THE POTENTIAL OF OSTEOPATHIC MANIPULATIVE TREATMENT IN REGULATING MYOFIBROBLAST ACTIVITY

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ABSTRACT

Myofibroblasts are cells that have characteristics of fibroblasts and smooth muscle cells. They play an important role in wound healing because of their ability to contract. When they get deregulated they can lead to hypertrophic scars or diseases like fibrosis, which are often seen in practice of osteopaths. This thesis is based on a literature research. First, factors that lead to myofibroblast differentiation, regulation and termination are elucidated, explained in detail and consequently this is discussed and set in context to known effects of different osteopathic manipulative treatments. In this work factors that are most promising to be able to interact with myofibroblast activity, like the stiffness of the extra cellular matrix and the possible influence on TGF-β1, represent the focus of discussion. Further, the ability of myofibroblasts for mechanosensing and their interaction with the effects of osteopathic manipulative treatments is an important part of this thesis. The limitations are that a lot of literature is based on animal and/or in vitro studies and it is questionable, whether the data is transferable to the in vivo situation without further amendments or not. In conclusion it can be said that theoretically osteopathic manipulative treatments are a promising option to interact with myofibroblasts because of their ability to release tissue tension, but further studies are needed to confirm this in vivo.

Keywords: Myofibroblast, fibroblast, osteopathic manipulative treatment, manual therapy, myofascial release

ABSTRACT

Myofibroblasten sind Zellen die sowohl Eigenschaften von Fibroblasten als auch von glatten Muskelzellen aufweisen. Auf Grund ihrer Fähigkeit zu kontrahieren sind sie in der Wundheilung von großer Bedeutung. Fehlfunktion der Myofibroblasten kann zu hypertrophen Narben oder Krankheiten wie Fibrose führen, die in der osteopathischen Praxis häufig vorkommen. In dieser Literaturarbeit werden zunächst die Faktoren, welche zur Myofibroblasten-Differenzierung, -Regulation und -Termination führen, im Detail erklärt. Danach werden diese in Bezug auf die bekannten Effekte von verschiedenen osteopathisch manipulativen Techniken diskutiert. In dieser Arbeit wird der Fokus auf Faktoren gelegt, die am wahrscheinlichsten Einfluss auf Myofibroblasten haben, beispielsweise die Steifheit der extrazellulären Matrix und die mögliche Beeinflussung von TGF-β1. Es stellte sich heraus, dass die Fähigkeit der Myofibroblasten zum „Mechanosensing“ hierfür eine wichtige Rolle spielt. Limitationen der Arbeit sind, dass die verwendete Literatur auf Tier und/oder in vitro Studien basiert und es fraglich ist ob die Ergebnisse ohne weitere Zusätze in die in vivo Situation übertragen werden können. Schlussfolgernd kann gesagt werden, dass osteopathisch manipulative Behandlungen theoretisch eine vielversprechende Möglichkeit darstellen um Myofibroblasten zu beeinflussen. Zum Beispiel durch Reduktion der Gewebespannung, auf welche Myofibroblasten durch „Mechanosensing“ reagieren können. Weitere Studien sind nötig um die Ergebnisse in vivo zu bestätigen.

Schlüsselwörter: Myofibroblast, Fibroblast, osteopatisch manipulative Therapie, Manualtherapie, fasciales unwinding

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1 Introduction

Myofibroblasts (MFB) were first described in wound healing by Gabbiani in 1971 (Gabbiani, Ryan, & Majne, 1971). They have characteristics of fibroblasts (FB) and smooth muscle cells (Desmoulière, Chaponnier, & Gabbiani, 2005).

MFBs play a major role in dermal wound closure and for restoring mechanical stability of injured organs (Hinz, 2010). If their activity becomes deregulated and chronic it can lead to tissue deformation by contracture and impedes organ function (Hinz, 2010). These tissue contractures can lead to hypertrophic scars or diseases like organ fibrosis (Tomasek, Gabbiani, Hinz, Chaponnier, & Brown, 2002).

One of the reasons for MFBs to be activated is mechanical stress (Gabbiani, 2004; Hinz, 2006; Hinz et al., 2012; Tomasek et al., 2002). One aim of osteopathic manipulative therapy (OMTh) is to improve physiologic function and/or support homeostasis that has been altered by somatic dysfunction (Treffer, Ehrenfeuchter, & Cymet, 2011). So if tension in the tissue leads to a somatic dysfunction osteopathic manipulative treatments (OMT) like indirect myofascial release, fascial unwinding or indirect methods work in the direction of ease to reduce tissue tension (Treffer et al., 2011).

Scars, hypertrophic scars and the resulting decreased mobility of the tissue are problems that confront osteopaths often in their everyday work. MFBs are significantly involved in wound healing (Tomasek et al., 2002) and also when they get deregulated for example in hypertrophic scars (Hinz, 2010). Until now there is no evidence in literature for the effect of OMT in complications and diseases that are triggered by MFBs, like hypertrophic scars or fibrosis. This thesis gives an overview about the mechanisms of MFB activity, the regulating factors that could be influenced by OMTh and if there are OMTs that are promising. This shall bring a different perspective to the treatment of hypertrophic scars and fibrosis than the usually investigated treatment options, like pharmacological methods and it gives suggestions to osteopaths which treatments to choose.

Up to this point no literature regarding the influence of OMT on MFBs has been found. The goal of this thesis is to i) elucidate how the MFB works and how it can be influenced and ii) to which extent it is possible to influence MFBs by OMT.

2 Aim of the thesis

The objective of this thesis is to elucidate the possibility of influencing MFB regulation by OMTh. The following research question was raised.

2.1 Research Question

Are there certain components in MFB regulation (activation, life cycle, termination) that are most likely to be manipulated by OMThs and which OMT is most promising to influence MFB activity and why?

2.2 Presumptions

Presumption one

The component most likely to be manipulated by OMTh is the mechanical tension in the extra cellular matrix (ECM).

Presumption two

Indirect methods like fascial unwinding (FU) and indirect myofascial release (IMFR) are the most promising strategies for influencing MFB activity because they focus on the point of ease to release the tension in the myofascial tissue.

3 Methodology

This thesis about the influence of MFB regulation by OMT represents basic research. For answering the research question data base driven literature research has been used. The articles were analyzed and set into context to be able to answer the research question in a qualitative, meaning narrative, way.

3.1 Literature research

The literature research was conducted using the data base PubMed (www.ncbi.nlm.nih.gov/pubmed) and the following journals Journal of Bodywork and Movement Therapies, Journal of the American Osteopathic Association, Osteopathische Medizin and International Journal of Osteopathic Medicine and www.ostmed-dr.com. The literature research started in April 2015 and repeatedly updated until January 2016.

Key words were: myofibroblast, fibroblast, myofibroblast and force, osteopathic medicine (OM), osteopathic manipulative treatment (OMT), osteopathic manipulative therapy (OMTh), myofascial release (MFR), fascial unwinding (FU), indirect method, tissue tension, tissue stiffness, connective tissue, ECM, effect, fascia, manual therapy

The keywords were used individually as well as in combination using the operator “and”. The combinations used are listed in Chapter 3.3.

Up to now no research work has been found in this field in Osteopathic Research Web, neither in a data base for diploma thesis and master thesis, nor any of the following data bases:

http://www.ncor.org.uk National Council for Osteopathic Research

http://www.hsc.unt.edu The Osteopathic Research Center

http://www.forcedo.org Foundation for Osteopathic Research and Continuous Education

http://www.corpp.org Commission for Osteopathic Research, Practice and Promotion

3.2 Inclusion and exclusion criteria

3.2.1 Inclusion criteria

- Inclusion criteria A (IC-A):

All literature that could be found by the key word driven data based research or

- Inclusion criteria B (IC-B):

Literature that is relevant for the thesis that is listed in the references of articles found by inclusion criteria A or

- Inclusion criteria C (IC-C):

Literature that was found by a different way without fulfilling inclusion criteria A or B described in section 3.3

3.2.2 Exclusion criteria

- Exclusion criteria A (EC-A):

Literature that is, after scanning, not relevant for answering the research question or

- Exclusion criteria B (EC-B):

Literature that is not in German or English or

- Exclusion criteria C (EC-C):

It is not possible to get access to the literature by purchasing it and or writhing to the author for full access.

3.3 Results of the literature research

The literature research was divided in two parts. First part to find literature about MFBs, how they can be activated and influenced. As there is a lot of literature about MFBs available, 9638 hits in the data base PubMed (20.01.2016), the keyword force was added. The results of the

literature research for the first part are shown in Figure 1. Due to the fact that no articles were added by IC-C it is not shown here.

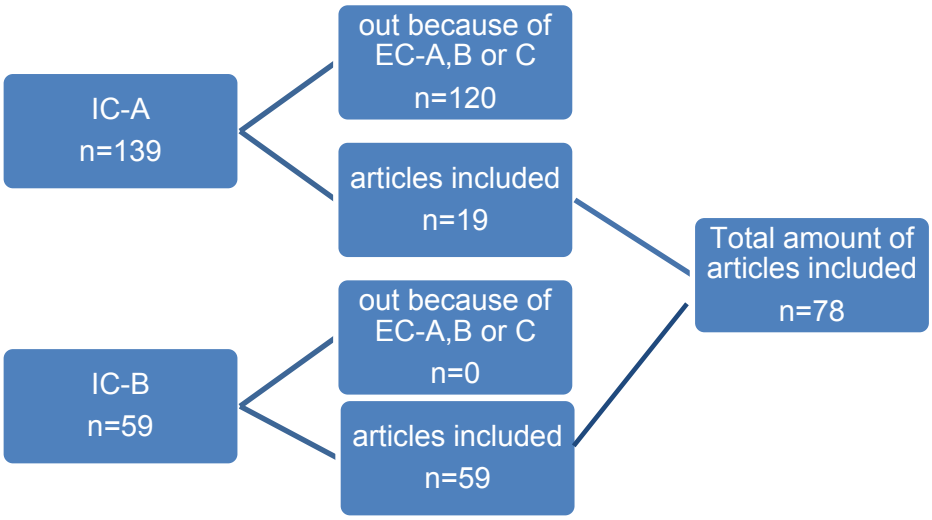

Figure 1: Results for the literature research for the first part of this thesis

To find a way to answer the research question the second part of the literature research used a lot of key words. The aim was to find out the effects of OMTs on cellular level as well as the tissue stiffness. The following keywords and their combinations were used to cover a broad spectrum.

- OMT and MFB
- Osteopathic medicine (OM) and MFB
- OMT and FB
- OM and FB
- OM and effect connective tissue (CT)
- OMT and CT
- OMT and ECM
- OMT and cellular effects
- OMT and tissue stiffness
- Fascia and manual therapy
- MFR and effects
- FU
- Tissue tension and manual therapy

Table 1 gives an overview on the amount of results and articles that could be found for the different key words using IC-A and all exclusion criteria.

Table 1:Overview of the amount of results of IC-A for the research for the second part of the literature research

Keywords	IC-A	EC-A	EC-B	EC-C	Doublets	Articles used
OMT and MFB	1	0	0	0	0	**1**
OM and MFB	5	4	0	0	1	**0**
OMT and FB	7	1	0	0	1	**5**
OM and FB	5	4	0	0	1	**0**
OM and effect CT	8	8	0	0	0	**0**
OMT and CT	9	4	0	0	2	**3**
OMT and ECM	0	0	0	0	0	**0**
OMT and cellular effect	12	8	0	0	3	**1**
OMT and tissue stiffness	1	1	0	0	0	**0**
Fascia and manual therapy	128	117	0	0	4	**7**
MFR and effects	56	53	0	0	3	**0**
FU	3	2	0	0	0	**1**
Tissue tension and manual therapy	74	72	0	0	2	**0**
Results	**309**	**274**	**0**	**0**	**17**	**18**

As Table 1 shows after the keyword based search 18 articles could be found that were relevant for answering the research question after using IC-A and all of the exclusion criteria. Additional articles were included by using IC-B and IC-C. IC-C lead to the finding of a master thesis in osteopathic research web on the effect of manually applied forces on connective tissue (Jung Adams, 2011). One new article could be found this way; the others were already covered by the research concerning IC-A. Two articles could be added by searching in the Journal of Bodywork and Movement Therapies with the keyword tissue stiffness. In total three articles were added by inclusion criteria C. Figure 2 gives an overview on the articles added for the second part of the literature research.

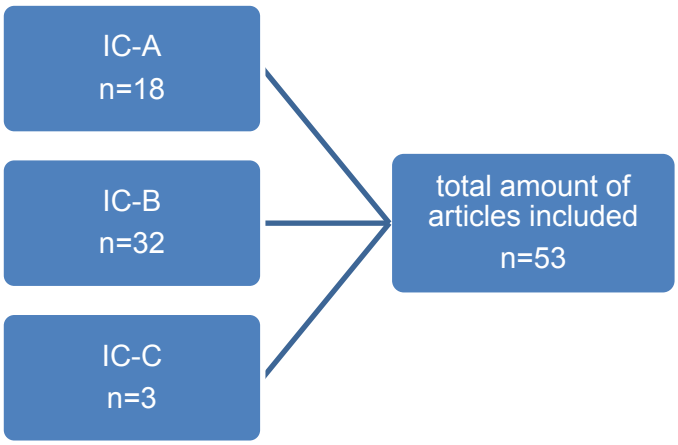

Figure 2: Results of the articles included by the second part of the literature research

The literature found for part one and two did not show any doublets. In total the literature found by the research and used in this thesis sums up to 131 articles.

4 Myofibroblasts

4.1 Characteristics of myofibroblasts

Myofibroblasts were first described in a study by Gabbiani and co-workers in 1971. A myofibroblast is a differentiated fibroblast that develops elements that are structurally and functionally similar to smooth muscle cells and plays a significant role in connective tissue contraction (Gabbiani, Hirschel, Ryan, Statkov, & Majno, 1972; Gabbiani et al., 1971). Intensive research over the past 45 years has led to an increase in the knowledge about this cell-type and to more precise and detailed definitions. Hinz published in 2007 that MFBs are fibroblast-like cells that express α-smooth muscle actin (α-SMA) and play an important role in proliferative and remodeling phases of wound healing in producing extracellular matrix (ECM), including collagen (Hinz et al., 2007).

In comparison, Fibroblasts are spindle shaped cells that can be found in the majority of tissues and organs of the body. They are usually associated with extracellular matrix molecules and their characteristic features include expression of vimentin in the absence of desmin and α-smooth muscle actin (McAnulty, 2007).

Tomasek and co-workers introduced an intermediate form between a fibroblast and a myofibroblast, the proto-myofibroblast. The proto-myofibroblast is a different type of MFB. It has the same morphological characteristics of the MFB like stress fibers, focal adhesions and extracellular fibronectin fibrils but it does not express α-SMA (Hinz, Mastrangelo, Iselin, Chaponnier, & Gabbiani, 2001; Tomasek et al., 2002). Proto-MFBs are mainly found in vivo in certain adult tissues, for example the lung alveolar septa or early granulation tissue (Hinz et al., 2001). This shows that they can function and persist as an independent cell type (Tomasek et al., 2002). Proto-MFBs in early granulation tissue lay down the first collagen bundles and pre organize the ECM by exerting comparably small traction forces (Hinz, 2010). Under certain circumstances proto-MFBs can be induced to form α-SMA and develop into differentiated MFBs. MFBs can be distinguished from proto-MFBs by de novo expression of α-SMA, the increased expression of ED-A fibronectin, increased assembly of stress fibers and more complex focal adhesions (Tomasek et al., 2002). This differentiation into MFBs is related to the production of transforming growth factor β1 (TGF-β1) by inflammatory cells and possibly also by fibrocytes (Desmoulière et al., 2005; Desmoulière, Geinoz, Gabbiani, & Gabbiani, 1993).

4.2 Origin

A large number of different cells is able to develop the MFB phenotype upon activation. There is agreement in the literature that local recruitment from fibroblasts in the surrounding dermis and subcutaneous tissue as well as pericytes or vascular smooth muscle cells around vessels are possible sources of cells that can be activated to the MFB phenotype (Desmoulière et al., 2005; Gabbiani, 2004; Hinz, 2010; Hinz et al., 2012). Also fibrocytes and circulating precursor cells are discussed to be able to form MFBs (Desmoulière et al., 2005; Gabbiani, 2004; Hinz, 2007; Hinz et al., 2012). Fibrocytes are a subpopulation of bone marrow derived leukocytes with fibroblast characteristics (Abe, Donnelly, Peng, Bucala, & Metz, 2001).

Depending on the nature of the injured organ and the particular microenvironment, a variety of precursor cells contribute to the MFB population. In 2010 Hinz reported the following incomplete list of cell types, indicating that there might be even more cell types of importance for the development of the MFB phenotype: chondrozytes, osteoblasts, hepatic stellate cells, smooth muscle cells, pericytes, mesenchymal stem cells, epithelial cells undergoing epithelial to mesenchymal transition, and possibly astrocytes (Hinz, 2007, 2010). Desmoulière et al. and Gabbiani highlight that the major source for recruitment of cells is performed by chemotaxis and subsequent migration from surrounding connective tissue (Desmoulière et al., 2005; Gabbiani, 2004). Figure 3 gives an overview on the different cell types that are able to transform into MFBs.

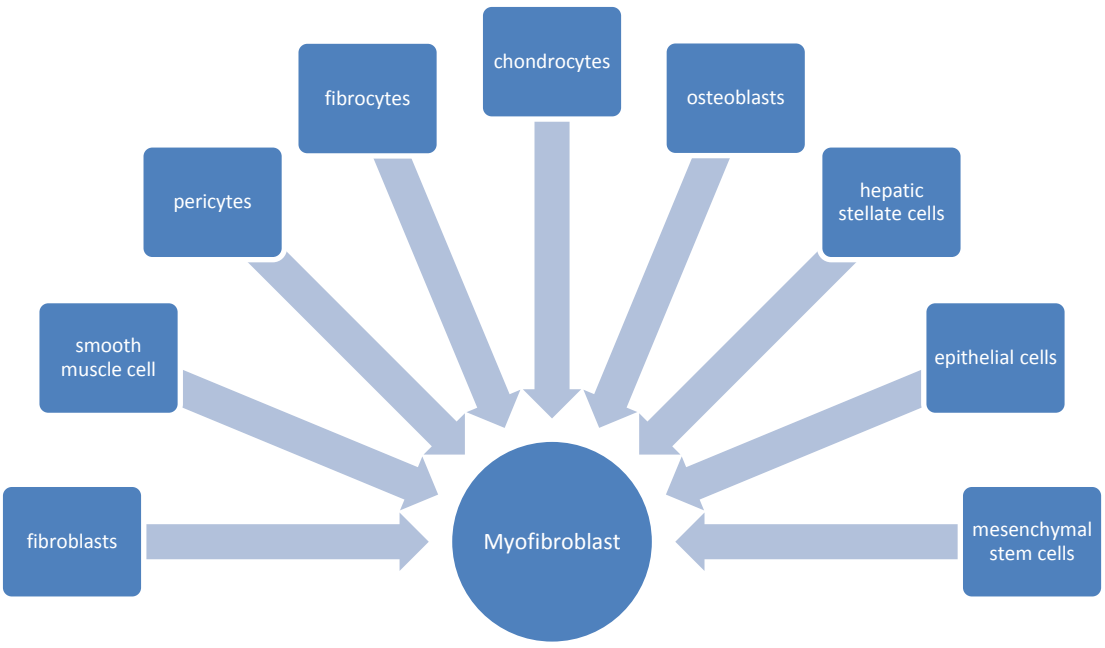

Figure 3: Cells that can develop the myofibroblast phenotype

4.3 Structure of the MFB

MFBs are characterized by developing contractile structures which are represented by stress fibers that contain bundles of actin microfilaments with associated contractile proteins such as non-muscle myosin and the extensive endoplasmatic reticulum of synthetically active fibroblasts (Gabbiani, 2004; Gabbiani et al., 1971; Tomasek et al., 2002). Actin stress fibers display a broad functionality in MFB biology. They are involved in contraction, focal adhesion maturation, extracellular matrix reorganization as well as transducing mechanical force into biochemical signals. Further they play an important role in the transcriptional control of genes, which are involved in locomotion, contraction and matrix reorganization and the localized regulation of messenger RNA translation. Special to the MFB in comparison to the FB is that the large bundles of microfilaments run parallel with the long axis of the cell (Sandbo & Dulin, 2011).

