THE PATHOPHYSIOLOGY OF POSTOPERATIVE PERITONEAL ADHESIONS- OSTEOPATHY AS A TREATMENT OPTION?

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Iris Lackner

Department for Health Science and Biomedicine

at Danube University Krems

Advisor: Dr. Astrid Grant Hay

Advisor: Dr. Erich Mayer-Fally   Vienna, 17.05.2017

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ABSTRACT

Peritoneal adhesions are pathological bonds of fibrous tissue that can develop between the omentum, intraabdominal or pelvic organs and the abdominal wall. They can form after any kind of peritoneal irritation, that is produced by inflammation, trauma or most commonly after abdominopelvic surgery. The development of adhesions can be considered the pathological part of wound healing. The concerned patients often suffer from severe negative impacts, such as abdominopelvic pain, posture abnormalities and restrictions, digestion problems, small bowel obstruction, dyspareunia or infertility. If untreated, these symptoms usually persist throughout life and therefore pose a serious impairment in daily activities and a dramatic reduction of life quality. Current therapies mostly comprise several pharmaceutical or operative treatments. Even though there has been a lot of research on these kinds of therapies, none has yet been proven to be perfectly successful. Further adhesiolytic surgeries have even been shown to generate new adhesions. Considering this, osteopathic manipulative treatment (OMT) seems to be an interesting and promising non-invasive treatment option.

This thesis is based on literature research and inquires the possible effects of several OMT techniques, including their cellular effects, in the treatment of peritoneal adhesions. A variety of techniques will be reviewed and discussed to finally conclude on the most promising results. Cellular mechanisms and influencing factors of adhesion formation will be presented, in order to link these mechanisms to an appropriate therapeutic stimulus.

Keywords:

Peritoneal adhesions, mesothelial/ peritoneal wound healing, osteopathic manipulative treatment and cellular effects, peritoneal adhesions and manual therapy, peritoneal adhesions and manual treatment

ABSTRACT

Peritoneale Adhäsionen sind pathologische Strukturen aus fibrösem Gewebe, die sich zwischen dem Omentum, Bauch- oder Beckenorganen und der Bauchdecke bilden. Sie können sich nach Irritationen des Peritoneums entwickeln, die beispielweise durch Entzündungen, Traumata oder nach Operationen im Bauch- oder Beckenbereich hervorgerufen werden. Die Entstehung von Adhäsionen kann als pathologische Form der Wundheilung betrachtet werden. Die betroffenen Patienten leiden oft an gravierenden Symptomen, wie chronischen Bauch- oder Beckenschmerzen, Haltungsabnormitäten und -einschränkungen, Verdauungsproblemen, Darmverschluss, Dyspareunie oder Infertilität. Diese Beschwerden bestehen unbehandelt oft lebenslänglich und stellen somit für die Betroffenen eine beträchtliche Einschränkung in ihren täglichen Aktivitäten und eine drastische Verminderung von Lebensqualität dar. Gängige Therapiekonzepte sind diverse Pharmazeutika und operative Eingriffe zur Entfernung. Obwohl hinsichtlich dieser Therapiearten viel Forschung betrieben wird, hat sich bis jetzt noch keine als vollständig erfolgreich erwiesen. Weitere adhäsiolytische Eingriffe führen erwiesenermaßen sogar zur Entstehung neuer Adhäsionen. In Anbetracht dessen scheint die osteopathisch manipulative Behandlung eine interessante und vielversprechende, non-invasive Methode zu sein.

Diese These basiert auf einer Literaturrecherche und hinterfragt die möglichen Auswirkungen osteopathischer Techniken, einschließlich der zellulären Effekte, in der Behandlung peritonealer Adhäsionen. Eine Reihe von Techniken wird rezensiert und diskutiert werden, um dann einen Rückschluss auf die Vielversprechendsten ziehen zu können. Außerdem werden die zellulären Mechanismen und einflussnehmende Faktoren in Bezug auf Adhäsionen beschrieben, um eine Verknüpfung zu einem geeigneten therapeutischen Stimulus herstellen zu können.

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Schlüsselwörter:

Peritoneale Adhäsionen, mesotheliale/ peritoneale Wundheilung, osteopathisch manipulative Behandlung und zelluläre Effekte, peritoneale Adhäsionen und manuelle Therapie, peritoneale Adhäsionen und manuelle Behandlung

CONTENTS

1   Introduction

Intraabdominal or pelvic adhesions arise from mesothelial damage as a consequence of aberrant wound healing after peritoneal irritation, caused by inflammation, trauma or most common after abdominopelvic surgery with further influence from a variety of other factors (Brüggmann et al, 2010; Arung, Meurisse, & Detry, 2011). Their formation may be considered the pathological part of healing. Peritoneal wound healing differs from that of other tissues and the formation of adhesions is due to the peritoneum's specific response to injuries (diZerega & Campeau, 2001). Physiologically separated organs become attached to each other or to the abdominal wall, which can lead to major problems such as bowel obstruction, digestive problems, chronic abdominal, vertebral or pelvic pain, dyspareunia, infertility and following adhesiolysis and further operations (Brüggmann et al, 2010; Bove & Chapelle, 2012). The organs must be able to move and glide freely against each other and the abdominal wall to enable a good peristalsis. Adhesion formation after surgery can limit these movements, causing pain and other pathologies listed above (Bove & Chapelle, 2011).

