpm or less than 45 bpm, systolic blood pressure greater than 140 mmHg or less than 90 mmHg, or failure of heart rate to increase with passive tilt (50-degrees head-up). Long-distance runners and other conditioned athletes also were excluded;	We conducted a continuation project to further examine the association between OMT and autonomic nervous system activity as demonstrated by HRV, studying the hypothesis that cervical myofascial release increases vagal tone;	- Control group: no treatment; - Cervical MFR; - Sham treatment;	Predominantly parasympathetic responses were observed with subjects in the horizontal position, while a 50- degree tilt provided a significantly different measure of maximum sympathetic tone ($p < 0.001$);

describing the biomechanical parameters used in these maneuvers. Thus it is difficult to compare clinical relevance from different studies (Cao et al., 2013). Cao et al. (2013) therefore attempted to determine whether variations in modeled MFR strain magnitude or duration can induce different cellular responses. Differently from previous works this study used another in-vitro model: using 3-dimensional bioengineered tendons (BETs) to more closely mimic the physical environment found in-vivo. The authors investigated the dose-dependent effects of modeled MFR related to tissue function in the absence of RMS. The results showed an increase in total BET dry weight in higher-magnitude MFR treatment groups, caused by an increase in extracellular matrix production. Increasing strain magnitude and duration enhanced inflammatory cytokines and growth factors. These finding suggest that variations in manual therapy biomechanical parameters may differentially affect physiological responses in vivo.

Further, physiological experiments of modeled MFR have been directed towards wound healing. Fibroblasts play a key role in the wound healing process, involving secretions of necessary cytokines and extracellular matrix proteins that enhance proliferation, migration and angiogenesis (Tettamanti et al., 2004). Related to these statements, Cao et al. (2013a) investigated the effect of modeled MFR on fibroblast wound healing. Using an in vitro scratch wound strain model, the authors observed the effects in response to RMS and MFR. The results showed a reduction in wound closure rate as a result of RMS, partially attenuated by a single dose of MFR. To further these wound healing studies in 3-dimensional preparations, wound healing was assessed in MFR-strained BETs (Cao et al., 2014). The authors investigated the effects of different durations and magnitudes of MFR strain on wound healing. Results showed that greater magnitudes determine a significant reduction in the BET's width. Lower magnitudes (3%–6% beyond resting position) and longer durations (≥5 min) of MFR improved wound healing.

Only one study considered the biological effects of MFR related to the autonomic nervous system, in particular heart rate variability

(HRV) (Henley et al., 2008). The study was conducted on healthy subjects that acted as their own controls and received interventions of cervical myofascial OMT, touch-only sham OMT, and no-touch control. Results showed a significant decrease of LF/HF ratio (low frequency/high frequency) in comparison to control and sham groups. The data established a clear association between the effects of OMT and changes in autonomic activity (Henley et al., 2008).

Overall, MFR is thought to reduce inflammation, induce muscles regeneration and improve wound healing. Although these findings highlight the relevance of MFR, in vivo study results are necessary to translate the data to a clinical perspective.

3.1.3. Other direct manipulative techniques

Analyzing the literature we found four articles investigating the biological effects of fascial treatment using different techniques from those described previously (see Table 3). One study observed EMG variability induced by a massage technique (Kassolik et al., 2009). Muscles are indirectly connected by fascial system that transfers the tension due to the presence of collagen fibers (Woodhead-Galloway, 1980). These fibers are strain resistant and enable tension transfer for long distances without loss of the force which is produced by muscles during rest and activity (Kassolik et al., 2007a,b). The tensegrity principle states that increased tension in one element of a structure has to be balanced by increased tension in another element of the same structure to maintain its shape (Ingber, 1998). Based on tensegrity principle, the authors tested the effects of massage on electrical (EMG) and mechanical (MMG) activities of a muscle lying distant, but indirectly connected to, the massaged muscle (to record activity of the middle deltoid muscle, brachioradialis was massaged – for tensor fasciae latae, the peroneal muscles was massaged). EMG amplitude increased during massage only in tensor fasciae latae, while MMG amplitude increase in both muscles. Authors concluded that these results, induced by massage therapy, supports the tensegrity principle through electrical and mechanical connection.