These actin stress fibers are important for the mechano-transduction system of the MFB. The MFB has this mechano-transduction system in order to generate force by stress fibers that can be transmitted to the surrounding ECM and therefore extracellular mechanical signals can be transduced into intracellular signals. This mechano-transduction system consists of actin bundles that terminate in the fibronexus, which is a specialized adhesion complex that uses transmembrane integrins to link intracellular actin with extracellular fibronectin fibrils (Dugina, Fontao, Chaponnier, Vasiliev, & Gabbiani, 2001; Tomasek et al., 2002).

Actin stress fibers are composed of bundles of polymerized actin filaments (Sandbo & Dulin, 2011). The stress fibers of proto-MFBs contain only β and γ cytoplasmatic actins, whereas the stress fibers of MFBs also contain α-SMA (Desmoulière et al., 2005). The actin filaments are mainly held together by actin –crosslinking protein α-actinin, although other crosslinking proteins, such as fascin, espin and filamin, have also been identified. α-Actinin proteins are periodic along the fiber and alternate with bands of non-muscle myosin and tropomyosin (Pellegrin & Mellor, 2007). This composition is responsible for the augmentation of actin-filament bundling via cross linking and the generation of contractile force on actin-filaments via Rho-dependent signaling (Sandbo & Dulin, 2011).

The linkage between the cytoskeleton (actin stress fibers) and the ECM is formed by transmembrane molecules at focal adhesion sites. These transmembrane molecules are heterodimers of the α and β subunits of integrin molecules (Hynes, 2002). The heterodimers $\alpha_5\beta_1$or $\alpha_v\beta_3$ are the integrins involved in FBs and MFBs focal adhesion complex formation at cell contact sites associated with prominent stress fibers (Dugina et al., 2001; Geiger,

Bershadsky, Pankov, & Yamada, 2001; Hynes, 2002). The external surface of the cell, the matrix is anchored to the cell membrane by integrin receptors that bind to specific sequences found on matrix molecules such as fibronectin or vitronectin (Hynes, 2002). On the cytoplasmatic side a focal adhesion complex is rapidly assembled by the integrin attachment and clustering. This focal adhesion complex is built of several proteins in high abundance including talin, vinculin and paxillin, and multiple accessory proteins characterized by both, integrin- interacting and actin- interacting domains (Geiger et al., 2001; Hynes, 2002). The cytoplasmatic side of a forming focal adhesion complex serves as a nucleating site for actin filament formation. Together with the initial formation of the actin filaments several proteins closely associated with the cytoplamatic side cross-link the individual actin filaments. The most important of these cross linking proteins is α-actinin (Pellegrin & Mellor, 2007; Sandbo & Dulin, 2011).

Another feature of the MFB is that they are directly connected to each other by Gap junctions. Gap junctions are connections between two cells that consist of pores. These pores allow passage of molecules. Gap junctions are composed of several hemichannels in the plasma membrane that contain distinct but functionally related proteins called connexins. The fact that the MFBs form gap junctions indicates that MFBs potentially form multicellular contractile units during granulation tissue contraction (Tomasek et al., 2002).

In the transmembrane there are certain cell-cell adhesion proteins that are intracellularly linked to the actin cytoskeleton that are called cadherins. FBs do not express cadherins. Proto-myofibroblasts in early granulation tissue express small N-cadherine. When the MFB becomes differentiated by expressing a-SMA the N-cadherine becomes replaced by larger OB-cadherine which seems to play a functional role in coordinating MFB contraction in populations (Hinz, 2007).

4.4 Formation of the MFB phenotype

4.4.1 Formation from the FB to the proto-MFB

Up to now the modulation towards the proto-MFB is not completely understood and available data is mainly based on in vitro studies (Desmoulière et al., 2005; Tomasek et al., 2002). Mechanical tension is one of the most important factors to start the differentiation of the normal FB to the proto-MFB (Tomasek et al., 2002). This has been shown by splinting wounds with a plastic frame. FBs in splinted granulation tissue form stress fibers earlier than FBs in unsplinted healing wounds and therefore develop into proto-MFBs faster. This process is reversible, so if

there is a loss of tension it leads to a loss of stress fibers (Hinz et al., 2001). The maintenance of the proto-MFB needs continuous interaction between cell-generated stress and the reaction of a substratum that is sufficiently stiff to resist force (Tomasek et al., 2002).

A second factor that seems to be important for the formation of the proto-MFB during embryonic development and wound healing in adults is the platelet-derived growth factor (PDGF). Although PDGF is important for the development of the proto-MFB, it does not induce the formation of the differentiated MFB in vitro nor in vivo (Tomasek et al., 2002).

The proto-MFB develops in wound healing when FBs migrate into the wound and produce the first collagen and cellular fibronectin rich ECM. FBs and collagen fibers become orientated parallel to the wound bed and along the expected lines of stress that are apparently caused by tractional forces of the initially closing wound. These FBs show the proto-MFB phenotype (Hinz et al., 2001). In wound healing FBs increase the level of fibronectin production and they change the repertoire of fibronectin transcripts. They now include two splice segments, ED-A and ED-B, in their fibronectin messenger RNA, which are not included in normal dermis. This process leads to de novo expression of ED-A fibronectin in granulation tissue which is important to promote further MFB differentiation (Tomasek et al., 2002). ED-A fibronectin might lead to the formation of proto-MFB but it is also linked to altered cytomechanical environment because it is also expressed by normal FBs in granulation tissue (Hinz et al., 2001).

4.4.2 The development of the differentiated MFB

MFB differentiation is part of normal wound healing that is usually terminated when the tissues are repaired (Hinz, 2007). The development of the differentiated MFB depends on mechanical stress that develops within a given tissue and the local expression of growth factors, e. g. TGF-β1. Both factors induce an accumulation of α-SMA-containing stress fibers and other characteristics of the MFB phenotype, like enhanced or newly acquired cell contraction, migration, proliferation, cytokine production, ECM secretion and ECM degradation (Hinz, 2016; Tomasek et al., 2002). Tomasek and co-workers therefore postulate the MFB differentiation as a positive feedback loop in which tension facilitates TGF-β1 production and/or activation of α-SMA expression which in return increases force production and tension development. These relations might be important for the continued formation and sustained function of the MFBs (Tomasek et al., 2002).

4.4.2.1 The role of TGF-β1 in MFB differentiation

TGF-β1 is seen as a central regulator of the development from the proto-MFB to the differentiated MFB because of its capacity to promote accumulation of intracellular contractile proteins, high collagen density and the ED-A fibronectin splice variant (Serini et al., 1998; Vaughan, Howard, & Tomasek, 2000). Possible sources of TGF-β1 in damaged tissues could be platelets, white blood cells, particularly macrophages, or parenchymal cells (Vaughan et al., 2000). Injured epithelial cells are able to produce TGF-β1 and stimulate fibroblasts in a paracrine way. Fibroblasts themselves have an autocrine production of TGF-β1 this can preserve the fibrogenic action once the inflammatory stimulus has eased (Tomasek et al., 2002). To this point TGF-β1 is known to induce collagen synthesis by fibroblastic cells, it increases the production of plasminogen activator inhibitor-1 (PAI-1), the expression of cellular fibronectin (particularly ED-A), and the expression of α-SMA (Vaughan et al., 2000).

Although there is a lot of research in this field the molecular mechanism which integrates the differentiation of the MFB is not yet known (Tomasek et al., 2002). TGF-β1 is generally released in a latent form and stored within the ECM. Once it is in the ECM in its active form it binds to the TGF-β1 receptors type I and II in the transmembrane of the cell and forms a receptor complex with a protein, which has serine/threonine kinase activity (Massagué & Wotton, 2000). The subunit of type I activates so called Smads. Smads are transcription factor proteins that mediate TGF-β1 signals (Tomasek et al., 2002). These Smads form a transcriptional complex with co-Smads to translocate to the nucleus in order to activate the transcription of target genes. This signaling pathway is responsible for the increased expression of PAI-1 (Lund et al., 1987).

Smad3 is hypothesized to be responsible for increased expression on collagen type 1 and presumably does not induce α-SMA expression (Schnabl et al., 2001; Tomasek et al., 2002). Concerning the α-SMA expression there are controversial reports because Hinz 2007 reports a discrepancy in literature about the role of Smad3 and Smad2 in combination in the α-SMA expression. This discrepancy might be because of the different roles of Smad2 and Smad3 depending on the level of MFB differentiation. Hinz 2007 suggests to see Smad2/3 as modulators of the activity of other transcription factors (Hinz, 2007) (Figure 4).

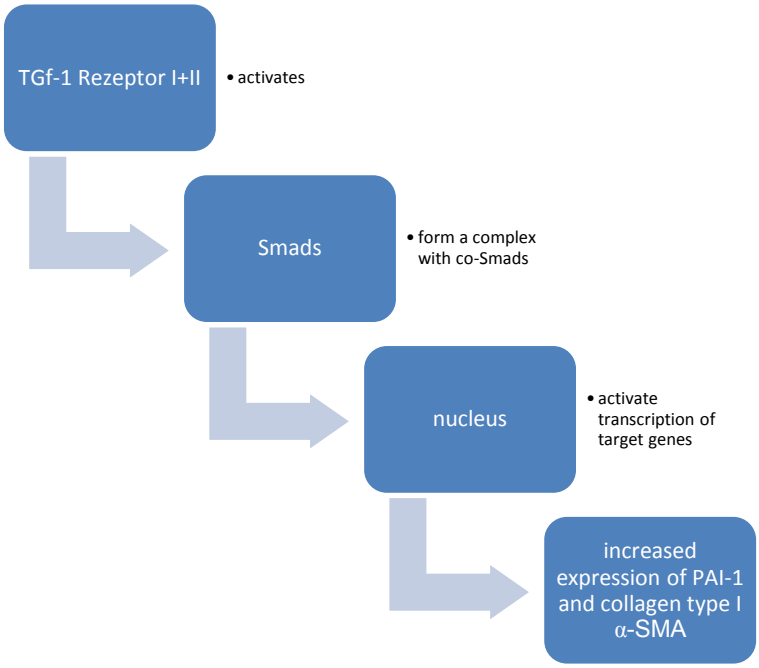

Figure 4: overview of signaling pathway, triggered by binding of TGF-β1 to its receptors in the cell membrane.

The combination of ED-A fibronectin and TGF-β1 stimulates the MFB differentiation and the expression of α-SMA (Serini et al., 1998). This effect only exists when there is mechanical tension. If the mechanical tension is removed the MFB phenotype is lost. Increased mechanical tension increases ED-A fibronectin and the α-SMA expression but it does not change the level of TGF-β1 (Hinz et al., 2001).

An additional role of TGF-β1 is that it enhances the assembly of stress fibers and the formation of the fibronexous adhesion complexes (Dugina et al., 2001; Vaughan et al., 2000) which are typical for the MFB phenotype. The formation of mature and 'supermature' focal adhesions in MFBs show de novo appearance of tensin, focal adhesion kinase and an important increase of vinculin and paxillin (Dugina et al., 2001).

Factors that support the action of TGF-β1 are connective tissue growth factor and the cell surface protein galectin-3 (Hinz, 2007). Connective tissue growth factor induces fibroblast proliferation and potentiates the action of TGF-β1 (Leask, Holmes, & Abraham, 2002). Although connective tissue growth factor and TGF-β1 cooperate the expression of α-SMA remains unchanged by the action of connective tissue growth factor. This means that it does not substitute for the action of TGF-β1 (Folger, Zekaria, Grotendorst, & Masur, 2001). Galectin-3 is upregulated in experimental kidney, liver and lung fibrosis and is obligatory for TGF-β1-

induced MFB differentiation of hepatic stellate cells in experiment. The mechanism how it cooperates with TGF-β1 is not yet understood but if it is knocked out hepatic fibrosis significantly reduces at unchanged levels of TGF-β1 and it inhibits MFB activation of cultured stellate cells in presence of TGF-β1 (Henderson et al., 2006).

Factors that antagonize the action of TGF-β1 are the inflammatory mediator IL-1 and interferon-γ, a cytokine produced by T-cells. Both factors inhibit TGF-β1 induced α-SMA expression (Higashi et al., 2003; Shephard et al., 2004). Additionally there are basic fibroblast growth factor (Cushing, Mariner, Liao, Sims, & Anseth, 2008) and TGF-β3 (Serini & Gabbiana, 1996) discussed to play a role in MFB activation by antagonizing TGF-β1. Although the effect of TGF-β3 seems to be variable depending on cell culture or animal model (Hinz, 2016).

Factors that can induce MFB differentiation without TGF-β1 are interleukin (IL) -6, nerve growth factor and a protein called Fizz1 that is found in the inflammatory zone (Hinz, 2007). In absence of exogenous TGF-β1 IL-6 increases α-SMA transcription in FB cultures from IL-6-null mice (Gallucci, Lee, & Tomasek, 2006). Likewise, the α-SMA expression is enhanced in cultured skin and lung FBs by nerve growth factor (Micera et al., 2001). Nevertheless, a cooperation of IL-6 and nerve growth factor with autocrine TGF-β1 cannot be ruled out because they were not tested simultaneously with the inhibition of TGF-β1 (Hinz, 2007). The molecular mechanism of Fizz1 remains elusive but it induces α-SMA expression in absence of TGF-β1 (Liu et al., 2004). Further, angiotensin-II (Swaney et al., 2005), endothelin-1 (Shi-Wen et al., 2004) and thrombin (Bogatkevich et al., 2003) induce α-SMA expression in vitro but it is unclear whether endogenous TGF-β1 contributes to this effect or not. Additionally, in a recent review by Boris Hinz, Wnt/b-catenin signaling, Hyaluronic acid and transient receptor potential vanilloid membrane channels are mentioned and discussed to contribute to the MFB activation (Hinz, 2016).

4.4.2.2 Mechanical aspects of MFB differentiation

Usually FBs are shielded from mechanical stress by the surrounding ECM and do not develop a contractile apparatus (Hinz, 2006). When the FB gets exposed to mechanical stress, e.g. as a result of tissue injury, ECM remodeling by their own activity makes the ECM stiffer. This action leads to changes in the cell morphology, they shift from a dendritic to a bipolar/stellate phenotype and the punctuate contacts grow into mature focal adhesions in conjunction with the formation of stress fibers (Tamariz & Grinnell, 2002). This cell type now represents the proto-MFB. With further remodeling and increasing of ECM rigidity the expression of α-SMA starts, which marks the phenotype of the differentiated MFB (Tomasek et al., 2002).

Mechanical tension is another key element for MFB differentiation and function. A mechanical feedback loop controls the MFBs' activity to secrete and organize ECM to reestablish the tissue integrity (Hinz, 2010). The differentiated MFB depends on mechanical tension. This tension is controlled by the continuous interaction between the intracellular contractile activity and the rigidity of the ECM (Hinz & Gabbiani, 2003; Hinz et al., 2001).

The mechanical property influences the function and morphology of the MFB. It interacts with synthetic (Kessler et al., 2001), proteolytic (Mudera et al., 2000), contractile activities (Hinz et al., 2001) and the secretion of ECM proteins (Chiquet, 1999; Hinz & Gabbiani, 2003). Tissue stiffness can be expressed as the Young's elastic modulus E. The Young's modulus (in Pa) is defined as force per area (stress) that is required to induce a length change (strain) in an elastic material (Janmey, Georges, & Hvidt, 2007). The Young's modulus of normal tissues and organs has been measured by force spectroscopy and it reasonably well characterizes ECM stiffness at small length changes induced by single cells although tissues are not true elastic materials (Hinz, 2016).

In 2010 Hinz published a stiffness map for the MFB. It describes formation of the MFB according to the stiffness of the ECM. The first provisional ECM of a normal healing wound has a Young's modulus of about 10-1000 Pa. This can be compared to in vitro conditions of fibroblasts growing in two dimensional soft polyacrylamide gels and or in three dimensional soft collagen gels, where the development of stress fibers is suppressed and they form only small immature adhesion sites called focal complexes, that are about 1µm long (Galbraith, Yamada, & Sheetz, 2002; Tamariz & Grinnell, 2002). For the expression of the proto- MFB the substrates need to be stiffer with a Young's modulus of at least 3000 Pa. Until a stiffness of about 18000 Pa the granulation tissue of rat wounds is still mainly populated with proto-MFBs. Within this range of stiffness, they form α-SMA negative stress fibers and terminate into so called mature focal adhesions, which are bigger with a length of about 2-6 µm (Dugina et al., 2001; Geiger et al., 2001; Yeung et al., 2005). A Young´s modulus of about 20000 Pa and higher is required for the development of the differentiated MFB and therefore for the expression of α-SMA and so called supermature focal adhesions with a length of around 8/10-30 µm (Dugina et al., 2001; Goffin et al., 2006). To set these numbers in context, the stiffness of fibrotic tissue and contracting granulation tissue have shown to exhibit a Young's modulus of 25000-50000 Pa (Hinz, 2009, 2010).

Focal adhesion complexes in MFBs are fixing points to the matrix. They provide a stable linkage from matrix to cell in order to transmit intracellular contractile forces to the surrounding

matrix and therefore tissue contraction can occur (Sandbo & Dulin, 2011). Further, focal adhesions function as a nucleation site for the formation and strengthening of actin stress fibers and are also a foundation for associated signaling molecules (Burridge & Chrzanowska-Wodnicka, 1996; Hynes, 2002).

The formation of focal adhesions is a complex process. It gets started by the ligation and clustering of transmembrane integrins and signaling via rhoGTPases. This leads to actomyosin contractility and actin stress fiber formation that results in generation of intracellular tension, which is required for focal adhesion formation (Burridge & Chrzanowska-Wodnicka, 1996). Another factor that is mandatory for this formation is the integrity of the actin cytoskeleton (Dugina et al., 2001). This sums up with the current model which says that the formation and enlargement of focal adhesions depends on the transmission of tension via the actin cytoskeleton and actomyosin contraction (Galbraith et al., 2002).