Peritoneal adhesions were first described at a post-mortem examination of a patient with peritoneal tuberculosis in 1836 (Arung et al., 2011). In literature the number of intraabdominal adhesions after abdominal or pelvic surgery varies from 95% (Stanciu & Menzies, 2007), 50% to 100% (Brüggmann et al., 2010) or from 70% to 85% (Molinas, Binda, Manavella, & Koninckx, 2010; Weibel & Majno, 1973) and thereby pose the most frequent and severe post-surgical complication. Postoperative abdominopelvic adhesions are the reason for up to 6% of all re-admissions and the related problems concern all ages, mostly persisting lifelong (Stanciu & Menzies, 2007). Even though there has been a profound progress in surgical techniques over the last years, the burden of adhesion-associated intricacies have stayed the same (Parker et al., 2007). Various clinical and experimental studies have examined peritoneal adhesions' pathophysiology different operation techniques and various pharmalogical treatments for their prevention, but only a restricted amount of literature can be found on the non-operative treatment of arising or existing adhesions (Arung et al., 2011). The treatment of re-operative adhesiolysis remains the leading therapy, although adhesions reform in most cases and further re-operations lead to new scarring and adhesion formation (Parker et al., 2007). With regard to manual treatment, however, no literature has been found to indicate that it has a negative impact or causes any further tissue damage. Therefore, manual treatment poses a very interesting treatment option.

As osteopaths are quite often confronted with adhesion-related symptoms in daily practice, it has to be a main target to optimize peritoneal wound healing conditions and to improve

visceral movements again. Some studies could be found investigating the potential effects of fascial techniques, such as myofascial release (MFR) and fascial unwinding (FU), on tissue restrictions and inflammatory processes. As these techniques are used frequently by osteopaths, they will be reviewed and discussed explicitly (Meltzer et al., 2010).

2   Aim of the thesis

In the course of her osteopathic practice, the author has experienced that therapists are often confronted with problems such as the ones mentioned before and with patients that present themselves with a history of surgeries and the eventually resulting complications. The clinical experience how far-reaching the impact of peritoneal adhesions can be and how effectively an appropriate manual treatment influences adhesion-related symptoms and restrictions, inspired this thesis.

The aim of this thesis is to display the recent results in literature, under consideration of the pathogenesis, the correlating factors and the resulting problems. The author will give an overview of possible manual treatment options, in particular the osteopathic manipulative treatment, in order to provide a theoretical basis on the treatment of peritoneal adhesions.

2.1   Research Question:

Is osteopathic manipulative treatment (OMT) a suitable method to influence the development of postoperative abdominal adhesions and already existing adhesions and have there been specific methods mentioned that seem effective?

2.2   Hypotheses

2.2.1   Hypothesis I

OMT is able to influence both the development of and the already existing postoperative abdominal adhesions.

2.2.2   Hypothesis II

Visceral and fascial techniques, such as myofascial release (MFR) and fascial unwinding (FU) have been noted, and seem to be promising in the treatment of postoperative abdominal adhesions.

3   Methodology

This thesis is a literature review. Keyword-driven literature research was conducted in the international database PubMed (www.ncbi.nlm.nih.gov/pubmed). The thereby found literature was qualitatively analyzed and finally set in context to build up an individual synthesis on the research question.

3.1   Literature research

The research included relevant literature published until April 2017 found in PubMed and the following journals: *World Journal of Gastroenterology, Journal of Surgical Research, Journal of Bodywork and Movement Therapy, Journal of the American Osteopathic Association* and *International Journal of Osteopathic Medicine.*

To date no research work can be found in this field in the Osteopathic Research Web or any of the following databases:

http://www.orcedo.org   Foundation   for   Osteopathic   Research   Continuous
http://www.ncor.org.uk   National   Council   for   Osteopathic
Research http://www.osteopathicresearch.org   Osteopathic
Research Web

3.2   Sorting of data and its analysis

The studies found by the database-driven literature research were sorted and doublets were removed. Consecutively the studies were analyzed and set in context with the research question.

Keywords: Peritoneal adhesions, mesothelial cell, mesothelial/ peritoneal wound healing, (postoperative) abdominal/ peritoneal adhesions and manipulative treatment, abdominal/ peritoneal adhesions and manipulative therapy, abdominal/ peritoneal adhesions and manual therapy/ treatment, abdominal scars and manual therapy/ osteopathic manipulative medicine, tissue changes and manual treatment, tissue tension and manual treatment, osteopathic manipulative treatment and cellular effects, myofascial release and effects, fascial unwinding and effects

The keywords were used separately and combined using the term "AND".