Bertolucci (2008) investigated the effects of a precise manual technique named: "Muscle Repositioning" (MR), in order to interact with EMG. This kind of fascial treatment seems to evoke neurological reactions, such as involuntary motor activity (Bertolucci, 2008). The results showed involuntary tonic cervical erector action during MR associated with involuntary eye movements. The author proposes MR as a novel manual technique that produces unique palpatory sensations for the therapist, sense of firmness to touch and the integration of bodily segments into a single block. Bertolucci hypothesized that MR activates the central nervous system eliciting neural reactions.

Pohl (2010), using a high-frequency ultrasound, observed tissue changes consequent to a manual technique (Sensory-Motor Body Therapy). The author found significant differences in the structure of the collagen matrix in the dermis before and after treatment. Pohl explained these results on the basis of changes in the mechanical forces of fibroblasts and increased microcirculation.

Roman et al. (2013) proposed a 3-dimensional mathematical model to explore the relationship between 3 manual therapy motions (costant sliding, perpendicular vibration, and tangential oscillation) and the flow characteristics of hyaluronic acid (HA) below the fascial layer. This mathematical model suggests that perpendicular vibration and tangential oscillation may increase HA pressure and providing positive clinical effects observed in manual therapy techniques.

Overall, these different forms of manipulation seem to affect EMG activity, connective tissue structures and HA pressure.

Table 3
Other Direct Manipulative Techniques.

Articles	Study type	Sample (n)	Subject characteristics	Outcome	Intervention	Significance
Kassolik et al., 2009	Observational study;	n = 33;	Healthy subjects mean age 20.1 ± 1.1;	EMG and MMG of Medium Deltoid and TFL;	Record the activity of the middle deltoid muscle the brachioradialis was massaged, and for the tensor fasciae latae the peroneal muscles were massaged;	- ICC values for the EMG were 0.985 for the middle deltoid muscle (very good reproducibility) and 0.960 for the tensor fasciae latae (very good reproducibility). - MMG were 0.810 for the middle deltoid muscle (good reproducibility) and 0.766 for the tensor fasciae latae (acceptable reproducibility) - RMS (root mean square) EMG at rest and during massage in the middle deltoid muscle ($P < 0.584$) while the RMS EMG was greater during massage compared to rest in the tensor fasciae latae ($P < 0.019$). RMS MMG value increased significantly during massage, compared to the rest value, in the middle deltoid muscle ($P < 0.011$) and in the tensor fasciae latae ($P < 0.003$)
Bertolucci, 2008	Observational study;	n = 6;	Healthy subjects mean age 24.67	EMG observation pre and post treatment	Muscle Repositioning for 10 –15 min to cervical extensor muscles;	Not indicated
Pohl, 2010	Observational study;	n = 30;	Symptomatic patients free of trigger points or muscle tenderness under pressure;	High resolution high frequency ultrasound (22 MHz) reaching 4 mm beneath the surface to measure skin thickness;	Treatment via sensory motor body therapy consisting of several hands-on techniques;	Highest peak $p < 0.0001$ Skin thickness $P < 0.01$;
Roman et al., 2013	Mathematical model;	–	–	Explore the relationship between the 3 manual therapy motions and the flow characteristics of HA below the fascial layer.	Navier-Stokes equations;	The fluid pressure of HA increased substantially as fascia was deformed during manual therapies.

3.2. Description of the studies analyzing the biological effect of indirect manipulation

3.2.1 Strain and counterstrain (SCS)

Literature review shows several studies based on the effectiveness of indirect manipulation, however studies related to basic science remain insufficient. Our search identified three articles eligible for the inclusion criteria, all tested the Strain and Counterstrain technique (see Table 4).