The size of the focal adhesions seems to play a part in expressing α-SMA and the differentiation from the proto-MFB to the MFB. If MFBs are grown in circumstances that only allow the formation of mature adhesions, they lose α-SMAs from their stress fibers. When the adhesion size gets enlarged from 6 μm to 8 μm they reintegrate α-SMA. This enlargement by the same applied stretch at an adhesion size from 4 μm to 5.4 μm has no effect on the α-SMA. This suggests that the change in focal adhesion size is enough in mediating this mechano-response, probably by controlling the level of intracellular tension (Hinz, 2006).

The difference between mature and supermature focal adhesions also manifests in the significantly higher resistance to extracellular strain and the higher transmission of intracellular forces to the ECM by supermature focal adhesions (Hinz, 2006). For mature focal adhesions a linear correlation between the size and the force exerted could be demonstrated with a mean stress of 2.0-5.5 nN/μm² (Beningo, Dembo, Kaverina, Small, & Wang, 2001). According to Hinz supermature focal adhesions of MFBs do not follow this linear regime. They are able to exert a 2-4-fold higher stress (6.6- 12.6 nN/μm²) compared to the mature focal adhesions of proto-MFBs (~ 3 nN/μm²). Due to these findings Hinz and co-workers propose that because of changes in the ECM organization and/or compliance the shift from mature to supermature focal adhesions permits the generation of a critical tension within stress fibers. This could increase the affinity of a so far unknown binding partner for α-SMA in stress fibers (Hinz, 2006, 2010).

The molecular composition of mature and supermature focal adhesions are different. Mature focal adhesions contain vinculin, paxillin, talin, α-actinin, focal adhesion kinase (FAK), cytoplasmic β- and γ- actins and αvβ3 integrin (Geiger et al., 2001). Supermature focal

adhesions add tension, α-SMA, α5β1 integrin and extracellular ED-A fibronectin to the list of components (Dugina et al., 2001).

4.4.2.3 Activation from latent TGF-β1 to the active form

TGF-β1 is secreted by fibroblasts non- covalently associated with its latency associated pro-peptide (LAP). This complex covalently binds to the latent TGF-β1 binding protein (LTBP-1). This binding protein is a component of the ECM and its function is to store and present latent TGF-β1 for later activation (Worthington, Klementowicz, & Travis, 2011; Zilberberg et al., 2012). There are different mechanisms and factors that promote the activation from L TGF-β1 to its active form that are still focus of current research (Hinz, 2016). Binding of αv integrin is one that is currently intensively studied. Another for this thesis more interesting factor is that the mechanical resistance of the ECM has an influence on TGF-β1 activation. There are two ways how mechanics can play a role in activating latent TGF-β1. One is via intracellular actin/myosin mediated cell contraction force that is transmitted to an RDG binding site in LAP. This force leads to a conformational change that liberates TGF-β1 from the LAP (Hinz, 2013, 2015). This process only works in connection to the second way, when the LAP is bound to the LTBP-1 and therefore connected to a mechanical resistant ECM. The mechanical state of the ECM at the time of cell pulling on LAP has a direct impact on the activation of TGF-β1. This is a mechanical checkpoint in tissue repair (Hinz, 2015; Klingberg et al., 2014). Hinz published a figure showing the activation from latent TGF-β1 in analogy of pulling a candy out of its wrapper. The state of the ECM is represented by a rubber band. The candy (TGF-β1) is tied to the rubber band on one side and on the other side the integrins (finger) pull on the wrapper (LAP). The rubber band needs to be strained for the candy to get unwrapped by pulling. This means that there is the need of a pre-strain in the ECM proteins to reach the trigger point for the TGF-β1 activation (Hinz, 2015). The amount of stiffness in the ECM needed for the mechanical activation of TGF-β1 is according to Wipff and colleagues ≥ 5 kPa Young's modulus (Wipff, Rifkin, Meister, & Hinz, 2007). This amount is still lower than the minimal stiffness required for the expression and incorporation of α-SMA into stressfibers (myofibroblast differentiation). This means that the mechanical activation of TGF-β1 and its ability to translate the level of ECM stiffness into a pro-fibrotic signal can provide a first control point in the progression of tissue remodeling (Hinz, 2010).

Mechanical stress can directly modulate α-SMA protein expression. There is a possibility of a connection between the levels of mechanical stress and MBF differentiation that is not regulated by the amount of active TGF-β1 (Hinz, 2010). This is because the differentiation of

MFBs does not occur in early stages of wound healing although the amount of TGF-β1 is high (Werner & Grose, 2003). One explanation for this phenomenon could be the insufficient mechanical conditioning of the ECM. Goffin and co-workers showed that the growth on soft substrates suppresses the differentiation of the MFB even when there is active TGF-β1 present (Goffin et al., 2006). Interestingly mechanical stress alone is not sufficient to activate differentiation into MFBs without TGF-β1 (Hinz et al., 2001).

It seems that for the MFB differentiation both, mechanical stress of the ECM and TGF-β1 are required. Both factors also collaborate to upregulate expression of collagen (Lindahl et al., 2002).

4.5 Contraction

MFBs can produce and sustain contractile force over a long period of time (Tomasek et al., 2002). This force is regulated by myosin light- chain (MLC) phosphorylation, which is similar to how smooth muscle cells work (Chrzanowska-Wodnicka & Burridge, 1996). This phosphorylation is regulated by two different kinase systems, the calcium dependent MLC kinase (MLCK) and the Rho/Rho-kinase (Burridge & Chrzanowska-Wodnicka, 1996). These two systems lead to different types of contraction of MFBs compared to smooth muscle cells (Desmoulière et al., 2005).

The contraction of smooth muscle cells is Ca^{2+} dependent, which means it works because of intracellular changes of the Ca^{2+} level. This makes it rapid, short lived and reversible. The MFB contraction is regulated by the Rho/Rho kinase (Bogatkevich et al., 2003; Katoh et al., 2001). The activated Rho kinase has two possibilities to increase MLC phosphorylation. One is the direct phosphorylation of the MLC similar to the Ca^{2+} dependent MLCK, but with lower catalytic efficiency. The other one inactivates myosin phosphatase by phosphorylating the myosin binding subunit of this enzyme complex (Tomasek et al., 2002). This promotes the contraction to isolated stress fibers which provides an energetically more favorable mechanism for maintaining sustained isometric tension (Katoh et al., 2001) and is needed by the MFB as they need to continuously generate force over long periods of time (Tomasek et al., 2002).

This contractile activity of the MFBs together with ECM synthesis and degradation leads to connective tissue remodeling. This results in irreversible and long contractures in a process that can last weeks, month or even years (Follonier Castella, Gabbiani, McCulloch, & Hinz, 2010). It remains to be shown how MFBs stabilize contractions that occur on a cellular or subcellular level to counteract the stress present in a tissue undergoing remodeling (Hinz et al.,

2012). One possibility indicated by in vitro studies is that MFBs use a lockstep or ratchet mechanism of cyclic and incremental contractile events. This mechanism combines strong (micronewtons) and far- ranging (tens of micrometers) contractions which are mediated by the Rho/Rho kinase and weak (approximately 100 pN) and short ranging (approximately 0.4 μm) cyclic contractions promoted by changes in the intracellular calcium concentrations (Castella, Buscemi, Godbout, Meister, & Hinz, 2010). Because of these two mechanisms a model is proposed, in which the stressed collagen initially develops a slack caused by the strong isometric contraction. These tension-released fibrils get straightened by the weak, repeated subcellular contractile events which leads to shortened and repeatedly stressed collagen fibrils that stabilize the status quo of the ECM and a new MFB contraction cycle can begin. It might be possible that the level of stress, meaning the resistance of the collagen fibers to pulling, may determine which mechanism of contraction will be engaged (Hinz, 2015; Hinz et al., 2012).

4.6 Function

4.6.1 Wound healing

Myofibroblasts play an important role in wound healing. Physiological wound healing consists of three phases, inflammation, proliferation and remodeling. In the proliferation phase cell-rich granulation tissue is formed and contraction occurs. This wound contraction has positive and negative effects. The positive effect is that it narrows the wound margins which leads to wound closure. The negative effect is when it gets excessive and causes formation of undesirable contracture or scarring leading to cosmetic and functional problems (Li & Wang, 2011).

Fibroblasts maintain tissue homeostasis by regulating the turnover of the ECM under normal conditions (Li & Wang, 2011) and they are stress shielded by the surrounding ECM (Tomasek et al., 2002). When the tissue gets injured there are changes in its mechanical property, the composition and organization of the ECM and locally released cytokines activate the fibroblast to differentiate into the MFB and start with the secretion of ECM components and contracting so the wound can heal (Hinz, 2007; Tomasek et al., 2002). When the tissue is repaired, MFBs usually disappear by apoptosis leaving behind a scar. In pathological conditions MFB activity persists and leads to tissue deformation which is seen in hypertrophic scars, after a burn injury, scleroderma or Dupuytren's disease. This excessive remodeling and contraction can also affect vital organs like liver, kidney, lung and heart and cause fibrosis which impedes organ function (Hinz, 2007, 2010). MFBs also play a role in the stroma reaction of tumors by creating a stimulating environment for epithelial tumor cells (Desmoulière, Guyot, & Gabbiani, 2004;

Hinz, 2007). Figure 5 gives an overview of the functions of MFBs and fibroblasts and the pathologies that can result when they are deregulated.

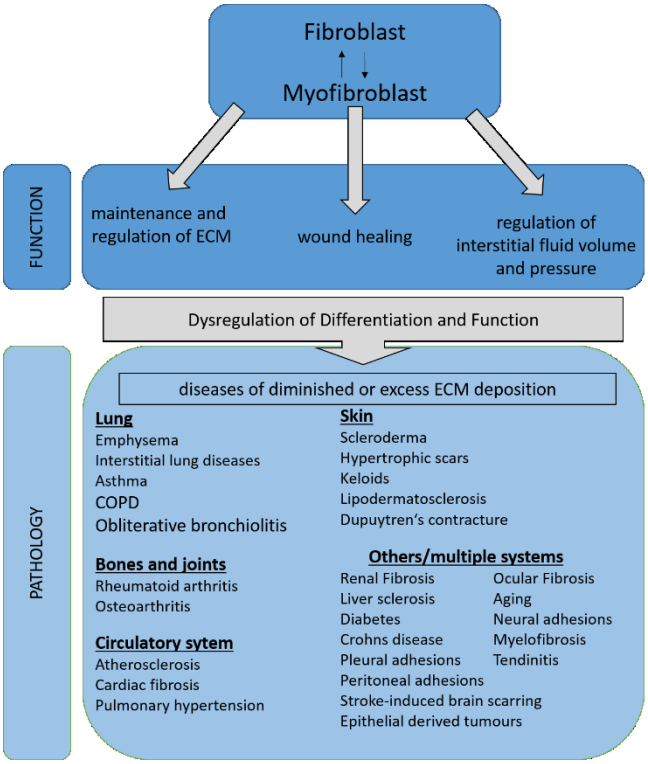

Figure 5: adapted from (McAnulty, 2007) shows the functions and pathologies that can be linked to a deregulation of MFBs and fibroblasts.

4.6.2 Contracture and Contraction

The function of MFBs to generate and maintain contractile force is clear. How this force translates into tissue shortening and leads to contractures is not completely understood. To clarify this, it is important to distinguish between muscle-based contraction and connective-tissue contracture as they are different processes. Muscle contraction is dominated by intracellular events and energy expenditure, is rapid and reversible shortening of tissue. Connective-tissue contracture is dominated by extracellular events such as matrix remodeling; it is a low energy, slow, semi-permanent shortening process that involves matrix-dispersed cells. Tomasek and co- workers defined some basic mechanisms in a working hypothesis for a model of extracellular matrix remodeling phase of matrix contractures.

This model says that because the MFBs stress fibers are connected to the surrounding collagen fibrils of the ECM by fibronexous adhesion complexes, actions from either inside or

outside the cell can lead to local matrix shortening and bundling of the surrounding pericellular collagen network. At first this is just a local process, but new matrix components are added to stabilize the new collagen organization, which can potentially increase collagen density and orientation. When the MFB now repeats this process, surrounding MFBs also contract; the small local process of matrix remodeling can lead to tissue contracture (Tomasek et al., 2002).

4.6.3 ECM

The differentiated MFB produces components of the ECM like collagen type I, III, IV, V and VI (Groma, 1998). Other than collagen-specific products, the MFB produces ED-A fibronectin (Serini et al., 1998) and glycoprotein tenascin-C (Chiquet-Ehrismann & Chiquet, 2003). According to Powell and colleagues MFBs synthesize collagen types I- VI and XVIII, glycoproteins, and proteoglycans for normal growth, differentiation and wound repair, as well as matrix molecules including laminin and thrombospondin, glycosaminoglycan, hyaluronic acid, heparan sulfate and matrix-modifying proteins such as matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) (Li & Wang, 2011; Powell et al., 1999).

The ECM plays an important role in the MFB life-cycle. This is because there is a constant mechanical feedback that the cells receive from the ECM where stiffer matrix leads to higher MFB contraction and ECM secretion, which leads to further ECM stiffening (Hinz, 2010). The mechanical characteristic of the environment appears to modulate its action to induce either migration or contraction (Grinnell & Ho, 2002; Hinz, 2007).

Klingberg and co-workers propose a model where changes in the ECM organization produced by fibroblasts over days, weeks and months in fibrotic lesions will augment the bioavailability of TGF-β1. They show that the mechanical state of the ECM can prime latent TGF-β1 for activation similar to the loading of a mechanical spring. Therefore, they suggest that excessive remodeling activity of fibroblastic cells in the early stages of tissue repair can set stage for the development of fibrosis by regulating the mechanical trigger point for latent TGF-β1 activation (Klingberg et al., 2014).

4.6.4 Mechano sensing

The MFB has a mechano-transduction system in order to generate force by stress fibers that can be transmitted to the surrounding ECM and therefore extracellular mechanical signals can be transduced into intracellular signals. This mechano-transduction system consists of actin bundles that terminate in the fibronexus, which is a specialized adhesion complex that uses

transmembrane integrins to link intracellular actin with extracellular fibronectin fibrils (Dugina et al., 2001; Tomasek et al., 2002). This adhesion complex provides the MFB with information about substrate mechanics and chemistry (Hinz, 2010). The relation between size maturation of focal adhesions and the capability of cells to sense stress are the yin and yang of mechanosensing (Hinz, 2010).

MFBs can sense the gradually increasing change in matrix stiffness that goes along with tissue remodeling. There are three possible ways of mechano sensing according to Hinz (Katsumi, Orr, Tzima, & Schwartz, 2004; Martinac, 2004; Vogel & Sheetz, 2006). One is represented by mechano-sensitive ion channels in the plasma membrane another one is integrin-mediated stress perception and the third one is described by geometry changes that reveal cryptic signaling domains in proteins of the ECM. Because of the fact that MFBs in vivo differentiate in response to matrix changes that occur over days and months, it demands a 'slow' mechano sensor. One possibility for this could be the very slow interstitial flow that accompanies inflammation and tissue regeneration (Hinz, 2007; Ng, Hinz, & Swartz, 2005).

4.7 Termination

4.7.1 Apoptosis

Until this point it is known that at the end of wound healing the synthesizing and contractile activity of MFBs is usually terminated and the cell number is dramatically reduced by apoptosis (Desmoulière, Redard, Darby, & Gabbiani, 1995). If this apoptosis does not occur it can lead to excessive contractile and synthesizing activity, sometimes over years and this leads to chronic pathological tissue contractures (Desmoulière et al., 2005). There are two possible mechanisms to account for MFBs disappearance at the end of physiological wound healing. One reason for the MFBs to undergo apoptosis is that the ECM is re-constituted and takes over the mechanical load and thereby releasing the MFBs form stress. This means that stress release can induce MFB apoptosis (Hinz, 2007; Hinz & Gabbiani, 2003). The other reason is the increase in the formation of specific cell-cell contacts that are accumulated in late granulation tissue and show a decrease of the expression of α-SMA and de-differentiate into α-SMA negative fibroblasts (Hinz, 2007).

Although it is clear that the cells undergo apoptosis, little is known about the factors regulating this phenomenon. Factors that might play a role are reduction in the concentration of local trophic factors, the remodeling of the ECM by MMPs, the balance between MMPs and TIMPs favors in early stages of wound healing ECM production. In later stages it is possible that this

balance changes and matrix degradation occurs. Another factor that can contribute to the apoptotic phenomena is a change in the physical stress caused by stretch of granulation tissue (Desmoulière et al., 2005; Grinnell, 2003).

4.7.2 De-activation

Another possibility for the termination of the MFB activity might be de-activation (Hecker, Jagirdar, Jin, & Thannickal, 2011; Hinz, 2016; Kloxin, Benton, & Anseth, 2010). Hecker and colleagues investigated the role of MyoD, a transcription factor in MFB activation and deactivation (Hecker et al., 2011). This hypothesis is supported by the work of Kloxin and co-workers. It shows MFB de-activation in response to a substrate mechanical stimulus. The results show that MFBs can be deactivated solely by changing the modulus of the underlying substrate (Kloxin et al., 2010). This means that the decrease of the stiffness of the ECM can de-activate MFBs.

5 Effects of osteopathic manipulative treatment

Osteopathic manipulative treatment (OMT) is defined as 'the therapeutic application of manually guided forces by an osteopathic physician (U.S. usage) to improve physiologic function and/or support homeostasis that has been altered by somatic dysfunction' (Treffer et al., 2011, p 28).

OMTs can be divided in three main categories: (i) direct, (ii) indirect and (iii) direct-indirect (Treffer et al., 2011). All OMT techniques have in common that they were designed to bring change in cellular function by addressing affected or at least associated tissues via biomechanical stimuli (Johnson & Kurtz, 2003).

For this thesis OMTs are covered and discussed that were found in literature regarding to myofibroblasts, fibroblasts, fascia, connective tissue, extracellular matrix, tissue tension, tissue stiffness addressing their cellular effects. There are no articles that investigated any effect of OMTs on MFBs. One study focused on the effect of brief stretching on TGF-β1, which is one of the most important factors for FBs to turn into MFBs. Most of the literature is available on the effect of OMTs on FBs.

There is a discrepancy in literature concerning the terms FB and MFB. Some authors, like Helene Langevin, differentiate between MFBs and FBs; in other articles, for example Manal Zein-Hammoud's review of 2015, it is not quite clear whether the effects are specific to FBs or can be addressed to MFBs as well. In a detailed review about the neuro-fasciagenic model of somatic dysfunction, Paolo Tozzi states that FBs and MFBs are both highly responsive to various effects of MFR and other OMTs (Tozzi, 2015). Langevin et al 2013 highlight the problem that 'There is a tendency in the literature to attribute myofibroblast behaviors (e.g. stress fiber formation, sustained matrix traction), to normal fibroblasts.' (Langevin, Nedergaard, & Howe, 2013, p5).

The functions of fibroblasts and MFBs are very similar and include the synthesis and degradation of a variety of glycoproteins, which build up the specialized ECMs of tissues and organs of the body. Another function is the regulation of the interstitial fluid volume, pressure and appropriate levels of tissue contraction for optimum function through cell-matrix interactions (McAnulty, 2007). Since MFBs and FBs have a lot of their functions in common it is likely that the effects of OMTs that are known to interact with FBs are also valid for the interaction with MFBs. Nevertheless, this part of the thesis focusses on the effects of OMTs that can be linked to the special functions of MFBs that are described in chapter 1.