3.3 Inclusion and exclusion criteria

3.3.1 Inclusion criteria (IC)

A.)   Inclusion criterion A (ICA):

Literature that could be found via the listed database, driven by the keywords OR

B.)   Inclusion criterion B (ICB):

Literature that was listed in the references of reviewed articles

3.3.2 Exclusion criteria:

A.) Exclusion criterion A (ECA)

Literature that was found, but not relevant for the thesis OR

B.) Exclusion criterion B (ECB)

The found literature was neither in English nor in German OR

C.) Exclusion criterion C (ECC)

The found literature was not available by purchasing

3.4 Results of literature research

The literature research was conducted to retrieve articles and studies about peritoneal wound healing and adhesion formation and the non-invasive manual treatment of peritoneal or abdominal adhesions. In the first step the keywords were used separately and then in combination. Using the terms peritoneal AND abdominal adhesions 9715 results were shown (November 2016). For the combination postoperative peritoneal adhesions AND treatment 1390 articles were found, whereof most comprised pharmaceutical or surgical treatments. In the next research step therefore the keywords manipulative therapy OR treatment AND manual therapy OR treatment were added. The search using the terms peritoneal adhesions AND manipulative treatment provided no results. Of the total amount of retrieved and used articles 24 doublets were sorted out. In total 140 articles were used for this master thesis, whereof 77 articles were found by the keyword driven literature research and the remaining 63 articles were retrieved via listed references. Figure 1 shows the results of the literature research, including the listed inclusion and exclusion criteria.
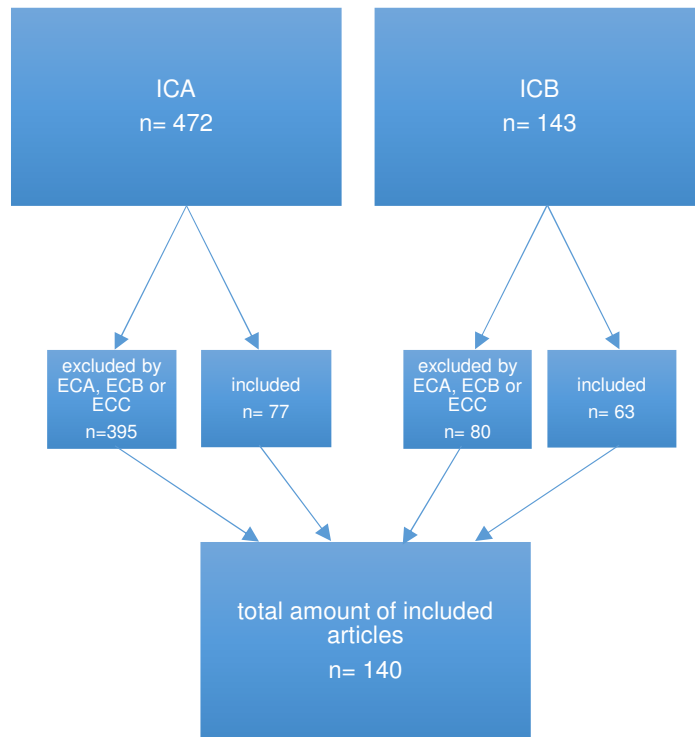
Figure 1 results of literature research

4 Postoperative peritoneal adhesions

4.1 Description and terminology

Peritoneal adhesions are pathological, fibrous tissue bonds that can develop between the omentum, loops of bowel or other intra-abdominal or pelvic organs and the abdominal wall (Arung, Meurisse, & Detry, 2011; Sandoval et al., 2016). These bonds may be a thin film of connective tissue, a thick fibrous bridge containing blood vessels and nerve tissue or a direct contact between two organ surfaces and the peritoneum. Originating between serous membranes they are accompanied by active myofibroblasts, extracellular matrix deposition and amounts of incompletely degraded fibrin (Sandoval et al., 2016). According to the study of Herrick et al. adhesions are not passive scar tissue, but highly cellular and contain dynamic regenerating structures (Herrick et al., 2000). The peritoneal wound healing differs from that of skin, as the entire injured area becomes epithelialized simultaneously and not gradually from the borders as in epidermalization of skin wounds (diZerega & Campeau, 2001).

Considering their etiology, peritoneal adhesions may be classified as congenital or acquired, either post-inflammatory or postoperative (the most frequent) (Brüggmann et al., 2010). This thesis focuses on acquired postoperative peritoneal adhesions.

4.2 Peritoneal structures involved in adhesions

4.2.1 The peritoneum

The peritoneum is the largest serous membrane in the human body and consists of two layers, the parietal and the visceral peritoneum. The inner surface of the abdominal wall is lined by the parietal peritoneum, whereas the visceral peritoneum covers all abdominal and pelvic organs, except the ovary, and integrates with the outer serosal layers of the organs (diZerega & Campeau, 2001; van Baal et al., 2016). It therefore forms a closed sac in male bodies and an open sac in female bodies through the uro-gynaecological tract (J.-J. Duron, 2007).