Strain Counterstrain (SCS), also known as positional release therapy (D'Ambrogio and Roth, 1997), is a passive positional technique aimed at relieving musculoskeletal pain and dysfunction through indirect manual manipulation (Jones et al., 1995). The mechanisms explaining the effects of SCS reported in clinical practice remain largely theoretical. Suggested factors in SCS intervention include aberrant neuromuscular activity mediated by muscle spindles and local circulation or inflammatory reactions influenced by the sympathetic nervous system (Chaitow, 2007).

Two studies tested the effects of SCS related to the proprioceptive theory of Irwin Korr, an American physiologist who defined the characteristics of "osteopathic lesions". A trial of patients with Achilles tendonitis found that Achilles tendon stretch reflex amplitudes reduced after SCS, but the H-reflex, which bypasses the muscle spindles, remained unchanged, suggesting that SCS might affect the stretch reflex by altering muscle spindle activity (Howell et al., 2006). A randomized controlled crossover study of patients with plantar fasciitis found that SCS treatments did not change reflex amplitudes but reduced pain and increased plantar flexion reflex torque (Wynne et al., 2006).

One laboratory study, (Meltzer and Standley, 2007), investigated human fibroblast proliferation and interleukin secretory profiles in response to modeled Repetitive Motion Strain (RMS, typical injury profile) and modeled indirect osteopathic manipulative techniques (IOMT). Fibroblasts were seeded onto membranes prestrained to 10% beyond resting length, subjected to the RMS profile and then subjected to IOMT profile, return to resting length for 60s of positional release. This physiological experiment aimed to investigate possible cellular and molecular mechanisms that could explain clinical outcomes associated to injury and osteopathic manipulative treatments. The SCS profile showed lower levels of interleukin-6 secretion, important for mediating inflammatory healing after acute injury, compared with the RMS group. Translate these data to clinical practice, and a cellular mechanism of understanding may explain the efficacy of SCS? (Meltzer and Standley, 2007).

Table 4
Strain and counterstrain.

Articles	Study type	Sample (n)	Subject characteristics	Outcome	Intervention	Significance
Meltzer and Standley, 2007	In vitro physiological experiment;	n = 120,000 cells per well using 6 wells per treatment group with 50–60% confluency;	Normal human dermal fibroblasts;	- Cell proliferation; - interleukin recognition using imagery of cytokine volumes quantification; - Interleukin secretion analysis via ELISA of 9 different interleukins;	Cell exposure to in vitro EQUI strain profiles using a computer-based system (Flexercell FX-4000 Tension Plus) using vacuum to strain cells: - Control: no strain profiles; - RMS: 8 h RMS then sampled immediately; - 24 RMS: 8 h RMS then sampled 24 h later; - 24 IOMT: 60s IOMT then sampled 24 h later; - 24 RMS + IOMT: 8 h RMS followed by 3 h rest and then 60s IOMT sampled 24 h post IOMT;	Defined as $P < 0.05$; - Proliferation: yes ($P < 0.05$) except RMS and 24IOMT; - Interleukin imagery: yes ($P < 0.05$) for RMS, 24 RMS (IL-1α) and No (other ILs); No for IOMT; - Interleukin secretions: yes ($P < 0.05$) 24IOMT IL-3 and IL-6 24RMS + IOMT;
Howell et al., 2006	Case-control;	n = 16 experimental group; n = 15 control group;	- Experimental group: subjects with Achilles tendinitis; - Control group: healthy subjects;	- Reduction of amplitudes of stretch reflex and H reflex in the triceps surae muscles; - Clinical outcome: soreness, stiffness, and swelling;	Control group: sham treatment; Experimental group: Strain and Counterstrain technique;	- The use of OMT produced a 23.1% decrease in the amplitude of the stretch reflex of the soleus ($P < 0.05$) in subjects with Achilles tendinitis; - The H-reflex was not significantly affected by OMT; - Control: neither reflex was significantly affected by sham manipulative treatment; - OMT subjects indicated significant clinical improvement in soreness, stiffness, and swelling;
Wynne et al., 2006	Single-blind, randomized controlled trial of crossover design;	n = 20 subjects with plantar fasciitis;	16 female and 4 male, age 20–66;	- Reduction of amplitudes of stretch reflex and H reflex in subjects with plantar fasciitis; - Clinical outcome: soreness, stiffness, and swelling;	Ten subjects (50%) were assigned to receive 3 weeks of Counterstrain treatment during phase 1 of the trial, while the other 10 subjects were given placebo capsules. After a 2- to 4-week washout period, phase 2 of the trial began with the interventions reversed;	Defined as $P < 0.05$; No significant changes in the electrically recorded reflexes were observed in SCS; Peak force and time to reach peak force both increased ($P < 0.05$) with the Counterstrain phase ($P < 0.05$).