The majority of the literature was found about fascial techniques. An increased density of MFBs could be found in the human lumbar fascia, suggesting that the fascia can actively contract in a smooth muscle-like manner (Schleip, Klingler, & Lehmann-Horn, 2005). Recent research suggests that OMT targets FBs and MFBs by manipulating the fascia (Zein-Hammoud & Standley, 2015). Fascial techniques aim to release tensions in the tissue/fascia (Tozzi, 2012). These facts make fascial techniques interesting for the effects of OMTs on MFBs.

The description and discussion of techniques is assembled as follows: (i) OMTs that can be linked to cellular effects, (ii) effects to techniques that are not specific to osteopathic medicine but have specific effects, and (iii) effects than cannot be linked to one specific technique but can be achieved by OMT. The last chapter is about (iv) fascial techniques that are commonly used.

5.1 OMTs and their cellular effect

5.1.1 Myofascial release

Myofascial Release (MFR) can be performed in a direct or indirect way and uses continual palpatory feedback to achieve release of myofascial tissues. MFR balances the structure in three planes of motion and makes positional corrections to lead the tissue to relaxation. This can be done either directly where the tissue is brought to the restrictive barrier and loaded with constant force to achieve the relaxation or indirectly where the tissues are guided along the path of least resistance (Treffer et al., 2011). In literature MFR is usually referred to as a direct technique (Tozzi, 2012; Zein-Hammoud & Standley, 2015).

MFR is a well-studied technique and a large amount of literature is available. Most of the literature is about the effect on fibroblasts but as MFBs are activated FBs some effects can be linked to address MFBs as well. Sometimes MFR and acyclic long duration strain refer to the same technique in literature (Zein-Hammoud & Standley, 2015).

FBs and MFBs are both highly responsible to direction (Eagan, Meltzer, & Standley, 2007), magnitude (Cao, Hicks, Campbell, & Standley, 2013), duration and frequency (Meltzer & Standley, 2007) of a therapeutic load, and can regulate cell apoptosis, activity or proliferation (Meltzer et al., 2010), mainly by influencing gene expression, secretion of inflammatory mediators and ion conductance (Tozzi, 2015).

5.1.1.1 Strain directions and magnitudes

Different strain directions seem to have different effects on FBs. FB morphology is for example affected by heterobiaxial but not equibiaxial strains. This different response is likely correlated to actin, which mediates strain induced cellular Ca^{2+} release (Eagan et al., 2007; Zein-Hammoud & Standley, 2015). This means that because of the direction of the strain actin is activated to induce cellular Ca^{2+}, which has an effect on the contractility of the cell. This again would have an effect on the tissue stiffness.

Heterobiaxial or cyclic short duration strains are able to produce inflammatory reactions and increased (occasionally reduced) FB proliferation. In comparison to that equibiaxial or acyclic long duration strains showed a completely reversed pattern and even a normalization of the apoptotic rate was found (Eagan et al., 2007; Meltzer et al., 2010). For the clinical use this could mean, that the fascial tissue possibly will respond better to balanced and sustained stretch rather than intermittent and unequal loads (Tozzi, 2015).

High magnitude (therapeutic) load (from 9% to 12% elongation) can produce upregulation of ECM products (Cao et al., 2013) and cause pro inflammatory responses (Yang, Im, & Wang, 2005). The combination of increased magnitude and duration (1-5 minutes) triggers cytokine and growth factor secretions (Cao et al., 2013). Upregulation of ECM products as well as increased inflammatory responses can be seen either as a positive effect regarding wound healing or as negative effect when it comes to fibrosis or hypertrophic scarring.

FBs and MFBs have been revealed to express two cannabinoid receptors, CB1, CB2 and endocannabinoid ligand-metabolizing enzymes. Modeling MFR with cyclic equiaxial stretching showed a doubling in the expression of the cannabinoid receptor CB1 in FBs. The endocannabinoid system decreases FB secreted metalloproteinase enzymes and is thereby able to damp cartilage destruction. It also affects FB remodeling through lipid rafts associated with focal adhesions (McPartland, 2008). The system can be upregulated by OMT (MFR, muscle energy technique and thrust technique) (McPartland et al., 2005). This upregulation leads to effects like reduced inflammation in myofascial tissues and plays a role in fascial reorganization (McPartland, 2008). This would be beneficial for treatment of fibrosis or hypertrophic scars or processes in the body where constant inflammation leads to problems.

Another effect of MFR is the breakdown of abnormal collagen cross-links and increased matrix hydration for re-organization and remodeling of collagen fibers after myofascial work (Martin, 2009). This could be used to treat fibrosis or hypertrophic scars.

5.1.1.2 Effects of MFR on wound healing in vitro

It could be shown that long duration (≥ 5 minutes) and low magnitude (3% - 6%) of MFR were most effective in improving wound healing in vitro. The effect could be attributed to gene activation and changes in the ECM, like collagen synthesis, secretion and architecture that might result from MFR applied for longer than two minutes. A possible explanation for this could be that by holding MFR for more than two minutes mechanical stress signals are generated by MFBs that trigger early matrix remodeling. Another possibility could be that due to the strain it comes to a decrease in cell/extracellular matrix attachment (Cao, Hicks, Zein-Hammoud, & Standley, 2015; Zein-Hammoud & Standley, 2015). This article indicates that the effect of MFR is able to interact with MFBs not just FBs.

5.1.2 Strain & Counterstrain

Strain & Counterstrain (CS) and indirect OMT refer to the same technique (Zein-Hammoud & Standley, 2015). Both are indirect techniques. Tender points in the tissue are assessed to take the tissue to the point of ease. The operator is able to localize the exact position of ease by palpating the tender point and asking the patient for feedback concerning the level of tenderness. When the tenderness disappears the point of ease is found and the patient is held in this position for up to 90 seconds. Then the patient is very slowly returned back to a neutral position (Tozzi, 2012).

Tender points develop in shortened muscles, which are a result of their own protection. The effect of CS is due to muscle shortening and the position of ease, obtained by shortening tissues, is central in CS (Zein-Hammoud & Standley, 2015).

Again the components of various types of strain lead to a different respond in FBs by changing their cellular morphology, proliferation, and cytokine and nitric oxide secretions (Zein-Hammoud & Standley, 2015). CS can reverse the delayed inflammatory response and reduction in cellular proliferation caused by repetitive motion strain (Meltzer et al., 2010; Meltzer & Standley, 2007). Furthermore, IL-1β and IL-6 could be reduced by indirect OMT (CS). Both interleukins are known growth inhibitors of FBs. The inflammatory interleukins could be decreased by indirect OMT (Meltzer & Standley, 2007). This means that the timing of the inflammatory response and the cellular proliferation can be effected in a way that would improve wound healing, whereas the inflammatory interleukins could be decreased, which would be beneficial for the termination of a repair process.

A minimum and maximum threshold, which affects cellular viability and physiologic change, could be established by using different strain magnitudes. The results indicate that the cellular shape is a product of both strain magnitude and duration (Dodd et al., 2006).

5.1.3 Harmonic technique

Many fascial techniques involve application of oscillations and vibrations as activating forces (Tozzi, 2015). Harmonic technique for example uses vibrations to improve ranges of movement. It can be applied in a direct or indirect way. This approach is used as a diagnostic and a therapeutic tool. It works through the oscillatory nature of the body's tissues. In case of dysfunction the body's inherent oscillatory property is dampened. The maximal effect is produced when the frequency of the driving force matches the natural frequency of the segment. When this takes place, a constant amplitude of oscillation is maintained and the resonance frequency of the system is reached. Ease can be achieved with minimal oscillatory traction when applied to the fascia with a functional intent (Tozzi, 2012).

At a cellular level fascial tissue seems to show a physiological oscillatory behavior. The contractions of MFBs show periodic oscillation periods of a half-life of approximately 100 s (1 c.p.100 s), which are modulated by intracellular calcium oscillations (Castella et al., 2010). These calcium oscillations are mediated via adherence junctions. This is a possible explanation of the increase of calcium oscillation frequencies in MFBs due to the transmission of an increased mechanical load via such intercellular junctions (Follonier Castella et al., 2010). This in turn also leads to reactive changes in the contractile cell behavior (Godbout et al., 2013). Such reactive changes in the contractile behavior could influence the stiffness of the ECM.

5.2 Effects of general manual techniques

5.2.1 Passive stretch

The duration of the load is likely to be a significant factor. Brief passive 20-30% tissue stretch can attenuate the increase in soluble TGF-β1 in vitro and Type I procollagen in vivo following a tissue injury. The study tested the effect of brief passive stretching on TGF-β1 by an in vitro model with mouse subcutaneous tissue that was stretched 20% strain for 10 min once and an in vivo model where mice underwent unilateral subcutaneous microsurgical injury on the back and then stretched 20-30% strain for 10 min two times a day for seven days. The results showed that brief (10 min) static tissue stretch attenuates TGF-β1-mediated new collagen

deposition in response to injury. According to the authors amount, timing and duration of therapeutically applied stretch is likely to be critical to obtain beneficial anti-fibrotic effects (Bouffard et al., 2008). This means that by decreasing the action of TGF-β1 the additional production of collagen is reduced and therefore the risk of fibrosis or scarring is reduced (Tozzi, 2015).

Apparently brief passive stretch (minutes to hours) seems to affect FBs but does not activate them to become MFBs. This only happens when the stretch is continuous over days or weeks (Langevin et al., 2006).

5.2.2 Direct manual approach

Direct manual approach can be used to treat scars that generate a pain syndrome by relieving the involved connective tissue (Kobesova & Lewit, 2000). This should be applied in the first twelve hours following surgery to reduce the risk of adhesion formation and inflammatory reactions (Chapelle & Bove, 2013).

5.2.3 Soft tissue technique of the skin

Ultrasound measurements before and after manual intervention where performed to explain the felt differences in tissue texture experienced by therapists. The manual intervention is described as a connective tissue technique of the skin using rolling and pressing movements of the fingers. This technique is used to treat only a very localized area going back and forth in all directions. It showed highly significant differences in the collagen fiber density and orientation of the ECM in the dermis after treatment. These changes reflect the differences in regularity, softness and tension that can be felt before and after treatment. As cause of these changes it can be speculated that an interaction of increased microcirculation and relaxation of FBs. It is a possible explanation that before treatment there is a contracture of FBs in the skin, which can be seen in the ultrasound as higher densities of collagen fibers and felt as tension in the tissue. It might also exert a pressure on receptors in the skin which can cause processes that lead to feelings of discomfort. Manual treatment of the skin is probably able to influence these receptors and therefore leading to relaxation of FBs. Microcirculation might be improved when FBs are relaxed because there is a high concentration of FBs around blood vessels (Pohl, 2010). This treatment could be related to a direct technique on the skin that is used by osteopaths as well.

5.3 Effects that cannot be related to one specific technique

5.3.1 Changes in tissue tension or mechanosensing

Fibroblasts may be able to rapidly change their contractile apparatus and remodel their cytoskeleton in reaction to modulate changes in tissue tension. In this process their focal adhesions might be changed along the direction of tissue stretch within minutes (Ciobanasu, Faivre, & Le Clainche, 2013; Geiger, Spatz, & Bershadsky, 2009). Another possibility is that they build up an equal counter tension to maintain tensional homoeostasis by expanding the actomyosin activation and the microtubule network (Eastwood, McGrouther, & Brown, 1998). This means that FBs and MFBs can react to tension changes in the tissue, therefore they can react to forces applied by OMT. This fact would be beneficial for all techniques, as due to manual work on the tissues a change in tissue tension is likely to be achieved.

An in vitro study suggested that isometrically stretched fascia followed by subsequent rest leads to an increased stiffness. According to the authors this effect is produced by a super-compensation of matrix hydration leading to increase in stiffness (Schleip et al., 2012). A second in vitro study investigated the relationship of varying mechanical stresses and dynamic deformations of human fascia lata and plantar fascia in manual therapy. The results show that fascia lata and plantar fascia show similar behavior and to produce a constant deformation in the tissue a duration of up to 60 seconds should be applied (Chaudhry et al., 2007). This study investigated extension along the axis and not a common fascial technique.

5.3.2 Mechanical stretching and different styles of stress

Some articles investigated the effects of mechanical stretching and different styles of stress, e.g. static stress versus intermittent stress, on the behavior of FBs. Their results are listed here.

Cyclic (0.5 Hz per 4 h) uniaxial small magnitude stretching produced anti -inflammatory reactions in FBs (Yang et al., 2005). FBs respond better to cyclical stress than static stress, shown by increasing the production of collagenase by 200% (Carano & Siciliani, 1996). Collagenase has the potential to break cross linking peptide bonds and thereby preventing excessive connective tissue formation, as occurs during wound healing (Tozzi, 2015).

FBs have been shown to be able to dynamically modulate the viscoelastic behavior of areolar connective tissue through Rho-dependent cytoskeletal mechanisms by changing shape. Static

tissue stretch of areolar connective tissue (~20-25 %) causes fibroblast cytoskeletal remodeling via activation of focal adhesion complexes and initiate signaling pathways mediated by Rho kinase. Counter tension is developed by remodeling of the cell's focal adhesions and actomyosin activation. This counter tension makes it possible for the surrounding tissue to relax further and achieve a lower level of resting tension (Langevin et al., 2011). This does not count for dense connective tissue so to create this effect a technique should focus on the superficial layers of fascia or connective tissue. Apparently brief, moderate, balanced, static or slow cyclic strains, appropriately applied to the fascia, may be sensed at a cellular level and transduced in normalizing tissue structure and function (Tozzi, 2015).

5.3.3 Changes in the epigenetic level

Tensional loads appear to be sensed at the nuclear level. In vivo and in vitro studies showed that FBs respond within minutes to mechanical stretching by dynamically remodeling their cytoskeleton with perinuclear redistribution of α-actin (Langevin, 2006). Mechanical forces seem to be able to affect gene regulation at an epigenetic level and therefore regulate cell behavior and tissue differentiation. This effect on the epigenetic level can be passed on to daughter cells (Arnsdorf, Tummala, Castillo, Zhang, & Jacobs, 2010). This effect could probably be induced by a therapeutic mechanical load as well (Tozzi, 2015). Apparently there is a basis for improving connective tissue function and reducing tissue adhesions by using vibration as mechanical signal and thereby changing a critical epigenetic factor in regulating the microenvironment of the ECM (Kutty & Webb, 2010).

5.3.4 Effect on fluid dynamics

Fascial techniques seem to have an impact on interstitial flow. The effect of the three therapy motions defined as constant sliding, perpendicular vibration and tangential oscillation were investigated regard ing to their relationship to the interstitial flow characteristics of hyaluron acid below the fascial layer. Apparently the most effective way to increase interstitial flow might be during perpendicular vibration and tangential oscillation with respect to the fascial layer instead of constant sliding or back and forth motion (Chaudhry, Bukiet, Roman, Stecco, & Findley, 2013; Roman, Chaudhry, Bukiet, Stecco, & Findley, 2013).

In tissue with increased interstitial flow (6 µm/s or roughly 1-3 dyn/cm² shear stress) FBs proliferate, differentiate into MFBs and align parallel to each other. These actions display features that are usually observed in tissues of fibrotic phenotype like scar tissue or desmoplastic stroma around tumors (Ng et al., 2005). On the other side, increased interstitial

flow may improve drainage of inflammatory mediators, which could lead to a decrease of chemical irritation and nociceptive stimulation to nerve endings. These effects could further lead to a reset of aberrant reflexes underlying somatic dysfunction (Tozzi, 2015). This may have an effect on tissue stiffness.

5.4 Techniques that aim to release tensions

Apart from the techniques that have already been described above, many other techniques claim to be able to reduce tension in the tissues. The aspect of decreasing tension in the tissue is important for this thesis so that the most commonly used fascial techniques will be discussed in this section. The techniques are reduced to fascial techniques as fascia is the tissue where MFBs can be found. The following has no claim on being complete but highlights the most important techniques.

It seems to be common knowledge that tissue tension can be reduced by the discussed techniques. There are several different theories on the mode of action and the mechanisms explaining effects of the techniques, but studies investigating the stiffness before and after treatment are very rare.

5.4.1 Fascial unwinding

Fascial unwinding (FU) is a commonly used technique with the intention to release fascial restrictions and restore tissue mobility and function. It is an indirect technique where the operator puts the restricted tissues/joint in a position by using all vectors of the dysfunctional pattern that are inherent in the fascial motion. This may lead to a complex three-dimensional pattern including a shearing, torsional or rotational component, that needs to be acknowledged, amplified and unwound until release is felt. (Tozzi, 2012). According to a hypothetical model of Budiman Minasny, the relaxing effects of FU are based on a neurobiologic process employing the self-regulation dynamic system theory (Minasny, 2009).

5.4.2 Still technique

Still technique is a combined, direct- indirect technique where the dysfunctional tissues are further manipulated in the malposition and then brought into a position of ease until relaxation occurs. First, the position of ease for the restricted fascial element has to be determined. Secondly, a compressive force has to be introduced and maintained in the tissue (direct part). This is followed by the application of force to follow the tissue as it unwinds along its wandering

pathway toward, and through, the position of initial restriction (indirect part). Still technique uses the natural recoil of the structures from their exaggerated position to beneficial use the motion to release tension and loosen adhesions (Tozzi, 2012).

5.4.3 Balanced ligamentous tension release

Balanced ligamentous tension release (BLT) is an indirect technique. It starts with an initial disengagement of the joint, which is followed by exaggeration of the dysfunctional vectors of the somatic dysfunction until a ligamentous tensional compromise is achieved. This point is kept, while the tensional and neurological information is elaborated up to when a release is recognized. At the end of the treatment eventually there is a releasing stage, where a palpable change in the structure being treated occurs. This change can either be muscular or fascial tension, the temperature of the skin or articular range of mobility (Tozzi, 2012).

5.4.4 Effects of OMT on fascial mobility

One study was found that compared the effects of MFR and hot pack therapy on fascial gliding and flexibility of the vastus lateralis muscle. This in vivo study was performed on twelve healthy males. It tested the effect of 4 min MFR on deep fascial motion measured by B-mode ultrasound as well as muscle stiffness measured by real time elastography and a durometer before and after treatment. MFR could effectively improve the gliding function and flexibility of muscle and fascia (Ichikawa, Takei, Usa, Mitomo, & Ogawa, 2015).

Another study investigated the effects of FU and MFR on non-specific neck and low back pain patients. Dynamic ultra sound evaluation was used to assess effective sliding motion of fascial layers in vivo apart from other outcome measures not relevant for this thesis. According to these findings FU and MFR appear to be effective in improving and even restoring normal tissue mobility and function. Dynamic ultra sound was also considered to be a valid non-invasive instrument for assessing effective sliding motion of fascial layers (Tozzi, Bongiorno, & Vitturini, 2011).

Out of all the described effects, the following list of techniques and strain directions could lead to a decrease in tissue tension.