The parietal peritoneum is sensitive to pressure, pain, temperature, and laceration. Whereas the visceral peritoneum is not receptive for these sensations, it is sensitive to stretch or tension and chemical irritation (diZerega & Campeau, 2001). The peritoneum enables movements of the intraabdominal organs and minimizes friction, enables passive transport of fluids by hydrostatic and osmotic pressure and maintains the homeostasis in the abdominal cavity. The peritoneum participates to a high degree in inflammation, antigen presentation and tissue repair (Steven E Mutsaers, 2004; van Baal et al., 2016). It contributes to fibrotic adhesion formation subsequent to infection and surgery and it also plays an important role in the

development of peritoneal diseases such as endometriosis, mesothelioma and peritoneal carcinomatosis. It is involved in almost every intraabdominal condition and therefore disruption of the balances leads to a variety of symptoms. Recurrent diseases often result in obstructive or paralytic ileus of the bowel, with high morbidity and mortality rates (van Baal et al., 2016).

4.2.1.1 Anatomy of the peritoneum

With a surface of around 10,000 cm^2 in adults the peritoneum can be seen as the biggest human organ. Long microvilli on the apical surface magnify the functional surface area for secretion and absorption (diZerega, 1997; Molinas et al., 2010). The structures of the visceral and the parietal peritoneum are very similar. Their structure is either defined as a single-cell layer of mesothelial cells (Melichar and Freedman, 2002) or as a three-layered tissue, consisting of three layers, the mesothelium, a basal lamina and the submesothelial stroma (Michailova and Usunoff, 2006). The mesothelium and basal lamina are of similar appearance throughout the abdomen, whereas the submesothelial stroma differs in its thickness. The mesothelial cells are sustained by a scaffold of connective tissue (CT) (diZerega & Campeau, 2001). The anatomical nomenclature of peritoneal structures starts at the abdominal cavity and ends at the abdominal wall. This sub-mesothelial connective tissue comprises extra-cellular matrix, which is made of glycoproteins, glycosaminoglycans, proteoglycans, fiber bundles of different collagen types and various cell types, such as scattered fibroblasts, macrophages, mast cells and a diversifying amount of fat (J.-J. Duron, 2007; Eskeland, 1964, 1966; Eskeland & Kjaerheim, 1966; Raftery, 1973b). The peritoneum's functional surface is enlarged by plentiful and long microvilli, which are observable at the upper side of mesothelial cells. The visceral and parietal peritoneum are both covered with a lining of an anti-sticking agent, called surfactant, on their mesothelial sheet (diZerega & Campeau, 2001; J.-J. Duron, 2007; K. N. Michailova, 1995; K. Michailova, Wassilev, & Wedel, 1999).

4.2.1.2 Peritoneal fluid

Physiologically a volume of 5–20 ml of peritoneal fluid is present in the abdominal cavity. The daily production of peritoneal fluid of around one liter is necessary to keep the visceral surfaces moisturized and exchange substances and immune cells between the peritoneal fluid and plasma. The fluid contains many plasma proteins, amongst them fibrinogen which is crucial in the healing and peritoneal adhesion formation process and a rich variety of immune cells, such as macrophages, natural killer cells, lymphocytes, eosinophils, mesothelial cells and mast cells (J.-J. Duron, 2007; Gazvani & Templeton, 2002; van Baal et al., 2016). Moreover, it contains chemical mediators, such as interleukins, interferon-c (ILc), tumor necrosis factor-α (TNF-α), transforming growth factor- β (TGF-β) and vascular endothelial growth factor (VEGF) (J.-J.

Duron, 2007; Maciver, McCall, & James Shapiro, 2011). These cellular mediators and fibrinogen present in the peritoneal fluid have an active and important part in the process of peritoneal healing and adhesion formation (J.-J. Duron, 2007). As serous membranes are receptive to bacterial invasions, the anti-inflammatory role of the peritoneum and its fluid is crucial to prevent infections that may potentially cause a severe peritonitis. Furthermore, the peritoneum's ability to create adhesions is basically a vital function, as bacteria on damaged, leaking serosal walls can cause life-threatening conditions. When the balance between peritoneal fluid secretion and drainage is disrupted, ascites is the consequence, which is an accumulation of fluid in the peritoneal cavity (van Baal et al., 2016).

4.2.1.3 The Mesothelial cell (MC)

4.2.1.3.1 Anatomical description of MCs

Mesothelial cells cover the three serous cavities (pleural, pericardial and peritoneal) and the organs contained within. They form a monolayer of specialized pavement-like cells, which is called the mesothelium. The cells' morphology is either flattened, dome-shaped or cubic, with an approximate diameter of 25μm and a thin basement membrane supported by connective tissue stroma of variable thickness (J.-J. Duron, 2007; Steven E Mutsaers, 2004). Mesothelial cells have round or oval nucleus and contain microtubules and microfilaments, glycogen, vesicles and vacuoles, a few mitochondria, a poorly developed Golgi apparatus and a few rough endoplasmatic reticula (RER). In addition, the cells have a microvillous border with random cilia on their luminous surface. Mesothelial boundaries are twisted with adjacent cells often overlapping. Their cell–cell junctions are highly developed, including tight junctions, adherens junctions, gap junctions and desmosomes. Especially the tight junctions are necessary for the development of cell surface polarity and the maintenance of a semi-permeable diffusion barrier.