4. Discussion

The present review identified a range of studies describing the biological effects resulting from direct or indirect manipulation of the fascial system. The results of this review highlight different cellular mechanisms responsible for beneficial outcomes related to OMT and in general manual therapy.

Biophysical perturbation of tissues - whether resulting from injury, somatic dysfunction, or OMT – affects range of motion, pain, and local inflammation (Elkiss et al., 2003; Sucher, 1994). Therefore, somatic dysfunction and OMT are both characterized by various biophysical strains placed on the microenvironments of cells and their surrounding tissues (Dodd et al., 2006). This interesting connection provided the authors' with the idea [MFR and SCS paragraphs] of investigating, through in-vitro studies, how RMS (injury model) and OMT might affect cellular physiology.

The first data (Dodd et al., 2006) confirmed the hypothesis that biomechanical stimuli had profound and clinically relevant effect on several cellular processes. In particular, different intensity of acyclic strains, a kind of stimulation that could mimic some forms of postural injury and OMT, differentially influence cellular shape, proliferation and cytokines secretion (Dodd et al., 2006). These results suggest a beneficial use of dose-dependent manipulation in patient care (Zein Hammoud and Standley, 2015), but did not identify the optimal dosage of manual treatment? Observing clinical practice the authors proposed that changing MFR magnitude and duration induced unique fascial tissue responses (Cao et al., 2013). Results showed that by varying parameters of manual techniques it is possible to activate or inhibit an inflammatory process, mediated by fibroblasts, and that only higher magnitudes and longer durations (6% beyond resting length and 5 min duration) of MFR up-regulate cytokine secretions (Cao et al., 2013). These findings suggest that dose-dependent and prophylactic MFR may potentially regulate inflammation and wound healing responses in patients. 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. Additionally, the potential prophylactic effect of MFR (and perhaps other OMT modalities) may prevent injury in individuals with risk factors for musculoskeletal injury (Cao et al., 2013). However, limitations still exist in correlating these data with clinical outcomes; in-vitro studies cannot reproduce many aspects produced clinically, such as pressure, changes in tissue temperature, stimulation of sensory nerves, and so, the research of optimal dose of treatment is still under investigation (Cao et al., 2013).

Manual therapies have been shown to be effective treatment for alleviating systemic and localized acute inflammation (Teodorczyk-Injeyan et al., 2006; Smith et al., 1994). However, the mechanism as to how manual therapy regulates inflammation remains elusive. Therefore, the authors hypothesized that the human fascial system inducse pro-inflammatory mediators in response to RMS; and that direct (MFR model) or indirect (SCS model) manipulation, reduces such secretions (Meltzer and Standley, 2007; Meltzer et al., 2010). These studies were designed to create a possible clinical scenario in which patients with a repetitive motion injury were treated with OMT. Results showed that RMS increases several pro-inflammatory mediators, such as interleukins, and both therapeutics models reverse these effects. These data could explain the clinical efficacy of OMT in increasing patients' range of motion, alleviating pain and promoting the body's natural self-healing ability (Cao et al., 2013).