- MFR by breaking down collagen cross links (Martin, 2009)
- MFR, especially heterobiaxial strain via the effect on intracellular Ca^{2+} (Eagan et al., 2007)

- Brief passive stretch via the influence on TGF-β1(Bouffard et al., 2008)
- Cyclic stress because it activates collagenase (Carano & Siciliani, 1996)
- Static stretch via the effect on FBs that leads to tissue relaxation (Langevin et al., 2011)
- Oscillations and vibration via their effect on the interstitial flow (Roman et al., 2013)

6 Synthesis

In this chapter the effects of OMTs that can be linked to influence MFBs will be discussed.

6.1 Environmental sensing, focal adhesions and biotensegrity

6.1.1 Mechanotransduction and focal adhesions

There are different words or phrases to describe the phenomenon that cells can sense and respond to a wide range of external signals. These signals can either be chemical or physical and can be integrated by the cell. After analyzing the information, the cell can change its morphology, dynamics, behavior and eventually fate (Geiger et al., 2009). Terms used to describe these facts are “environmental sensing” or, when it focuses more on the mechanical aspect, “mechanosensing” or “mechanotransduction”. The fact that cells are able to react to external forces is important and interesting for osteopaths or manual therapists, because it can be a basis on how OMTs are integrated in the body and lead to reactions.

Environmental sensing on cellular level is complex. As described above the most important factor for sensing are focal adhesions in the cell membrane. They connect the cytoskeleton of the cell (inside) to the ECM (outside) via special integrins (proteins). Different cells have different integrins in their membrane which are activated by different stimuli and activate different processes within the cell as a respond. In adhesion mediated environmental sensing there is a focus on the surface chemistry as well as the effect of mechanical force in regulating the stages of focal adhesion assembly (Geiger et al., 2009). As mentioned in the chapter of MFBs and focal adhesions, in MFBs for example αvβ3 and α5β1 integrins could be found in the cell membrane (Dugina et al., 2001; Geiger et al., 2001). Differential activation of these integrins can lead to major differences in initiation and progression of the adhesion process (Geiger et al., 2009). In this case this means that αvβ3 is found in mature focal adhesions, which are present in the proto MFB and α5β1 integrin is found in super mature focal adhesions, which are present in differentiated MFBs (Dugina et al., 2001; Geiger et al., 2001; Wipff & Hinz, 2009). So amongst other factors, mechanical force can influence the way the focal adhesions are assembled which makes a great difference in the reaction of the cell. Mechanical force is also the part that is very likely altered by OMTs. So from this point of view OMT could be able to interact with MFBs. Additional articles investigating this theory are not available yet.

6.1.2 Focal adhesions, fibronectin and mechanical stress

Another factor that can be influenced by mechanical stress is fibronectin (FN). Fibronectin is an ECM protein. It is believed that the assembly to a FN matrix is induced by a conformational change through binding of FN to α5β1 integrin on the cell surface. This binding triggers a lot of processes within the cell like stress fiber formation (Steward, Cheng, Ye, Bellin, & LeDuc, 2011). Integrins and FN are important in MFB differentiation as discussed in Chapter 4.4.2. It has been shown that mechanical stress stimulated a revealed localization of FN around the cell periphery and an increase in FN fibril formation. Mechanical stimulation is able to directly affect FN reorganization and recruitment (Steward et al., 2011). This has an impact on MFBs and on the ECM. This would be another factor where a mechanical stimulus is able to produce cellular effects. This mechanical stimulus could possibly be induced by OMT. To support this theory further studies would be needed.

6.2 TGF-β1

The fact that TGF-β1 is crucial for the differentiation of the MFB and that it can be decreased by passive stretch (Bouffard et al., 2008) shows that this technique is a possible way to influence MFBs. Although passive stretch is not a classical OMT, while working manually with tissues in different ways, the tissue is likely to be stretched. It might be good to know this effect when dealing with patients with hypertrophic scars or fibrosis, because according to the authors duration and timing of the strain is important for the effect. The authors proposed a model of healing of connective tissue where the soluble TGF-β1 levels are reduced because of passive stress, which in turn leads to less collagen deposition and reduced tissue adhesion. This would mean a decrease in the fibrotic response (Bouffard et al., 2008). It has to be mentioned that these findings are based on in vitro and in vivo mouse tissue experiments so further studies on humans are needed.

To support this theory different facts should be investigated. First, it is necessary to validate that it is possible to create the required brief passive stretch of the tissue by using manual techniques in vivo. Second, it has to be shown that passive stretch, applied to healing wounds or existing scars, can influence the physiological effect positively. Timing, frequency and indication of the treatment need to be elucidated in advance.

6.3 Endocannabinoid System

One study showed that FBs and MFBs express cannabinoid receptors and that the endocannabinoid system can be influenced by OMT. Therefore it is suggested that via OMTs and the effect on the endocannabinoid system MFBs can be influenced (McPartland, 2008). For more details see (McPartland et al., 2005) and (McPartland, 2008).

6.4 Interstitial flow

Interstitial flow can be induced by fascial techniques as discussed earlier (Roman et al., 2013). This can be seen as a positive fact when the focus is on the transport of metabolic and messenger substances (Meert, 2012). Then it could play a role in restoring homoeostasis, where it has been compromised (Tozzi, 2015). When it comes to MFBs this is a bit different because interstitial flow can be seen as shear stress and induces FB to MFB differentiation, which can be beneficial concerning tissue repair or harmful in case of development of fibrosis (Ng et al., 2005). Due to the fact that numbers of how much fascial techniques induce the interstitial flow are missing it is difficult to compare these findings.

6.5 Tissue stiffness

The rigidity or stiffness of the ECM is a crucial factor for MFB differentiation (Tomasek et al., 2002). Many manual techniques, including OMTs like MFR, FU, Oscillations, SC, BLT and many more, claim to be able to reduce tissue stiffness as discussed in the previous chapter. Here, again the problem is to find actual numbers of the effects these techniques have on the tissue to make it possible to compare them to the numbers that are available for MFB differentiation. So one can only speculate that the effect of OMT on tissue stiffness can influence MFB behavior.

7 Discussion

7.1 Discussion of the research question and presumptions

The object of this thesis was to elucidate whether it is possible to influence MFBs by OMT or not. Therefore, the following research question was raised:

Are there certain components in MFB regulation (activation, life cycle, termination) that are most likely to be manipulated by OMTh and which OMT is most promising to influence MFB activity and why?

Before answering the question, it is important to note, that this thesis is literature based so no new results are shown, which makes the answers partly speculative and further studies will be necessary to confirm or object the proposed theories given here. Nevertheless, after working through the literature the following statements can be made about the components of MFB regulation.

Factors that activate MFBs are:

- Mechanical tension in the ECM
- TGFβ-1
- ECM rigidity (tissue stiffness)
- IL-6
- Nerve growth factor
- Fizz-1 (protein)
- Hyaluronic acid
- Wnt/b catenin

It is important to note that those factors usually need to be combined in one or the other way to stimulate FBs to differentiate into MFBs.

Factors that can terminate or deactivate MFBs:

- Reduced mechanical load because of reconstituted ECM (stress release)
- Reduced expression of α-SMA because of cell-cell contacts at the end of wound healing
- Release in the tissue tension

There is not much known about the termination of MFBs; these are the facts that are most likely to have an influence but further studies are needed. Out of these factors, and the effects of OMTs that are described in literature, it should be possible to influence the following:

- Mechanical tension in the ECM via fascial techniques
- TGF-β1 via passive stretch (Bouffard et al., 2008)
- ECM rigidity via fascial techniques (Ichikawa et al., 2015; Pohl, 2010; Tozzi et al., 2011)
- IL-6 via fascial techniques (Eagan et al., 2007; Meltzer & Standley, 2007)

This leads to the discussion of the first presumption:

Presumption one: The mechanical tension in the ECM is the component most likely to be manipulated by OMT.

It is difficult to verify or falsify this because there are only few articles in literature about the effect of OMTs on mechanical tension. It seems to be common knowledge that OMTs or fascial techniques are able to reduce the tension in the tissue but this is not supported by studies that investigated the tissue stiffness before and after treatment. There are no numbers in how much a technique is able to reduce the tension in a given tissue. This makes it impossible to compare OMT effects to the numbers that are available on the tissue stiffness that is required for FBs to turn into MFBs. This would be necessary to propose that OMT can influence mechanical tension and thereby MFB differentiation.

There were two studies that investigated the palpable changes in the subcutaneous tissue that occur after manual therapy. One with ultrasound on 30 patients (Pohl, 2010) and another one with ultrasound and elastography in a case study (Luomala, Pihlman, Heiskanen, & Stecco, 2014). Both report beneficial effects that can be seen by the ultrasound and request further study in this area.

Two other studies (Ichikawa et al., 2015; Tozzi et al., 2011) investigated the effect of MFR and other fascial techniques on fascial gliding. According to Paolo Tozzi myofascial techniques are effective in releasing area of impaired fascial gliding and dynamic ultrasound is a reliable instrument to measure these. Ichikawa states that MFR could improve fascial gliding and the flexibility of the vastus lateralis muscle. These are important findings but it is not said that the effects can be used to connect them to MFB behavior as they did not specifically investigate the ECM rigidity.

TGF-β1 could be influenced by static passive stretch over 10 min (Bouffard et al., 2008). The critical point about this fact is that it is unlikely that there is an osteopathic technique, which uses 10 min static stretch. Nevertheless, knowing about the effect could be beneficial because it would be worth a try in dealing with hypertrophic scars or fibrosis but further studies to investigate these effects in vivo in humans are missing.

IL-6 can be influenced by OMT but as the other two are the main factors in MFB differentiation it is unlikely that the change of IL-6 alone has an effect. But together with the possibility of changing TGFβ1 levels and mechanical tension it could be beneficial. So the first presumption seems to be right but it cannot be supported by definitive numbers.

Presumption two: Indirect methods like fascial unwinding and indirect MFR are most promising to influence MFB activity because they focus on the point of ease to release the tension in the myofascial tissue.

This presumption cannot be verified or falsified by the literature available at present. When it comes to the treatment of fibrosis, where too much MFB activity is harmful it can be speculated that release of tension in the tissue is a factor, which influences MFB termination. Techniques that use the point of ease are more effective in treating such conditions. But this very speculative thought would need further research.

7.2 Aim of treatment

When it comes to interacting with MFBs by OMT the first thing that needs to be clarified is whether the goal is to improve wound healing or to treat fibrosis. This is important because for wound healing MFBs are “good”. So in order to use the beneficial properties of MFBs, the goal would be to activate them. Apparently, as already discussed in the chapter about the effects of MFR on wound healing, wound healing is highly dependent on duration and magnitude of MFR strain. Lower magnitude and longer duration (more than two minutes) of strain seem to be most effective in reducing wound size. These findings are based on an in vitro model of three dimensional bioengineered tendons (Cao et al., 2015). Further studies to see if these data are clinically translatable are necessary.

The fact that larger magnitude of strains resulted in enlarging wound size and exacerbating the site of injury (Cao et al., 2015) could speculatively lead to the assumption that this would deactivate MFBs and matrix remodeling and therefore probably be beneficial for treatment of fibrosis. In the case of fibrosis or hypertrophic scars the action of MFBs gets excessive and

leads to tissue deformation (Tomasek et al., 2002). To treat these conditions by interacting with MFBs the aim would be to stop their action by interacting with the factors that are known to lead to MFB termination, like release of the tension in the tissue. As already discussed in the chapter about OMT there are several available.

One article developed a three dimensional mathematical model for deformation of human fascia in manual therapy. According to their results, forces needed to create deformations in dense fascia (fascia lata or plantar fascia) created by compression or shear, are beyond the human physiologic range. But it might be possible to create deformation in softer tissues, like the superficial nasal fascia (Chaudhry et al., 2008). The authors state that the reports of tissue release are rather the result of reflexive changes in the tissue or changes in twisting or extension forces (O'Connell, 2011). Further the forces generated by OMT may stimulate fascial mechanoreceptors, which may lead to change in the muscle tone that can be felt or the fascia might alter tonus regulation by itself because of MFB- facilitated active tissue contractility (Schleip et al., 2005). The authors of the article require further research for these theories (Chaudhry et al., 2008). This would support the theory that the effects of OMT on MFBs or other cells might be because of changes in the ECM that trigger further reactions.

7.3 Theoretical model for explaining OMT effects

There are a lot of models that try to explain the effects of OMTs. One of them is the biotensegrity model, which explains how MFBs could be influenced by externally applied mechanical stress. This model can be used to explain the effects of OMTs. It gives a possibility to explain how mechanical stress, applied to the whole organism, can be transmitted to individual cells and transduced into a biochemical signal (Chen & Ingber, 1999). This model is of interest for this thesis because MFBs are cells that are sensitive to mechanical tension and the tension of the surrounding ECM is crucial for their differentiation. This model can give an explanation for the way mechanically applied forces (meaning effects of various OMTs) are able to make a difference on a cellular and molecular level, which is important for MFBs. According to this model it should be possible to interact or influence MFBs by OMTs. Swanson gives a detailed review on the biotensegrity model and its application in osteopathic manipulative medicine (Swanson, 2013). A short introduction to the model and the most important facts will be highlighted here.

The tensegrity model comes from architecture and sees structures as stabilized by continuous tension with discontinuous compression. This allows the structures to be strong efficient and lightweight. Because of the level of pre-stress and triangulation tensegrity structures are

intrinsically self-stabilized. The self-stabilization makes it possible for tensegrity systems to transfer applied forces throughout their structures with high flexibility and minimal damage. They also return immediately back to prior shape when the force is ceased. This principle used to describe the architecture of biological organisms i.e. biotensegrity and can be applied to molecular, cellular, tissue organ and organ system levels (Swanson, 2013).

7.3.1 The mechanotransduction system in light of biotensegrity

Focal adhesions (FA) are the mechanical link between ECM and the cytoskeleton (Swanson, 2013) as already discussed in the chapter about MFBs. FAs are formed by intergrins and bind to proteins of the ECM and the cytoskeleton (Geiger et al., 2009). This makes FAs to the key components of cellular biotensegrity and regulators of mechanotransduction (Swanson, 2013). The cytoskeleton is again linked to the nucleus by microfilaments and intermediate filaments (Maniotis, Chen, & Ingber, 1997). Because of this connection the nucleus undergoes predictable deformation when extracellular forces are applied to focal adhesions in cultured cells (Ingber, 2006; Wang, Tytell, & Ingber, 2009). According to the research in this area it has been proposed that by regulating the opening and closing of nuclear pore complexes, inducing chromatin remodeling, or melting of selected regions of DNA, mechanical forces could directly affect genetic expression (Wang et al., 2009). These facts lead to the possibility that changes in the ECM or within the cell could lead to varied mechanotransduction and ultimately result in disease (Swanson, 2013).

7.3.2 Biotensegrity and OMT

Swanson proposes that biotensegrity is the vital missing link in understanding fascial architecture and how mechanical forces can lead to fascial restrictions and their release, which is claimed to be done by OMTs. In this model the cellular components of the tissue, meaning FBs and MFBs, are important (Swanson, 2013). According to the work of Paul Standley (Dodd et al., 2006; Eagan et al., 2007; Meltzer et al., 2010; Meltzer & Standley, 2007) and Helene Langevin (Bouffard et al., 2008; Langevin, Bouffard, Badger, Iatridis, & Howe, 2005; Langevin et al., 2006; Langevin et al., 2010) that are already discussed in the previous chapter, FBs of irregular connective tissue are pre-stressed biotensegrity cells. They are linked hierarchically with the ECM and through changes in gene expression they are capable of responding to mechanical forces (Swanson, 2013). Although MFBs are the main cells in connective tissue that generate contractile force (Tomasek et al., 2002) also FBs play a role in generating pre-stress and transferring it and increase the stress in response to mechanical forces (Matthews, Overby, Mannix, & Ingber, 2006). Additionally, the results of Langevins study discussed in the

previous chapter, where they state that superficial fascia can be relaxed because of FBs altering their shape are interesting (Langevin et al., 2011). This leads to the hypothesis that FBs and MFBs may be important for the results of OMTs, like MFR and FU. The explanation is that the release during these techniques may result from FBs sensing the mechanical forces, being applied by means of mechanotransduction. This would lead to changes in the FB pre-stress, which would reduce the pre-stress within the fascia. Additionally, cell-cell contacts between FBs within the fascia could potentially add to numerous FBs changing their level of pre-stress. This could cause a more global change in the pre-stress in the fascia leading to a palpable release. Due to this release the physiologic motion would be restored within the tissue and the FBs would be able to return to their normal resting pre-stress (Swanson, 2013).

7.4 Limitations of the thesis

Based on the available literature, this thesis has its limitations in the discussion of some aspects, where further research is necessary.

7.4.1 Limitations concerning MFBs

Although there is a lot of literature available about MFBs a lot of things remain still elusive like the termination or deactivation process. A lot of the information known, is based on in vitro animal studies and it is therefore difficult to say whether it is possible to translate these findings in in vivo studies on humans or not.

7.4.2 Limitations concerning the effect of OMTs

The major problem, as mentioned above, is that there is not a lot of literature available and the literature found is not specific regarding the effects of FBs or MFBs. A great part of the information available is based on in vitro animal studies and the translation in a clinical situation has not been elucidated yet. Because of these limitations parts of this thesis are speculative, but this work is a basis for further research, raising questions that can be seen as the starting point for important future studies.

8 Conclusion

This thesis critically summarizes, discusses and evaluates available literature on the possibility to use OMTs to interact with MFBs. A closer look at MFBs shows that the main factors to activate them are TGF-β1 and mechanical stress. Especially the fact that they can be influenced by mechanical stress makes them interesting for OMTs. MFBs can sense the tension in the surrounding tissue and react to it by mechanosensing. Different OMTs are able to interact with the tension in the tissue. Theoretically it should be possible that via the connection of tissue tension MFBs and OMT interact. Techniques that are likely to be beneficial are techniques that release the tension in the tissues, for example fascial techniques, as MFBs are also found in fascia.

The practical use of the findings of this thesis is to elaborate a scientific basis for treating conditions like wound healing, hypertrophic scars or fibrosis because MFBs are involved in these conditions (Hinz, 2010; Tomasek et al., 2002). Osteopaths are commonly confronted with the treatment of hypertrophic scars and fibrosis as they are often the cause of somatic dysfunction and can lead to a broad variety of symptoms. From the theoretical point of view, it would be beneficial to treat hypertrophic scars or fibrosis with OMTs that focus on relaxing the tissue. What has been found so far is that duration, magnitude and direction of strain make a difference in cell behavior. It can be stated that apparently brief, moderate, balanced, static or slow cyclic strains, appropriately applied to the fascia, may be sensed at a cellular level and transduced in normalizing tissue structure and function (Tozzi, 2015). According to Helene Langevin brief passive stretch (minutes to hours) is not efficient enough to activate FBs to become MFBs. For this the stretch would need to be continuous over days or weeks (Langevin et al., 2006). This leads to the suggestion that it should not make a difference whether a direct or indirect technique is applied, as at the end of treatment, release of the tension is the goal of both techniques. On the other hand, it would be interesting to see, whether it would make a difference to use a direct or an indirect approach in an in vitro study. It could be possible that MFBs react quicker or more efficient when the treatment already starts with reduction of the tissue tension. This theory would need further research.