The embryological origin of mesothelium is mesodermal tissue and in humans it starts to develop around day 14 of gestation. The cells line the coelomic cavities and their shape changes progressively from round to elongated flattened cells (Steven E Mutsaers, 2004). Although mesothelial cells are derived from mesoderm they express the mesenchymal intermediate filaments vimentin and desmin, but also cytokeratins that are epithelial cell intermediate filaments (Ferrandez- Izquierdo, Navarro-Fos, Gonzalez-Devesa, Gil-Benso, & Llombart-Bosch, 1994).

4.2.1.3.2 Functions of MCs

The main function of the mesothelium is to provide a slippery, non-adhesive and protective secrete to shield the serosal surfaces from abrasion, infection or tumor cell adhesion. This secrete contains glycosaminoglycans, proteoglycans and phospholipids. Moreover, the MCs are able to synthesize cytokines, growth factors, chemokines and matrix components to control inflammation, cell proliferation, differentiation and migration. Mesothelial cells enable the transport of fluid and cells, antigen presentation, inflammation and tissue repair, coagulation and fibrinolysis and therefore regulate the healing process (Molinas et al., 2010). Basically the mesothelium is slow in renewing, with 0.16% to 0.5% of cells undergoing mitosis at any one time, but the mitotic activity can be significantly increased by accurate stimulation. 30% to 80% of the mesothelial cells start to synthetize DNA at the wound margins and the opposing surface within 48 hours after serosal damage. Hereafter cells from the wound borders start to migrate to the wound center and free-floating cells from the serosal fluid get attached and incorporated to the whole wound surface (Steven E Mutsaers, 2004; Sandoval et al., 2016; Yang, Chen, & Lin, 2003).

There are a number of possible origins for the rise of new mesothelial cells, such as transformed peritoneal cells, metaplasia of subperitoneal connective tissue cells, maturing of mesenchymal stem cells or contiguous peritoneum. However, clear identification of the cells' source remains difficult, as it is hard to distinguish between the different cells in their several phases of development (diZerega & Campeau, 2001).

Mesothelial cells are able to change their phenotype, similar to changes in epithelial-to-mesenchymal transition (EMT) (Steven E Mutsaers, 2004). This affects regular tissue repair as well as pathological processes. Under pathological circumstances stimulated mesothelial cells gradually lose their epithelial phenotype and their cytokeratin expression and adopt a fibroblast-like or myofibroblast-like phenotype (Steven E Mutsaers, 2004; Sandoval et al., 2016; Yang et al., 2003). Transforming growth factor-β_1 (TGF-β_1), which is a profibrotic wound mediator, stimulates EMT in mesothelial cells and upregulates smooth muscle actin and type I collagen production via myofibroblast activity (Yang et al., 2003). These aspects state that the mesothelium itself is a possible origin of fibrotic cells during the process of serosal wound healing and during the pathological development of adhesions. Normally the converted cells should be able to regain their epithelial-like phenotype after completed mesothelium restoration (Foley-Comer et al., 2002; Steven E. Mutsaers, 2002). However, under pathological conditions this cell transformation and the impaired healing accompanied by diminished fibrinolytic activity are the origin of serosal adhesions formation, tissue fibrosis and malignant mesothelioma

(Steven E Mutsaers, 2004). In addition, the destruction of the mesothelial layer through trauma or surgery leads to reduced lubrication of the tissues surface, which is another factor enhancing the formation of adhesions (Brüggmann et al., 2010; Maciver et al., 2011).

4.3   The extracellular matrix (ECM)

In the *Collins Dictionary of Medicine* the extracellular matrix is defined as material, which is secreted by cells and fills spaces between the cells in a tissue. It serves as protection for the cells and holds them together (Youngson Robert M., 2004).

On the one hand, the ECM offers passive mechanical support for the cells and therefore its structure and quality is a main factor for the determination of tissue characteristics (Agren & Werthen, 2007). On the other, hand the extracellular matrix is in continuous interaction with cells and provides the media for information transmission, as it connects cells and their cytoplasmic matrices within its network of weaves, struts, adhesives and gels (Agren & Werthen, 2007; S. E. Mutsaers, Bishop, McGrouther, & Laurent, 1997). Collagen is the major element of the ECM in most tissues. Of the at least 18 different collagen types, the fundamental interstitial collagen types are I, II and III. They build a system of fibers and connect cells with each other and other structures. Collagen type IV is the main element in basement membranes (Mayne & Brewton, 1993; S. E. Mutsaers, Bishop, et al., 1997).

ECM molecules act in concert with integrins and growth factors have the ability to transduce signals, which govern the cell processes of wound healing. The ECM is composed of several elements, which implement potent influences on cell structure and function, as the state of the ECM affects integrin expression, which then has effects on the cell's phenotype. Events such as cell adhesions, proliferation, differentiation and cell growth, as well as synthesis of ECM itself, are dependent on these interferences. Deregulation of cell-ECM interactions might be an actuator for the development of many disorders, as delayed wound healing might be due to deficient ECM remodeling.