RMS in combination with forceful movements increases risk of muscle tear and fibroblasts may influence muscle repair through paracrine cytokine activity (Cooper et al., 2004; Quinn et al., 1990). Could manual treatment accelerate muscle injury recovery? Results showed that RMS reduces muscle repair and MFR increase muscle differentiation and myoblast fusion efficiency into myotubes (Hicks et al., 2012). Fibroblasts activity not only participates in muscles repair but represents a key step in the wound healing process, involving secretions of necessary proinflammatory cytokines and extracellular matrix proteins that enhance proliferation, migration, and angiogenesis (Tettamanti et al., 2004). Studies included in this review showed the ability of the MFR model to reverse the negative effects of RMS on wound closure. It has been observed that lower magnitude (3%–6%) and longer duration (5 min) improved wound healing in-vitro. This mechanism could be attributed to changes in the extracellular matrix (eg, collagen synthesis, secretion, and architecture) that might result from MFR applied for longer than 2 min. If clinically translatable, these results suggest that valuable achievements might be acquired through the use of optimum MFR magnitude and duration to mediate wound repair in patients.

This review highlights that manual medicine treatments rely on biophysical techniques that may take many forms to treat injuries and somatic dysfunctions but the way to reach a standardized technique is not established. Strain direction plays an important role to obtain successful clinical results and it has been observed that only heterobiaxial strain affects fibroblast morphology but equibiaxial strain demonstrates the most beneficial results during the MFR studies previously described (Eagen et al., 2007).

Stretching is a well-known direct approach to tissues with the aim of improving performance and reducing injuries. Studies included in this review showed that fibroblasts change shape in response to sustained stretching; such changes are associated with a large-scale relaxation of the connective tissue. The role of cell remodeling could be explained by regulation of interstitial fluid pressure and flow. This mechanism might serve as protection against fluid stasis by keeping the matrix under tension equilibrium (Langevin et al., 2013a,b). Stiffening of connective tissue accompanies several pain syndromes and these studies might clarify the mechanisms how stretching improves range of motion and reduces pain.

These in-vitro models are not ideal, because they fail to consider other cell types and organ systems that are affected by the clinician during manipulation such as muscle, blood vessels, nerves, and so on, which may also contribute to the clinical outcomes (Cao et al., 2013). How in-vitro strain magnitudes reflect in-vivo magnitudes is also difficult to determine (Zein Hammoud and Standley, 2015). Furthermore, the present review contains some limitations; only English-language articles were included, which may have lead to the exclusion for other relevant studies. Additionally, a statistical analysis and quality assessment was not performed for the present review, which may weaken the interpretation of the results.

Future studies should expand these in-vitro results through in-vivo observation. Consequently, to obtain such results scientists need to find a technical instrumentation able to measure and observe how manual therapy affects the human body.

5. Conclusion

This present review provides an overview of studies in medical literature that show proof of concept that different manual techniques might uniquely affect fibroblasts function and consequently explain the beneficial results of OMT and manual medicine. Understanding the biological mechanisms by which manual techniques work could underpin their clinical efficacies and propel them to the class of evidence-based, first-line therapies (Zein Hammoud and Standley, 2015). In order to provide an evidence base to describe clinical effectiveness of OMT the goal should be to establish the characteristics (magnitude, duration, direction) of manual techniques. Is it really possible to standardize manual treatments? Chaitow (2014) noted that detail of a single manual

method might need to incorporate a dozen or more descriptors such as velocity, rhythm, range of motion, etc. So, precise replication of a single manual technique becomes extremely difficult, or impossible, to realize. Standley (2014), proposed to create a virtual "Rosetta Stone" of manual therapeutic methodology to achieve more comprehensive results related to the effectiveness and mechanisms involved in manual therapy.

Conflicts of interest

Authors declare no conflicts of interest.

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