Further studies to verify the suggestions made here would be necessary. One thing to investigate would be the effect of OMT on tissue stiffness. In vivo measurements of tissue stiffness is challenging. Apparently dynamic ultrasound, real time elastography or maybe Myoton could be helpful tools for measurements in vivo. The connection between fascial gliding and somatic dysfunction and how it can be evaluated is described in Leon Chaitows

editorial (Chaitow, 2014). This could be helpful for studies concerning MFBs. Further in vitro studies investigating the effect of techniques on MFB behavior as they are already existing for FBs could provide interesting data to further design in vivo studies with patients to see which technique and which parameters of the technique are crucial to treat fibrosis or hypertrophic scars more efficiently.

Additionally, there is a lot of research going on in the field of mechanosensing. Matteo Escude for example developed a self-consistent model for mechanosensitivy (Escudé, Rigozzi, & Terentjev, 2014). The results and further studies can be interesting for understanding how cells can feel changes in tissue tension and react to them, which is interesting for understanding how they can be influenced by OMT.

REFERENCES

Abe, R., Donnelly, S. C., Peng, T., Bucala, R., & Metz, C. N. (2001). Peripheral blood fibrocytes: differentiation pathway and migration to wound sites. *The Journal of Immunology, 166*(12), 7556-7562.

Arnsdorf, E. J., Tummala, P., Castillo, A. B., Zhang, F., & Jacobs, C. R. (2010). The epigenetic mechanism of mechanically induced osteogenic differentiation. *Journal of Biomechanics, 43*(15), 2881-2886. doi:10.1016/j.jbiomech.2010.07.033

Beningo, K. A., Dembo, M., Kaverina, I., Small, J. V., & Wang, Y. L. (2001). Nascent focal adhesions are responsible for the generation of strong propulsive forces in migrating fibroblasts. *The Journal of Cell Biology, 153*(4), 881-888.

Bogatkevich, G. S., Tourkina, E., Abrams, C. S., Harley, R. A., Silver, R. M., & Ludwicka-Bradley, A. (2003). Contractile activity and smooth muscle alpha-actin organization in thrombin-induced human lung myofibroblasts. *The American Journal of Physiology - Lung Cellular and Molecular Physiolology, 285*(2), L334-343. doi:10.1152/ajplung.00417.2002

Bouffard, N. A., Cutroneo, K. R., Badger, G. J., White, S. L., Buttolph, T. R., Ehrlich, H. P., . . . Langevin, H. M. (2008). Tissue stretch decreases soluble TGF-beta1 and type-1 procollagen in mouse subcutaneous connective tissue: evidence from ex vivo and in vivo models. *Journal of Cellular Physiology, 214*(2), 389-395. doi:10.1002/jcp.21209

Burridge, K., & Chrzanowska-Wodnicka, M. (1996). Focal adhesions, contractility, and signaling. *Annual Review of Cell and Developmental Biology, 12*, 463-518. doi:10.1146/annurev.cellbio.12.1.463

Cao, T. V., Hicks, M. R., Campbell, D., & Standley, P. R. (2013). Dosed myofascial release in three-dimensional bioengineered tendons: effects on human fibroblast hyperplasia, hypertrophy, and cytokine secretion. *Journal of Manipulative and Physiological Therapeutics, 36*(8), 513-521. doi:10.1016/j.jmpt.2013.07.004

Cao, T. V., Hicks, M. R., Zein-Hammoud, M., & Standley, P. R. (2015). Duration and magnitude of myofascial release in 3-dimensional bioengineered tendons: effects on wound healing. *Journal of the American Osteopathic Association, 115*(2), 72-82. doi:10.7556/jaoa.2015.018

Carano, A., & Siciliani, G. (1996). Effects of continuous and intermittent forces on human fibroblasts in vitro. *European Journal of Orthodontics, 18*(1), 19-26.

Castella, L. F., Buscemi, L., Godbout, C., Meister, J. J., & Hinz, B. (2010). A new lock-step mechanism of matrix remodelling based on subcellular contractile events. *Journal of Cell Science, 123*(Pt 10), 1751-1760. doi:10.1242/jcs.066795

Chaitow, L. (2014). Somatic dysfunction and fascia's gliding-potential. *Journal of Bodywork and Movement Therapies, 18*(1), 1-3. doi:10.1016/j.jbmt.2013.11.019

Chapelle, S. L., & Bove, G. M. (2013). Visceral massage reduces postoperative ileus in a rat model. *Journal of Bodywork and Movement Therapies, 17*(1), 83-88. doi:10.1016/j.jbmt.2012.05.004

Chaudhry, H., Bukiet, B., Roman, M., Stecco, A., & Findley, T. (2013). Squeeze film lubrication for non-Newtonian fluids with application to manual medicine. *Biorheology, 50*(3-4), 191-202. doi:10.3233/BIR-130631

Chaudhry, H., Huang, C., Schleip, R., Ji, Z., Bukiet, B., & Findley, T. (2007). Viscoelastic behavior of human fasciae under extension in manual therapy. *Journal of Bodywork and Movement Therapies, 11*(2), 159-167.

Chaudhry, H., Schleip, R., Ji, Z., Bukiet, B., Maney, M., & Findley, T. (2008). Three-dimensional mathematical model for deformation of human fasciae in manual therapy. *Journal of the American Osteopathic Association, 108*(8), 379-390.

Chen, C. S., & Ingber, D. E. (1999). Tensegrity and mechanoregulation: from skeleton to cytoskeleton. *Osteoarthritis and Cartilage, 7*(1), 81-94. doi:10.1053/joca.1998.0164

Chiquet, M. (1999). Regulation of extracellular matrix gene expression by mechanical stress. *Matrix Biology, 18*(5), 417-426.

Chiquet-Ehrismann, R., & Chiquet, M. (2003). Tenascins: regulation and putative functions during pathological stress. *The Journal of Pathology, 200*(4), 488-499. doi:10.1002/path.1415

Chrzanowska-Wodnicka, M., & Burridge, K. (1996). Rho-stimulated contractility drives the formation of stress fibers and focal adhesions. *The Journal of Cell Biology, 133*(6), 1403-1415.

Ciobanasu, C., Faivre, B., & Le Clainche, C. (2013). Integrating actin dynamics, mechanotransduction and integrin activation: the multiple functions of actin binding proteins in focal adhesions. *European Journal of Cell Biology, 92*(10-11), 339-348. doi:10.1016/j.ejcb.2013.10.009

Cushing, M. C., Mariner, P. D., Liao, J. T., Sims, E. A., & Anseth, K. S. (2008). Fibroblast growth factor represses Smad-mediated myofibroblast activation in aortic valvular interstitial cells. *The FASEB Journal, 22*(6), 1769-1777. doi:10.1096/fj.07-087627

Desmoulière, A., Chaponnier, C., & Gabbiani, G. (2005). Tissue repair, contraction, and the myofibroblast. *Wound Repair and Regeneration, 13*(1), 7-12. doi:10.1111/j.1067-1927.2005.130102.x

Desmoulière, A., Geinoz, A., Gabbiani, F., & Gabbiani, G. (1993). Transforming growth factor-beta 1 induces alpha-smooth muscle actin expression in granulation tissue myofibroblasts and in quiescent and growing cultured fibroblasts. *The Journal of Cell Biology, 122*(1), 103-111.

Desmoulière, A., Guyot, C., & Gabbiani, G. (2004). The stroma reaction myofibroblast: a key player in the control of tumor cell behavior. *The International Journal of Developmental Biology, 48*(5-6), 509-517. doi:10.1387/ijdb.041802ad

Desmoulière, A., Redard, M., Darby, I., & Gabbiani, G. (1995). Apoptosis mediates the decrease in cellularity during the transition between granulation tissue and scar. *The American Journal of Pathology, 146*(1), 56-66.

Dodd, J. G., Good, M. M., Nguyen, T. L., Grigg, A. I., Batia, L. M., & Standley, P. R. (2006). In vitro biophysical strain model for understanding mechanisms of osteopathic manipulative treatment. *Journal of the American Osteopathic Association, 106*(3), 157-166.

Dugina, V., Fontao, L., Chaponnier, C., Vasiliev, J., & Gabbiani, G. (2001). Focal adhesion features during myofibroblastic differentiation are controlled by intracellular and extracellular factors. *Journal of Cell Science, 114*(Pt 18), 3285-3296.

Eagan, T. S., Meltzer, K. R., & Standley, P. R. (2007). Importance of strain direction in regulating human fibroblast proliferation and cytokine secretion: a useful in vitro model for soft tissue injury and manual medicine treatments. *Journal of Manipulative and Physiological Therapeutics, 30*(8), 584-592. doi:10.1016/j.jmpt.2007.07.013

Eastwood, M., McGrouther, D. A., & Brown, R. A. (1998). Fibroblast responses to mechanical forces. *Proceedings of the Institution of Mechanical Engineers Part H - Journal of Engineering in Medicine, 212*(2), 85-92.

Escudé, M., Rigozzi, M. K., & Terentjev, E. M. (2014). How cells feel: stochastic model for a molecular mechanosensor. *Biophysical Journal - Cell, 106*(1), 124-133. doi:10.1016/j.bpj.2013.10.042

Folger, P. A., Zekaria, D., Grotendorst, G., & Masur, S. K. (2001). Transforming growth factor-beta-stimulated connective tissue growth factor expression during corneal myofibroblast differentiation. *Investigative Ophthalmology & Visual Science, 42*(11), 2534-2541.

Follonier Castella, L., Gabbiani, G., McCulloch, C. A., & Hinz, B. (2010). Regulation of myofibroblast activities: calcium pulls some strings behind the scene. *Experimental Cell Research, 316*(15), 2390-2401. doi:10.1016/j.yexcr.2010.04.033

Gabbiani, G. (2004). The evolution of the myofibroblast concept: a key cell for wound healing and fibrotic diseases. *Giornale Gerontologia, 52*(5), 280-282.

Gabbiani, G., Hirschel, B. J., Ryan, G. B., Statkov, P. R., & Majno, G. (1972). Granulation tissue as a contractile organ. A study of structure and function. *Journal of Experimental Medicine, 135*(4), 719-734.

Gabbiani, G., Ryan, G. B., & Majne, G. (1971). Presence of modified fibroblasts in granulation tissue and their possible role in wound contraction. *Experientia, 27*(5), 549-550.

Galbraith, C. G., Yamada, K. M., & Sheetz, M. P. (2002). The relationship between force and focal complex development. *The Journal of Cell Biology, 159*(4), 695-705. doi:10.1083/jcb.200204153

Gallucci, R. M., Lee, E. G., & Tomasek, J. J. (2006). IL-6 modulates alpha-smooth muscle actin expression in dermal fibroblasts from IL-6-deficient mice. *Journal of Investigative Dermatology, 126*(3), 561-568. doi:10.1038/sj.jid.5700109

Geiger, B., Bershadsky, A., Pankov, R., & Yamada, K. M. (2001). Transmembrane crosstalk between the extracellular matrix--cytoskeleton crosstalk. *Nature Reviews Molecular Cell Biology, 2*(11), 793-805. doi:10.1038/35099066

Geiger, B., Spatz, J. P., & Bershadsky, A. D. (2009). Environmental sensing through focal adhesions. *Nature Reviews Molecular Cell Biology, 10*(1), 21-33. doi:10.1038/nrm2593

Godbout, C., Follonier Castella, L., Smith, E. A., Talele, N., Chow, M. L., Garonna, A., & Hinz, B. (2013). The mechanical environment modulates intracellular calcium oscillation activities of myofibroblasts. *PLoS One, 8*(5), e64560. doi:10.1371/journal.pone.0064560

Goffin, J. M., Pittet, P., Csucs, G., Lussi, J. W., Meister, J. J., & Hinz, B. (2006). Focal adhesion size controls tension-dependent recruitment of alpha-smooth muscle actin to stress fibers. *The Journal of Cell Biology, 172*(2), 259-268. doi:10.1083/jcb.200506179

Grinnell, F. (2003). Fibroblast biology in three-dimensional collagen matrices. *Trends in Cell Biology, 13*(5), 264-269.

Grinnell, F., & Ho, C. H. (2002). Transforming growth factor beta stimulates fibroblast-collagen matrix contraction by different mechanisms in mechanically loaded and

unloaded matrices. *Experimental Cell Research, 273*(2), 248-255. doi:10.1006/excr.2001.5445

Groma, V. (1998). Demonstration of collagen type VI and alpha-smooth muscle actin in renal fibrotic injury in man. *Nephrology Dialysis Transplantation, 13*(2), 305-312.

Hecker, L., Jagirdar, R., Jin, T., & Thannickal, V. J. (2011). Reversible differentiation of myofibroblasts by MyoD. *Experimental Cell Research, 317*(13), 1914-1921. doi:10.1016/j.yexcr.2011.03.016

Henderson, N. C., Mackinnon, A. C., Farnworth, S. L., Poirier, F., Russo, F. P., Iredale, J. P., . . . Sethi, T. (2006). Galectin-3 regulates myofibroblast activation and hepatic fibrosis. *Proceedings of the National Academy of the Sciences U S A, 103*(13), 5060-5065. doi:10.1073/pnas.0511167103

Higashi, K., Inagaki, Y., Fujimori, K., Nakao, A., Kaneko, H., & Nakatsuka, I. (2003). Interferon-gamma interferes with transforming growth factor-beta signaling through direct interaction of YB-1 with Smad3. *Journal of Biological Chemistry, 278*(44), 43470-43479. doi:10.1074/jbc.M302339200

Hinz, B. (2006). Masters and servants of the force: the role of matrix adhesions in myofibroblast force perception and transmission. *Eurpean Journal of Cell Biology, 85*(3-4), 175-181. doi:10.1016/j.ejcb.2005.09.004

Hinz, B. (2007). Formation and function of the myofibroblast during tissue repair. *Journal of Investigative Dermatology, 127*(3), 526-537. doi:10.1038/sj.jid.5700613

Hinz, B. (2009). Tissue stiffness, latent TGF-beta1 activation, and mechanical signal transduction: implications for the pathogenesis and treatment of fibrosis. *Current Rheumatology Reports, 11*(2), 120-126.

Hinz, B. (2010). The myofibroblast: paradigm for a mechanically active cell. *Journal of Biomechanics, 43*(1), 146-155. doi:10.1016/j.jbiomech.2009.09.020

Hinz, B. (2013). It has to be the αv: myofibroblast integrins activate latent TGF-β1. *Nature Medicine, 19*(12), 1567-1568. doi:10.1038/nm.3421

Hinz, B. (2015). The extracellular matrix and transforming growth factor-β1: Tale of a strained relationship. *Matrix Biology, 47*, 54-65. doi:10.1016/j.matbio.2015.05.006

Hinz, B. (2016). Myofibroblasts. *Experimental Eye Research, 142*, 56-70. doi:10.1016/j.exer.2015.07.009

Hinz, B., & Gabbiani, G. (2003). Mechanisms of force generation and transmission by myofibroblasts. *Current Opinion in Biotechnology, 14*(5), 538-546.

Hinz, B., Mastrangelo, D., Iselin, C. E., Chaponnier, C., & Gabbiani, G. (2001). Mechanical tension controls granulation tissue contractile activity and myofibroblast

differentiation. *The American Journal of Pathology, 159*(3), 1009-1020. doi:10.1016/S0002-9440(10)61776-2

Hinz, B., Phan, S. H., Thannickal, V. J., Galli, A., Bochaton-Piallat, M. L., & Gabbiani, G. (2007). The myofibroblast: one function, multiple origins. *The American Journal of Pathology, 170*(6), 1807-1816. doi:10.2353/ajpath.2007.070112

Hinz, B., Phan, S. H., Thannickal, V. J., Prunotto, M., Desmoulière, A., Varga, J., . . . Gabbiani, G. (2012). Recent developments in myofibroblast biology: paradigms for connective tissue remodeling. *The American Journal of Pathology, 180*(4), 1340-1355. doi:10.1016/j.ajpath.2012.02.004

Hynes, R. O. (2002). Integrins: bidirectional, allosteric signaling machines. *Cell, 110*(6), 673-687.

Ichikawa, K., Takei, H., Usa, H., Mitomo, S., & Ogawa, D. (2015). Comparative analysis of ultrasound changes in the vastus lateralis muscle following myofascial release and thermotherapy: a pilot study. *Journal of Bodywork and Movement Therapies, 19*(2), 327-336. doi:10.1016/j.jbmt.2014.11.018

Ingber, D. E. (2006). Cellular mechanotransduction: putting all the pieces together again. *The FASEB Journal, 20*(7), 811-827. doi:10.1096/fj.05-5424rev

Janmey, P. A., Georges, P. C., & Hvidt, S. (2007). Basic rheology for biologists. *Methods in Cell Biology, 83*, 3-27. doi:10.1016/S0091-679X(07)83001-9

Johnson, S. M., & Kurtz, M. E. (2003). Osteopathic manipulative treatment techniques preferred by contemporary osteopathic physicians. *Journal of the American Osteopathic Association, 103*(5), 219-224.

Jung Adams, B. (2011). *Evidence on the effect of manually applied forces on the cell physiology in connective tissue; a systematic narrative review.* (Master Thesis), The British School of Osteopathy, 275, Borough High Street, London SE1 1JE.

Katoh, K., Kano, Y., Amano, M., Onishi, H., Kaibuchi, K., & Fujiwara, K. (2001). Rho-kinase--mediated contraction of isolated stress fibers. *The Journal of Cell Biology, 153*(3), 569-584.

Katsumi, A., Orr, A. W., Tzima, E., & Schwartz, M. A. (2004). Integrins in mechanotransduction. *Journal of Biological Chemistry, 279*(13), 12001-12004. doi:10.1074/jbc.R300038200

Kessler, D., Dethlefsen, S., Haase, I., Plomann, M., Hirche, F., Krieg, T., & Eckes, B. (2001). Fibroblasts in mechanically stressed collagen lattices assume a "synthetic" phenotype. *Journal of Biological Chemistry, 276*(39), 36575-36585. doi:10.1074/jbc.M101602200

Klingberg, F., Chow, M. L., Koehler, A., Boo, S., Buscemi, L., Quinn, T. M., . . . Hinz, B. (2014). Prestress in the extracellular matrix sensitizes latent TGF-β1 for activation. *The Journal of Cell Biology, 207*(2), 283-297. doi:10.1083/jcb.201402006

Kloxin, A. M., Benton, J. A., & Anseth, K. S. (2010). In situ elasticity modulation with dynamic substrates to direct cell phenotype. *Biomaterials, 31*(1), 1-8. doi:10.1016/j.biomaterials.2009.09.025

Kobesova, A., & Lewit, K. (2000). A case of a pathogenic active scar. *Australasian Chiropractic & Osteopathy, 9*(1), 17-19.