Cell-cell and cell-matrix contacts are established through adhesion/ ECM receptor molecules on the cell surfaces. These connections induce signals for migration, differentiation and maturation of cells (Shock and Laurent, 1996). The condition of the ECM and the type of adhesion/ ECM receptor molecule direct the type of signal. Integrins, which are cell-cell-surface receptors, play an elemental role in wound healing, as they control, in addition to cell proliferation and migration, also platelet aggregation, immune reactions, wound contraction and stimulate matrix production and deposition. They are the main structures of adhesion-complexes at the cell membrane, connecting the ECM to the cytoskeleton and thus

establishing a mechanical continuum by which the forces are conducted between the cell's inside and outside. Macrophages, lymphocytes, leucocytes, as well as resident cells produce and release cytokines ECM ligands for integrins comprise amongst others fibronectin and fibrinogen (Burridge & Chrzanowska-Wodnicka, 1996; D. E. Ingber et al., 1994).

The glycoprotein fibronectin (FN), which is a major element of most extracellular matrices, adjusts many cell activities via direct interaction with the integrin receptors of cell surface. FN is produced by various adherent cells which then integrate it into a fibrillary network. This assembly procedure is reliant on integrin-dependent and fibronectin–integrin interferences, inducing a stepwise process, which involves a conformational activation of fibronectin outside and an organization of the actin cytoskeleton inside. In the assembly process FN undergoes conformational alterations, which expose fibronectin-binding sites and stimulate intermolecular interactions that are needed for fibril formation. The FN matrix is essential for normal cell adhesion and growth (Mao & Schwarzbauer, 2005). Reduced expression and increased degradation of FN might compromise cellular migration and proliferation and is possibly responsible for morphological changes (Agren & Werthen, 2007; Mao & Schwarzbauer, 2005). Fibronectin fragments are able to initiate metalloproteinase enzymes (MMPs) (Agren & Werthen, 2007; Kapila, Kapila, & Johnson, 1996).

4.4   Connective tissue (CT)

Connective tissue is omnipresent in the body and supports, connects or separates the diverse tissues and organs. Van der Wal describes it as a continuous integrating matrix of the body (van der Wal, 2009). The main components are elastic and collagenous fibers, the ECM and cells, such as fibroblasts, macrophages, mast cells, leucocytes and adipocytes. The type of CT is determined by its density and cellularity. Fibroblasts are elemental for the supportive function of connective tissues.

The structure of connective tissue can be described as a fine web with thin branches permeating every tissue and with stronger trunks forming connective tissue planes, linking the several parts together (H. Langevin, Cornbrooks, & Taatjes, 2004). The essential functions are revealed through its architecture. In the body cavity, the connecting characteristic of connective tissue facilitates functional mechanical interactions between organs and other body parts, whereas its separating property of modeling space provides the ability for movement. Embryonically CT develops from mesoderm. Its functional development and the differentiation from mesenchyme follow two patterns. When considering the body cavities, the one is lining

and defining the intercellular space with mesothelium and thereby providing mobility and the other is the establishment of a connecting substratum, either using fibers or interstitial matrix.

As soon as the mobility and the movements of associated organs or structures are drastically diminished or lacking the peritoneum and the pleural membranes, which are both fascial layers, they are subjected to adherence. The same problem is encountered by joints after immobilization, which displays the functional parallels of the different tissues (van der Wal, 2009).

5   Tissue Repair

The peritoneal healing after tissue damage differs from the wound healing of skin. As already mentioned with regard to tissue repair of the peritoneum, the entire injured area becomes epithelialized diffusely and simultaneously, as opposed to skin defects that are gradually epithelialized from the wound margins. New mesothelium develops in the center of a large wound at the same time as it does in smaller ones (Raftery, 1973a; van Baal et al., 2016). Polymorphonuclear neutrophils are the first cells that are observed at the site of injured peritoneum, followed by macrophages, which differentiate from monocytes two days later. On day four to seven mesothelial cells start to occur und re-populate the area (J.-J. Duron, 2007; Raftery, 1973b, 1973a). Mutsaers and Foley-Comer et al. describe the process in which mesothelial cells which are at a distance from the wounded area detach from the basement membrane into the serosal fluid and settle on the denuded surface, where they proliferate and spread, to subsequently repopulate the injured zone (Foley-Comer et al., 2002; S. E. Mutsaers, Bishop, et al., 1997; Steven E Mutsaers, 2004). Damaged mesothelium requires about seven days to restore the injured layer, irrespective of the defect's size. A minor deviation in time considering healing of the visceral and parietal peritoneum exists, an exception is the peritoneum of the liver, which takes one day less, as it supplies a better substrate to new mesothelial cells (J.-J. Duron, 2007; Raftery, 1973b, 1973a).

The complete process of mesothelial restoration is still not clear, as the origin of the renewing colonizing cells cannot be determined definitely. However, it can be presumed that several mechanisms could interact in the process of peritoneal wound healing (J.-J. Duron, 2007). The peritoneum has a multi-step inflammatory response, in which immune cells are recruited, vascular perfusion is increased, macrophages and immune cells are accumulated and pro- and anti-inflammatory mediators are released. The peritoneal fibroblasts and the mesothelial cells are very active in peritoneal immune actions and react to any variations of their milieu (van

Baal et al., 2016). An exceeding immune reaction potentially causes angiogenesis, fibrosis and finally damage of the peritoneum (van Baal et al., 2016).

5.1 Phases of tissue repair

The whole process of peritoneal wound healing can be divided into three to four phases:

- hemostasis

- inflammation

- proliferation

- remodeling

The hemostasis and inflammation phases are either treated separately or described within one phase according to the different approaches in literature. In this work, it will be summed up within the inflammatory phase.

5.1.1 Inflammatory phase

Tissue damage is followed by capillary bleeding, platelet aggregation and clotting, which induces hemostasis. It is primarily the extravasation of blood components that lead to constriction of the surrounding blood vessels to reduce hemorrhage. Then agents from activated platelets induce vasodilation, increase the vascular permeability and cause a consequent exudation of fibrinogen. This induced inflammatory reaction and the release of various chemical messengers lead to an upturn of proteins and cells of the peritoneal fluid and thereby the fibrinous exudates and the formation of fibrin is induced. This action incepts the coagulation cascade, which leads to the cleavage of fibrin from fibrinogen, then to the bonding with fibronectin to close the defect and form a temporary wound bed. The provisoriliy formed fibrin plug serves as a scaffold for migration of fibroblasts and other cells and facilitates the recruitment of inflammatory cells and the ingrowth of new blood vessels (Molinas et al., 2010; S. E. Mutsaers, Bishop, et al., 1997; Steven E Mutsaers, 2004).

Leucocytes, including macrophages and neutrophils are essential in the early stages of wound healing (diZerega, 1997). Macrophages phagocytose debris preserves the injured tissue from the invasion of pathogenic organisms and recruit fresh mesothelial cells onto the wound surface. They act in collaboration with neutrophils and are an important source of chemoattractants and growth factors, such as the platelet-derived growth factor (PDGF),

transforming growth factor beta (TGF-β) and tumor necrosis factor alpha (TNF-α) (S. E. Mutsaers, Bishop, et al., 1997). During this phase the secretion and crosslinking of hyaluronan is amplified, which then facilitates leukocyte adhesion and migration. Further adhesion molecules, like vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1, are expressed on the mesothelial cell surface and interrelate with the recruited leukocytes (van Baal et al., 2016; Yung & Chan, 2012).

5.1.2 Proliferation phase

The newly formed granulation tissue is a provisional matrix, which contains a high density of macrophages, fibroblasts, ECM and blood vessels within a bulky meshwork of fibronectin, collagen and hyaluronic acid. The fibroblasts produce and deposit large amounts of matrix proteins, mainly collagen type I and III, to increase the tensile strength of the affected area. In the next step myofibroblasts, which are mesenchymal cells, start to reduce the wound size by contracting in a smooth, muscle-like manner, pulling the wound margins closer together.

The fibroblast's supply with oxygen and nutrients by the blood vessels is pivotal for vital granulation tissue. For an outright tissue to renew the endogenous fibrinolytic activity of mesothelial cells it has to accomplish a breakdown of the fibrin deposits within 72 hours (Brüggmann et al., 2010; S. E. Mutsaers, Bishop, et al., 1997).

5.1.3 Remodeling phase

During this phase there is a continuous production and degradation of connective tissue proteins, whereby the collagen synthesis exceeds the degradation. The production of plasminogen activator inhibitors (PAI) facilitates fibrin deposition, whereas the secretion of tissue plasminogen activator (tPA) and urokinase PA (uPA) supports fibrinolytic activity. The matrix composition constantly changes and collagen fibrils are organized tighter with intra- and intermolecular crosslinking (S. E. Mutsaers, Bishop, et al., 1997; Steven E Mutsaers, 2004).

The decisive step during wound healing is the resolution of scar tissue. There are several collagenase enzymes and metalloproteinases from granulocytes, macrophages, epidermal cells and fibroblasts, which regulate the degradation of wound collagen and other matrix proteins and therefore change the tissue's quality over the next months (Mignatti & Rifkin, 1996). The time course of scar tissue is subject to a broad individual variance. Mutsaers et al. submit that scar diminution might be inhibited by metalloproteinase concentrations or chronic scarring activity (S. E. Mutsaers, Bishop, et al., 1997).

The cycle of tissue repair is subject to an accurate time-dependent sequence of actions, such as the adjustment of cell contribution by recruitment, reproduction and matrix synthesis and degradation. The most important influences in this course of events is the regulation by mediators and the mechanical load to which the wound is exposed during wound contraction (M. Chiquet, 1999). Therefore, persistent peritoneal adhesions are the outcome of a disturbed enduring remodeling process.

5.2   Influencing factors

As mentioned before, the course of tissue repair is subject to a particular time-dependent sequence of processes, which involve the regulation of the cells' milieu through recruitment, reproduction and matrix synthesis and degradation. All these processes are coordinated by mediators derived from inflammatory and resident cells and blood. Internally generated, as well as externally applied mechanical loads strongly affect the production and action of these mediators and therefore influence the healing process of tissues (S. E. Mutsaers, Bishop, et al., 1997). The most important elements in the course of tissue repair will be reviewed shortly.