Kutty, J. K., & Webb, K. (2010). Vibration stimulates vocal mucosa-like matrix expression by hydrogel-encapsulated fibroblasts. *Journal of Tissue Engineering and Regenerative Medicine, 4*(1), 62-72. doi:10.1002/term.219

Langevin, H. M. (2006). Connective tissue: a body-wide signaling network? *Medical Hypotheses, 66*(6), 1074-1077. doi:10.1016/j.mehy.2005.12.032

Langevin, H. M., Bouffard, N. A., Badger, G. J., Iatridis, J. C., & Howe, A. K. (2005). Dynamic fibroblast cytoskeletal response to subcutaneous tissue stretch ex vivo and in vivo. *American Journal of Physiology - Cell Physiology, 288*(3), C747-756. doi:10.1152/ajpcell.00420.2004

Langevin, H. M., Bouffard, N. A., Fox, J. R., Palmer, B. M., Wu, J., Iatridis, J. C., . . . Howe, A. K. (2011). Fibroblast cytoskeletal remodeling contributes to connective tissue tension. *Journal of Cellular Physiology, 226*(5), 1166-1175. doi:10.1002/jcp.22442

Langevin, H. M., Nedergaard, M., & Howe, A. K. (2013). Cellular control of connective tissue matrix tension. *Journal of Cellular Biochemistry, 114*(8), 1714-1719. doi:10.1002/jcb.24521

Langevin, H. M., Storch, K. N., Cipolla, M. J., White, S. L., Buttolph, T. R., & Taatjes, D. J. (2006). Fibroblast spreading induced by connective tissue stretch involves intracellular redistribution of alpha- and beta-actin. *Histochemistry and Cell Biology, 125*(5), 487-495. doi:10.1007/s00418-005-0138-1

Langevin, H. M., Storch, K. N., Snapp, R. R., Bouffard, N. A., Badger, G. J., Howe, A. K., & Taatjes, D. J. (2010). Tissue stretch induces nuclear remodeling in connective tissue fibroblasts. *Histochemistry and Cell Biology, 133*(4), 405-415. doi:10.1007/s00418-010-0680-3

Leask, A., Holmes, A., & Abraham, D. J. (2002). Connective tissue growth factor: a new and important player in the pathogenesis of fibrosis. *Current Rheumatology Reports, 4*(2), 136-142.

Li, B., & Wang, J. H. (2011). Fibroblasts and myofibroblasts in wound healing: force generation and measurement. *Journal of Tissue Viability, 20*(4), 108-120. doi:10.1016/j.jtv.2009.11.004

Lindahl, G. E., Chambers, R. C., Papakrivopoulou, J., Dawson, S. J., Jacobsen, M. C., Bishop, J. E., & Laurent, G. J. (2002). Activation of fibroblast procollagen alpha 1(I) transcription by mechanical strain is transforming growth factor-beta-dependent and involves increased binding of CCAAT-binding factor (CBF/NF-Y) at the proximal promoter. *Journal of Biological Chemistry, 277*(8), 6153-6161. doi:10.1074/jbc.M108966200

Liu, T., Dhanasekaran, S. M., Jin, H., Hu, B., Tomlins, S. A., Chinnaiyan, A. M., & Phan, S. H. (2004). FIZZ1 stimulation of myofibroblast differentiation. *The American Journal of Pathology, 164*(4), 1315-1326. doi:10.1016/S0002-9440(10)63218-X

Lund, L. R., Riccio, A., Andreasen, P. A., Nielsen, L. S., Kristensen, P., Laiho, M., . . . Danø, K. (1987). Transforming growth factor-beta is a strong and fast acting positive regulator of the level of type-1 plasminogen activator inhibitor mRNA in WI-38 human lung fibroblasts. *The EMBO Journal, 6*(5), 1281-1286.

Luomala, T., Pihlman, M., Heiskanen, J., & Stecco, C. (2014). Case study: could ultrasound and elastography visualized densified areas inside the deep fascia? *Journal of Bodywork and Movement Therapies, 18*(3), 462-468. doi:10.1016/j.jbmt.2013.11.020

Maniotis, A. J., Chen, C. S., & Ingber, D. E. (1997). Demonstration of mechanical connections between integrins, cytoskeletal filaments, and nucleoplasm that stabilize nuclear structure. *Proceedings of the National Academy of Sciences U S A, 94*(3), 849-854.

Martin, M. M. (2009). Effects of the myofascial release in diffuse systemic sclerosis. *Journal of Bodywork and Movement Therapies, 13*(4), 320-327. doi:10.1016/j.jbmt.2008.04.042

Martinac, B. (2004). Mechanosensitive ion channels: molecules of mechanotransduction. *Journal of Cell Science, 117*(Pt 12), 2449-2460. doi:10.1242/jcs.01232

Massagué, J., & Wotton, D. (2000). Transcriptional control by the TGF-beta/Smad signaling system. *The EMBO Journal, 19*(8), 1745-1754. doi:10.1093/emboj/19.8.1745

Matthews, B. D., Overby, D. R., Mannix, R., & Ingber, D. E. (2006). Cellular adaptation to mechanical stress: role of integrins, Rho, cytoskeletal tension and mechanosensitive ion channels. *Journal of Cell Science, 119*(Pt 3), 508-518. doi:10.1242/jcs.02760

McAnulty, R. J. (2007). Fibroblasts and myofibroblasts: their source, function and role in disease. *The International Journal of Biochemistry and Cell Biology, 39*(4), 666-671. doi:10.1016/j.biocel.2006.11.005

McPartland, J. M. (2008). Expression of the endocannabinoid system in fibroblasts and myofascial tissues. *Journal of Bodywork and Movement Therapies, 12*(2), 169-182. doi:10.1016/j.jbmt.2008.01.004

McPartland, J. M., Giuffrida, A., King, J., Skinner, E., Scotter, J., & Musty, R. E. (2005). Cannabimimetic effects of osteopathic manipulative treatment. *Journal of the American Osteopathic Association, 105*(6), 283-291.

Meert, G. (2012). Fluid dynamics in fascial tissues *Fascia: The Tensional Network of the Human Body* (Vol. 1, pp. 177-182). Edinburgh: Churchill Livingston.

Meltzer, K. R., Cao, T. V., Schad, J. F., King, H., Stoll, S. T., & Standley, P. R. (2010). In vitro modeling of repetitive motion injury and myofascial release. *Journal of Bodywork and Movement Therapies, 14*(2), 162-171. doi:10.1016/j.jbmt.2010.01.002

Meltzer, K. R., & Standley, P. R. (2007). Modeled repetitive motion strain and indirect osteopathic manipulative techniques in regulation of human fibroblast proliferation and interleukin secretion. *Journal of the American Osteopathic Association, 107*(12), 527-536.

Micera, A., Vigneti, E., Pickholtz, D., Reich, R., Pappo, O., Bonini, S., . . . Levi-Schaffer, F. (2001). Nerve growth factor displays stimulatory effects on human skin and lung fibroblasts, demonstrating a direct role for this factor in tissue repair. *Proceedings of the National Academy of Sciences U S A, 98*(11), 6162-6167. doi:10.1073/pnas.101130898

Minasny, B. (2009). Understanding the process of fascial unwinding. *International Journal of Therapeutic Massage & Bodywork, 2*(3), 10-17.

Mudera, V. C., Pleass, R., Eastwood, M., Tarnuzzer, R., Schultz, G., Khaw, P., . . . Brown, R. A. (2000). Molecular responses of human dermal fibroblasts to dual cues: contact guidance and mechanical load. *Cell Motility and the Cytoskeleton, 45*(1), 1-9. doi:10.1002/(SICI)1097-0169(200001)45:1<1::AID-CM1>3.0.CO;2-J

Ng, C. P., Hinz, B., & Swartz, M. A. (2005). Interstitial fluid flow induces myofibroblast differentiation and collagen alignment in vitro. *Journal of Cell Science, 118*(Pt 20), 4731-4739. doi:10.1242/jcs.02605

O'Connell, J. A. (2011). *Myofascial Release Approach* (Third Edition ed.). Baltimore, MD: Williams & Wilkins.

Pellegrin, S., & Mellor, H. (2007). Actin stress fibres. *Journal of Cell Science, 120*(Pt 20), 3491-3499. doi:10.1242/jcs.018473

Pohl, H. (2010). Changes in the structure of collagen distribution in the skin caused by a manual technique. *Journal of Bodywork and Movement Therapies, 14*(1), 27-34. doi:10.1016/j.jbmt.2008.06.001

Powell, D. W., Mifflin, R. C., Valentich, J. D., Crowe, S. E., Saada, J. I., & West, A. B. (1999). Myofibroblasts. I. Paracrine cells important in health and disease. *American Journal of Physiology, 277*(1 Pt 1), C1-9.

Roman, M., Chaudhry, H., Bukiet, B., Stecco, A., & Findley, T. W. (2013). Mathematical analysis of the flow of hyaluronic acid around fascia during manual therapy motions. *Journal of the American Osteopathic Association, 113*(8), 600-610. doi:10.7556/jaoa.2013.021

Sandbo, N., & Dulin, N. (2011). Actin cytoskeleton in myofibroblast differentiation: ultrastructure defining form and driving function. *Translational Research, 158*(4), 181-196. doi:10.1016/j.trsl.2011.05.004

Schleip, R., Duerselen, L., Vleeming, A., Naylor, I. L., Lehmann-Horn, F., Zorn, A., . . . Klingler, W. (2012). Strain hardening of fascia: static stretching of dense fibrous connective tissues can induce a temporary stiffness increase accompanied by enhanced matrix hydration. *Journal of Bodywork and Movement Therapies, 16*(1), 94-100. doi:10.1016/j.jbmt.2011.09.003

Schleip, R., Klingler, W., & Lehmann-Horn, F. (2005). Active fascial contractility: Fascia may be able to contract in a smooth muscle-like manner and thereby influence musculoskeletal dynamics. *Medical Hypotheses, 65*(2), 273-277. doi:10.1016/j.mehy.2005.03.005

Schnabl, B., Kweon, Y. O., Frederick, J. P., Wang, X. F., Rippe, R. A., & Brenner, D. A. (2001). The role of Smad3 in mediating mouse hepatic stellate cell activation. *Hepatology, 34*(1), 89-100. doi:10.1053/jhep.2001.25349

Serini, G., Bochaton-Piallat, M. L., Ropraz, P., Geinoz, A., Borsi, L., Zardi, L., & Gabbiani, G. (1998). The fibronectin domain ED-A is crucial for myofibroblastic phenotype induction by transforming growth factor-beta1. *The Journal of Cell Biology, 142*(3), 873-881.

Serini, G., & Gabbiana, G. (1996). Modulation of alpha-smooth muscle actin expression in fibroblasts by transforming growth factor-beta isoforms: an in vivo and in vitro study. *Wound Repair and Regeneration, 4*(2), 278-287. doi:10.1046/j.1524-475X.1996.40217.x

Shephard, P., Martin, G., Smola-Hess, S., Brunner, G., Krieg, T., & Smola, H. (2004). Myofibroblast differentiation is induced in keratinocyte-fibroblast co-cultures and is antagonistically regulated by endogenous transforming growth factor-beta and interleukin-1. *The American Journal of Pathology, 164*(6), 2055-2066.

Shi-Wen, X., Chen, Y., Denton, C. P., Eastwood, M., Renzoni, E. A., Bou-Gharios, G., . . . Abraham, D. J. (2004). Endothelin-1 promotes myofibroblast induction through the

ETA receptor via a rac/phosphoinositide 3-kinase/Akt-dependent pathway and is essential for the enhanced contractile phenotype of fibrotic fibroblasts. *Molecular Biology of the Cell, 15*(6), 2707-2719. doi:10.1091/mbc.E03-12-0902

Steward, R. L., Cheng, C. M., Ye, J. D., Bellin, R. M., & LeDuc, P. R. (2011). Mechanical stretch and shear flow induced reorganization and recruitment of fibronectin in fibroblasts. *Scientific Reports, 1*, 147. doi:10.1038/srep00147

Swaney, J. S., Roth, D. M., Olson, E. R., Naugle, J. E., Meszaros, J. G., & Insel, P. A. (2005). Inhibition of cardiac myofibroblast formation and collagen synthesis by activation and overexpression of adenylyl cyclase. *Proceedings of the National Academy of Sciences U S A, 102*(2), 437-442. doi:10.1073/pnas.0408704102

Swanson, R. L. (2013). Biotensegrity: a unifying theory of biological architecture with applications to osteopathic practice, education, and research--a review and analysis. *Journal of the American Osteopathic Association, 113*(1), 34-52.

Tamariz, E., & Grinnell, F. (2002). Modulation of fibroblast morphology and adhesion during collagen matrix remodeling. *Molecular Biolology of the Cell, 13*(11), 3915-3929. doi:10.1091/mbc.E02-05-0291

Tomasek, J. J., Gabbiani, G., Hinz, B., Chaponnier, C., & Brown, R. A. (2002). Myofibroblasts and mechano-regulation of connective tissue remodelling. *Nature Reviews Molecular Cell Biology, 3*(5), 349-363. doi:10.1038/nrm809

Tozzi, P. (2012). Selected fascial aspects of osteopathic practice. *Journal of Bodywork and Movement Therapies, 16*(4), 503-519. doi:10.1016/j.jbmt.2012.02.003

Tozzi, P. (2015). A unifying neuro-fasciagenic model of somatic dysfunction - Underlying mechanisms and treatment - Part II. *Journal of Bodywork and Movement Therapies, 19*(3), 526-543. doi:10.1016/j.jbmt.2015.03.002

Tozzi, P., Bongiorno, D., & Vitturini, C. (2011). Fascial release effects on patients with non-specific cervical or lumbar pain. *Journal of Bodywork and Movement Therapies, 15*(4), 405-416. doi:10.1016/j.jbmt.2010.11.003

Treffer, K., Ehrenfeuchter, W., & Cymet, T. (2011). Glossary of Osteopathic Terminology. Revised 2011. Retrieved from http://www.aacom.org/news-and-events/publications/glossary-of-osteopathic-terminology

Vaughan, M. B., Howard, E. W., & Tomasek, J. J. (2000). Transforming growth factor-beta1 promotes the morphological and functional differentiation of the myofibroblast. *Experimental Cell Research, 257*(1), 180-189. doi:10.1006/excr.2000.4869

Vogel, V., & Sheetz, M. (2006). Local force and geometry sensing regulate cell functions. *Nature Reviews Molecular Cell Biology, 7*(4), 265-275. doi:10.1038/nrm1890

Wang, N., Tytell, J. D., & Ingber, D. E. (2009). Mechanotransduction at a distance: mechanically coupling the extracellular matrix with the nucleus. *Nature Reviews Molecular Cell Biology, 10*(1), 75-82. doi:10.1038/nrm2594

Werner, S., & Grose, R. (2003). Regulation of wound healing by growth factors and cytokines. *Physiological Reviews, 83*(3), 835-870. doi:10.1152/physrev.00031.2002

Wipff, P. J., & Hinz, B. (2009). Myofibroblasts work best under stress. *Journal of Bodywork and Movement Therapies, 13*(2), 121-127. doi:10.1016/j.jbmt.2008.04.031

Wipff, P. J., Rifkin, D. B., Meister, J. J., & Hinz, B. (2007). Myofibroblast contraction activates latent TGF-beta1 from the extracellular matrix. *The Journal of Cell Biology, 179*(6), 1311-1323. doi:10.1083/jcb.200704042

Worthington, J. J., Klementowicz, J. E., & Travis, M. A. (2011). TGFβ: a sleeping giant awoken by integrins. *Trends in Biochemical Sciences, 36*(1), 47-54. doi:10.1016/j.tibs.2010.08.002

Yang, G., Im, H. J., & Wang, J. H. (2005). Repetitive mechanical stretching modulates IL-1beta induced COX-2, MMP-1 expression, and PGE2 production in human patellar tendon fibroblasts. *Gene, 363*, 166-172. doi:10.1016/j.gene.2005.08.006

Yeung, T., Georges, P. C., Flanagan, L. A., Marg, B., Ortiz, M., Funaki, M., . . . Janmey, P. A. (2005). Effects of substrate stiffness on cell morphology, cytoskeletal structure, and adhesion. *Cell Motility and the Cytoskeleton, 60*(1), 24-34. doi:10.1002/cm.20041

Zein-Hammoud, M., & Standley, P. R. (2015). Modeled Osteopathic Manipulative Treatments: A Review of Their in Vitro Effects on Fibroblast Tissue Preparations. *Journal of the American Osteopathic Association, 115*(8), 490-502. doi:10.7556/jaoa.2015.103

Zilberberg, L., Todorovic, V., Dabovic, B., Horiguchi, M., Couroussé, T., Sakai, L. Y., & Rifkin, D. B. (2012). Specificity of latent TGF-β binding protein (LTBP) incorporation into matrix: role of fibrillins and fibronectin. *Journal of Cellular Physiology, 227*(12), 3828-3836. doi:10.1002/jcp.24094

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ABBREVIATIONS

BLT	balanced ligamentous technique
CB2	cannabinoid receptor 2
CB1	cannabinoidreceptor 2
CT	connective tissue
EC-A	exclusion criteria-A
EC-B	exclusion criteria-B
EC-C	exclusion criteria-C
ECM	extra cellular matrix
FU	fascial unwinding
FB	fibroblast
FN	fibronectin
FA	focal adhesions
IC-A	inclusion criteria-A
IC-B	inclusion criteria-B
IC-C	inclusion criteria-C
IL	interleukin
LAP	latency associated pro-peptide
LTBP	latent TGF-β1 binding protein
MMP	matrix metalloproteinase
MFR	myofascial release
MFB	myofibroblast
MLC	myosin light chain
MLCK	myosin light chain kinase
OMTh	osteopathic manipulative therapy
OMT	osteopathic manipulative treatment
OM	osteopathic medicine
PAI-1	plasminogen activator inhibitor1
PDGF	platelet- derived growth factor
CS	strain & counterstrain
TIMP	tissue inhibitor of metalloproteinase
TGF-β1	transforming growth factor β1
α-SMA	α smooth muscle actin

INDEX A (ARTICLE VERSION)

The potential of osteopathic manipulative therapy in regulating myofibroblast activity

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ABSTRACT

Myofibroblasts are cells that have characteristics of fibroblasts and smooth muscle cells. They play an important role in wound healing because of their ability to contract. When they get deregulated they can lead to hypertrophic scars or diseases like fibrosis, which are often seen in practice of osteopaths. This thesis is based on a literature research. First, factors that lead to myofibroblast differentiation, regulation and termination are elucidated, explained in detail and consequently this is discussed and set in context to known effects of different osteopathic manipulative treatments. In this work factors that are most promising to be able to interact with myofibroblast activity, like the stiffness of the extra cellular matrix and the possible influence on TGF-β1, represent the focus of discussion. Further, the ability of myofibroblasts for mechanosensing and their interaction with the effects of osteopathic manipulative treatments is an important part of this thesis. The limitations are that a lot of literature is based on animal and/or in vitro studies and it is questionable, whether the data is transferable to the in vivo situation without further amendments or not. In conclusion it can be said that theoretically osteopathic manipulative treatments are a promising option to interact with myofibroblasts because of their ability to release tissue tension, but further studies are needed to confirm this in vivo.

Keywords:

Myofibroblast, fibroblast, osteopathic manipulative treatment, manual therapy, myofascial release

Introduction

Myofibroblasts (MFB) were first described in wound healing by Gabbiani in 1971 (1). They have characteristics of fibroblasts(FB) and smooth muscle cells (2). MFBs are important in dermal wound closure and for restoring mechanical stability of injured organs. If their activity becomes deregulated and chronic it can lead to tissue deformation by contracture and impedes organ function (3). These tissue contractures can lead to hypertrophic scars or diseases like organ fibrosis(4).

One of the reasons for MFBs to become activated is mechanical stress (4-7). One aim of osteopathic manipulative therapy (OMTh) is to improve physiologic function and/or support homeostasis that has been altered by somatic dysfunction (8). If tension in the tissue leads to a somatic dysfunction osteopathic manipulative treatments (OMT), like indirect myofascial release, fascial unwinding or indirect methods work in the direction of ease to reduce tissue tension (8).

Scars, hypertrophic scars and the resulting decreased mobility of the tissue are problems that confront osteopaths often in their everyday work. MFBs are significantly involved in wound healing (4) and when they get deregulated, they can lead for example to the formation of hypertrophic scars (3). Until now there is no evidence in literature of the effect of OMT in diseases that are triggered by MFBs.

Methods

The information of this article was gathered by data bank based literature research. The literature research was conducted using the data base PubMed (www.ncbi.nlm.nih.gov/pubmed) and the following journals, Journal of Bodywork and Movement Therapies, Journal of the American Osteopathic Association, Osteopathische Medizin and International Journal of Osteopathic Medicine and www.ostmed-dr.com. The literature research was started in April 2015 and repeatedly updated until January 2016.

Key words: myofibroblast, fibroblast, myofibroblast and force, osteopathic medicine (OM), osteopathic manipulative treatment (OMT), osteopathic manipulative therapy (OMTh), myofascial release (MFR), fascial unwinding (FU), indirect method, tissue tension, tissue stiffness, connective tissue, extracellular matrix (ECM), effect, fascia, manual therapy

The keywords were used individually as well as in combination using the operator “and”.

Myofibroblasts

MFBs are fibroblast-like cells that express α-smooth muscle actin (α-SMA) and play an important role in proliferative and remodeling phases of wound healing in producing extracellular matrix (ECM), including collagen (9).

Structure

MFBs are characterized by developing contractile structures, which are represented by stress fibers that contain bundles of actin microfilaments with associated contractile proteins such as non-muscle myosin and the extensive endoplasmatic reticulum of synthetically active fibroblasts (1, 4, 7). Actin stress fibers display a broad functionality in MFB biology. They are involved in contraction, focal adhesion maturation, ECM reorganization as well as transducing mechanical force into biochemical signals. Further they play an important role in the transcriptional control of genes, which are involved in locomotion, contraction and matrix reorganization and the localized regulation of messenger RNA translation. Special to the MFB in comparison to the FB is that the large bundles of microfilaments run parallel with the long axis of the cell (10). Further, MFBs are directly connected to each other by gap junctions (11).

Differentiation

The development of the differentiated MFB depends on mechanical stress that develops within a given tissue and the local expression of growth factors, e. g. TGF-β1. Both factors induce an accumulation of α-SMA-containing stress fibers and other characteristics of the MFB phenotype, like enhanced or newly acquired cell contraction, migration, proliferation, cytokine production, ECM secretion and ECM degradation (4, 12). Tomasek and co-workers therefore postulate the MFB differentiation as a positive feedback loop in which tension facilitates TGF-β1 production and/or activation of α-SMA expression, which in return increases force production and tension development. These relations might be important for the continued formation and sustained function of the MFBs (4).

Mechanical aspects

Focal adhesion complexes in MFBs are fixing points to the matrix. They provide a stable linkage from matrix to cell in order to transmit intracellular contractile forces to the surrounding matrix and therefore tissue contraction can occur (10). The MFB has a mechano-transduction system in order to generate force by stress fibers that can be transmitted to the surrounding

ECM and therefore extracellular mechanical signals can be transduced into intracellular signals.

Contraction, function and termination

MFB contraction is regulated by the Rho/Rho kinase (13, 14). This contractile activity of the MFBs together with ECM synthesis and degradation leads to connective tissue remodeling. This results in irreversible and long contractures in a process that can last weeks, months or even years (15). Myofibroblasts play an important role in wound healing. They are responsible for wound contraction, which has positive and negative effects. It narrows the wound margins, which leads to wound closure, whereas when it gets excessive and causes formation of undesirable contracture or scarring this leads to cosmetic and functional problems (16). Stress release induces MFB apoptosis (11, 17). Also increased formation of specific cell-cell contacts that are accumulated in late granulation tissue and show a decrease of the expression of α-SMA and de-differentiate into α-SMA negative fibroblasts can start apoptosis (11).

Osteopathic manipulative treatment and MFBs

Osteopathic manipulative treatment (OMT) is defined as the therapeutic application of manually guided forces by an osteopath to improve physiologic function and/or support homeostasis that has been altered by somatic dysfunction (8). All OMT techniques have in common that they were designed to bring change in cellular function by addressing affected or at least associated tissues via biomechanical stimuli (18).

Aspects of MFBs that can be influenced by OMT

Mechanotransduction and focal adhesions

The fact that cells are able to react to external forces (environmental sensing) is important and interesting for osteopaths or manual therapists, because it can be a basis on how OMTs are integrated in the body and lead to reactions. Environmental sensing, on cellular level is complex. The most important factor for sensing are focal adhesions in the cell membrane. They connect the cytoskeleton of the cell (inside) to the ECM (outside) via special integrins. Different cells have different integrins in their membrane, which are activated by different stimuli and activate different processes within the cell as a respond.

Amongst other factors, mechanical force can influence the way the focal adhesions are assembled, which governs the reaction of the cell. Mechanical force is the part that is very likely altered by OMTs. From this point of view OMT could interact with MFBs.

TGF-β1

TGF-β1 is crucial for the differentiation of the MFB and it can be decreased by passive stretch (19), this indicates that this technique is a possible way to influence MFBs. Although passive stretch is not a classical OMT, while working manually with tissues in different ways, the tissue is likely to be stretched. It might be good to know this effect when dealing with patients with hypertrophic scars or fibrosis, because duration and timing of the strain is important for the effect. The authors proposed a model of healing of connective tissue where, because of passive stretch, the soluble TGF-β1 levels are reduced, which leads to less collagen deposition and reduced tissue adhesion. This would mean a decrease in the fibrotic response (19). These findings are based on in vitro and in vivo mouse tissue experiments, further studies on humans are needed.

Tissue stiffness

The rigidity or stiffness of the ECM is a crucial factor for MFB differentiation (4). Many manual techniques, including OMTs like MFR, FU, Oscillations, Strain and Counterstrain, Balanced ligamentous technique and many more, claim to be able to reduce tissue stiffness. Here, the problem is to find numbers of the effects these techniques have on the tissue to compare them to the numbers that are available for MFB differentiation. One can only speculate that the effect of OMT on tissue stiffness can influence MFB behavior.

Discussion

Object of this article was to find out whether it is possible to influence MFBs by OMT or not. It is common knowledge that OMTs or fascial techniques are able to reduce the tension in the tissue but this is not supported by studies that investigated the tissue stiffness before and after treatment. There are no numbers in how much a technique is able to reduce the tension in a given tissue. This makes it impossible to compare OMT effects to the numbers that are available on the tissue stiffness that is required for FBs to turn into MFBs. This would be necessary to propose that OMT can influence mechanical tension and thereby MFB differentiation.

Aim of treatment

First it needs to be clarified whether the goal is to improve wound healing or to treat fibrosis. This is important because for wound healing MFBs are “good”. In order to use the beneficial properties of MFBs, the goal would be to activate them. Apparently, wound healing is highly dependent on duration and magnitude of MFR strain. Lower magnitude and longer duration (more than two minutes) of strain seem to be most effective in reducing wound size. These findings are based on an in vitro model of three dimensional bioengineered tendons (20). Further clinical studies are necessary.

Larger magnitude of strains resulted in enlarging wound size and exacerbating the site of injury (20) could speculatively lead to the assumption that this would deactivate MFBs and matrix remodeling and therefore probably be beneficial for treatment of fibrosis. In fibrosis or hypertrophic scars the action of MFBs gets excessive and leads to tissue deformation (4). For treating these conditions, the aim is to induce apoptosis by interacting with the factors that are known to lead to MFB termination, like release of the tension in the tissue. As discussed in the chapter about tissue stiffness, there are several available.

Biotensegrity

There are different models that try to explain the effects of OMTs. One of them is the Biotensegrity model, which explains how MFBs could be influenced by externally applied mechanical stress. It tries to show how mechanical stress, applied to the whole organism, can be transmitted to individual cells and transduced into a biochemical signal (21). Swanson gives a detailed review on the biotensegrity model and its application in osteopathic manipulative medicine (22).

Swanson proposes that biotensegrity is the vital missing link in understanding fascial architecture and how mechanical forces can lead to fascial restrictions and their release, which is claimed to be done by OMTs. In this model the cellular components of the tissue, meaning FBs and MFBs, are important (22). According to the work of Paul Standley (23-26) and Helene Langevin (19, 27-29) FBs of irregular connective tissue are pre-stressed biotensegrity cells. They are linked hierarchically with the ECM and through changes in gene expression they are capable of responding to mechanical forces (22).

Limitations

Based on the available literature, this article has its limitations in the discussion of some aspects, where further research is necessary. A lot of the information is based on in vitro studies and in vitro animal studies. It is therefore difficult to say whether it is possible to translate these findings into in vivo studies on humans or not.

Conclusion

This article critically summarizes, discusses and evaluates available literature on the possibility to use OMTs to interact with MFBs. A closer look at MFBs shows that the main factors to activate them are TGF-β1 and mechanical stress. Especially the fact that they can be influenced by mechanical stress makes them interesting for OMTs. MFBs can sense the tension in the surrounding tissue and react to it by mechanosensing. Different OMTs are able to interact with the tension in the tissue. Theoretically it should be possible that via the connection of tissue tension MFBs and OMT interact. Techniques that are likely to be beneficial are techniques that release the tension in the tissues, for example fascial techniques like MFR or FU.

The practical use of the findings of this article is to find a scientific basis for treating conditions like wound healing, hypertrophic scars or fibrosis because in these conditions MFBs are involved (3, 4). Osteopaths are commonly confronted with the treatment of hypertrophic scars and fibrosis as they are often the cause of somatic dysfunction and can lead to a broad variety of symptoms. From the theoretical point of view, it would be beneficial to treat hypertrophic scars or fibrosis with OMTs that focus on relaxing the tissue. What has been found out so far is that duration magnitude and direction of strain make a difference in cell behavior. It can be said is that apparently brief, moderate, balanced, static or slow cyclic strains, appropriately applied to the fascia, may be sensed at a cellular level and transduced in normalizing tissue structure and function (30).

Further studies to verify the suggestions made here would be necessary. One thing to investigate would be the effect of OMT on tissue stiffness.

Disclosure

The author has no personal financial or institutional interest in any of the drugs, materials, or devices described in this article.

References

1. Gabbiani G, Ryan GB, Majne G. Presence of modified fibroblasts in granulation tissue and their possible role in wound contraction. Experientia. 1971;27(5):549-50. PubMed PMID: 5132594.

2. Desmoulière A, Chaponnier C, Gabbiani G. Tissue repair, contraction, and the myofibroblast. Wound Repair Regen. 2005;13(1):7-12. doi: 10.1111/j.1067-1927.2005.130102.x. PubMed PMID: 15659031.

3. Hinz B. The myofibroblast: paradigm for a mechanically active cell. J Biomech. 2010;43(1):146-55. doi: 10.1016/j.jbiomech.2009.09.020. PubMed PMID: 19800625.

4. Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C, Brown RA. Myofibroblasts and mechano-regulation of connective tissue remodelling. Nat Rev Mol Cell Biol. 2002;3(5):349-63. doi: 10.1038/nrm809. PubMed PMID: 11988769.

5. Hinz B. Masters and servants of the force: the role of matrix adhesions in myofibroblast force perception and transmission. Eur J Cell Biol. 2006;85(3-4):175-81. doi: 10.1016/j.ejcb.2005.09.004. PubMed PMID: 16546559.

6. Hinz B, Phan SH, Thannickal VJ, Prunotto M, Desmoulière A, Varga J, et al. Recent developments in myofibroblast biology: paradigms for connective tissue remodeling. Am J Pathol. 2012;180(4):1340-55. doi: 10.1016/j.ajpath.2012.02.004. PubMed PMID: 22387320; PubMed Central PMCID: PMCPMC3640252.

7. Gabbiani G. The evolution of the myofibroblast concept: a key cell for wound healing and fibrotic diseases. G Gerontol. 2004;52(5):280-2.

8. Treffer K, Ehrenfeuchter W, Cymet T. Glossary of Osteopathic Terminology 5550 Friendship Blvd Suite 550 Chevy Chase, MD 20815: American Association of Colleges of Osteopathic Medicine; 2011 [cited 2015 11.06.2015]. Revised 2011:[Available from: http://www.aacom.org/news-and-events/publications/glossary-of-osteopathic-terminology.

9. Hinz B, Phan SH, Thannickal VJ, Galli A, Bochaton-Piallat ML, Gabbiani G. The myofibroblast: one function, multiple origins. Am J Pathol. 2007;170(6):1807-16. doi: 10.2353/ajpath.2007.070112. PubMed PMID: 17525249; PubMed Central PMCID: PMCPMC1899462.

10. Sandbo N, Dulin N. Actin cytoskeleton in myofibroblast differentiation: ultrastructure defining form and driving function. Transl Res. 2011;158(4):181-96. doi: 10.1016/j.trsl.2011.05.004. PubMed PMID: 21925115; PubMed Central PMCID: PMCPMC3324184.

11. Hinz B. Formation and function of the myofibroblast during tissue repair. J Invest Dermatol. 2007;127(3):526-37. doi: 10.1038/sj.jid.5700613. PubMed PMID: 17299435.

12. Hinz B. Myofibroblasts. Exp Eye Research. 2016;142:56-70. doi: 10.1016/j.exer.2015.07.009. PubMed PMID: 26192991.

13. Bogatkevich GS, Tourkina E, Abrams CS, Harley RA, Silver RM, Ludwicka-Bradley A. Contractile activity and smooth muscle alpha-actin organization in thrombin-induced human lung myofibroblasts. Am J Physiol Lung Cell Mol Physiol. 2003;285(2):L334-43. doi: 10.1152/ajplung.00417.2002. PubMed PMID: 12665468.

14. Katoh K, Kano Y, Amano M, Onishi H, Kaibuchi K, Fujiwara K. Rho-kinase--mediated contraction of isolated stress fibers. J Cell Biol. 2001;153(3):569-84. PubMed PMID: 11331307; PubMed Central PMCID: PMCPMC2190572.

15. Follonier Castella L, Gabbiani G, McCulloch CA, Hinz B. Regulation of myofibroblast activities: calcium pulls some strings behind the scene. Exp Cell Res. 2010;316(15):2390-401. doi: 10.1016/j.yexcr.2010.04.033. PubMed PMID: 20451515.

16. Li B, Wang JH. Fibroblasts and myofibroblasts in wound healing: force generation and measurement. J Tissue Viability. 2011;20(4):108-20. doi: 10.1016/j.jtv.2009.11.004. PubMed PMID: 19995679; PubMed Central PMCID: PMCPMC2891362.

17. Hinz B, Gabbiani G. Mechanisms of force generation and transmission by myofibroblasts. Curr Opin Biotechnol. 2003;14(5):538-46. PubMed PMID: 14580586.

18. Johnson SM, Kurtz ME. Osteopathic manipulative treatment techniques preferred by contemporary osteopathic physicians. J Am Osteopath Assoc. 2003;103(5):219-24. PubMed PMID: 12776762.

19. Bouffard NA, Cutroneo KR, Badger GJ, White SL, Buttolph TR, Ehrlich HP, et al. Tissue stretch decreases soluble TGF-beta1 and type-1 procollagen in mouse subcutaneous connective tissue: evidence from ex vivo and in vivo models. J Cell Physiol. 2008;214(2):389-95. doi: 10.1002/jcp.21209. PubMed PMID: 17654495; PubMed Central PMCID: PMCPMC3065715.

20. Cao TV, Hicks MR, Zein-Hammoud M, Standley PR. Duration and magnitude of myofascial release in 3-dimensional bioengineered tendons: effects on wound healing. J Am Osteopath Assoc. 2015;115(2):72-82. doi: 10.7556/jaoa.2015.018. PubMed PMID: 25637613.

21. Chen CS, Ingber DE. Tensegrity and mechanoregulation: from skeleton to cytoskeleton. Osteoarthritis Cartilage. 1999;7(1):81-94. doi: 10.1053/joca.1998.0164. PubMed PMID: 10367017.

22. Swanson RL. Biotensegrity: a unifying theory of biological architecture with applications to osteopathic practice, education, and research--a review and analysis. J Am Osteopath Assoc. 2013;113(1):34-52. PubMed PMID: 23329804.

23. Dodd JG, Good MM, Nguyen TL, Grigg AI, Batia LM, Standley PR. In vitro biophysical strain model for understanding mechanisms of osteopathic manipulative treatment. J Am Osteopath Assoc. 2006;106(3):157-66. PubMed PMID: 16585384.

24. Eagan TS, Meltzer KR, Standley PR. Importance of strain direction in regulating human fibroblast proliferation and cytokine secretion: a useful in vitro model for soft tissue injury and manual medicine treatments. J Manipulative Physiol Ther. 2007;30(8):584-92. doi: 10.1016/j.jmpt.2007.07.013. PubMed PMID: 17996550.

25. Meltzer KR, Cao TV, Schad JF, King H, Stoll ST, Standley PR. In vitro modeling of repetitive motion injury and myofascial release. J Bodyw Mov Ther. 2010;14(2):162-71. doi: 10.1016/j.jbmt.2010.01.002. PubMed PMID: 20226363; PubMed Central PMCID: PMCPMC2853774.

26. Meltzer KR, Standley PR. Modeled repetitive motion strain and indirect osteopathic manipulative techniques in regulation of human fibroblast proliferation and interleukin secretion. J Am Osteopath Assoc. 2007;107(12):527-36. PubMed PMID: 18178762.

27. Langevin HM, Storch KN, Cipolla MJ, White SL, Buttolph TR, Taatjes DJ. Fibroblast spreading induced by connective tissue stretch involves intracellular redistribution of alpha- and beta-actin. Histochem Cell Biol. 2006;125(5):487-95. doi: 10.1007/s00418-005-0138-1. PubMed PMID: 16416024.

28. Langevin HM, Storch KN, Snapp RR, Bouffard NA, Badger GJ, Howe AK, et al. Tissue stretch induces nuclear remodeling in connective tissue fibroblasts. Histochem Cell Biol. 2010;133(4):405-15. doi: 10.1007/s00418-010-0680-3. PubMed PMID: 20237796; PubMed Central PMCID: PMCPMC2880391.

29. Langevin HM, Bouffard NA, Badger GJ, Iatridis JC, Howe AK. Dynamic fibroblast cytoskeletal response to subcutaneous tissue stretch ex vivo and in vivo. Am J Physiol Cell Physiol. 2005;288(3):C747-56. doi: 10.1152/ajpcell.00420.2004. PubMed PMID: 15496476.

30. Tozzi P. A unifying neuro-fasciagenic model of somatic dysfunction - Underlying mechanisms and treatment - Part II. J Bodyw Mov Ther. 2015;19(3):526-43. doi: 10.1016/j.jbmt.2015.03.002. PubMed PMID: 26118526.