5.2.1   Cytokines and growth factors

It has been shown that resident endothelial cells' interaction with inflammatory or immune cells promotes expression and release of the cytokines PDGF, TGF-β, interleukin 1, interleukin 6, interleukin 8, colony stimulating factors and platelet activating factor, as well as ECM and adhesion proteins, anticoagulation factors and vasoactive proteins (including endothelin, prostaglandin E, and prostacyclin). Endothelial cells therefore obviously represent an important part in arranging the particular events of wound healing. The released polypeptide growth factors (PDGF) and transforming growth factor beta (TGF-β) are stored by platelets and are released upon aggregation during the clotting process. Five isoforms of TGF-β have been identified, whereof TGF-β_{1-3} potentially encourage the production of ECM. TGFβ_1 is commonly expressed in latent, non-active form by platelets, white blood cells, especially macrophages or parenchymal cells and mesothelial cells following tissue damage. In this form it is stored in the ECM and after its activation it binds to the cell's TGF-β_1 receptors, forming a protein complex (Massagué & Wotton, 2000; S. E. Mutsaers, Bishop, et al., 1997). This triggers the activation of so called Smads, which are intracellular transcription factor proteins that transduce TGF-β_1 signals to the nucleus (James J. Tomasek, Gabbiani, Hinz, Chaponnier, & Brown, 2002). These Smads activate downstream gene transcription and control the expression of particular

genes. The augmented expression of plasminogen activator inhibitor-1 (PAI-1) is a result of this course of events. (S. E. Mutsaers, Bishop, et al., 1997).

Moreover TGF-β₁ increases tensile strength of healing wounds by activation of myofibroblasts via the differentiation of fibroblasts and stimulates the influx of inflammatory cells. Also the collagen deposition by fibroblasts is enlarged and expression of fibronectin and expression of alpha-smooth muscle actin (α-SMA) at the injured area is increased (S. E. Mutsaers, Bishop, et al., 1997; James J. Tomasek et al., 2002). Their fibrogenic capacity makes TGF-β and PDGF main players in the progress of tissue fibrosis and fibrotic disorders (S. E. Mutsaers, Bishop, et al., 1997). PDGF, epidermal growth factor (EGF) and fibroblast growth factor (FGF) are produced by one cell and control both the own cell's function (autocrine), as well as that of other cells (paracrine). Information from these cells might be engaged in the attraction of inflammatory cells, fibroblasts and other resident cells to the wound site and thus relevant for induction of the healing process. The multitude of cytokines and growth factors play therefore an important role in regulating cell and tissue function during wound healing (S. E. Mutsaers, Bishop, et al., 1997).

Growth factors and hormones coordinate the fibroblast's production and degradation of ECM components, which can occur intra- or extracellular. Procollagen degradation is connected to the intracellular pathway. Diverse metalloproteinases including collagenases, gelatinases and stromelysins facilitate the extra-cellular degradation. Both degradative pathways are fundamental in the remodeling phase and in the modulation of matrix deposition during the wound healing process. The different models suggest that the upregulation of collagen synthesis might be due to the production of existing cells or/and the growing amount of new cells (S. E. Mutsaers, Bishop, et al., 1997). These events are fundamental for connective tissue remodeling.

5.2.2 Blood-derived proteins

Fibrinogen and fibronectin are two important examples of proteins derived from blood. After tissue damage they move via circulation to the site of injury and start to bundle with extracellular molecules and cell surface proteins. They are chemoattractants and mitogens for fibroblasts and establish a matrix for cell proliferation and organization. Thrombin is another cell mitogen, which incites collagen synthesis in fibroblasts (S. E. Mutsaers, Bishop, et al., 1997).

5.2.3 Regulation by the mechanical environment

Mutsaers et al., Chiquet and many other authors assert that the quantity and quality of the ECM do not only regulated by endogenous cellular processes and growth factors, but also by the sort and extent of mechanical stress acting on the tissues. Mechanical load affects various cell functions such as reproduction, orientation, collagen synthesis and growth factor production itself and therefore is a potent regulator of cell phenotype (Butt, Laurent, & Bishop, 1995; M. Chiquet, 1999; S. E. Mutsaers, Bishop, et al., 1997). The fibroblasts encounter active tension from contraction and passive tension from pulling adjacent cells or the circumjacent ECM. Under healthy conditions the ECM protects fibroblasts from mechanical forces, so they can sustain tissue homeostasis and control the matrix turnover (Li & Wang, 2011; James J. Tomasek et al., 2002). The altered mechanical characteristics of injured tissue lead, via activation of locally released cytokines, to the conversion of fibroblasts to myofibroblasts, which then begin to synthesize ECM components for the wound contraction (Hinz, 2007; Tomasek et al., 2002). In particular they produce collagen types I-VI and XVIII, glycoproteins, and proteoglycans, 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 active restructuring of the cytoskeletal matrix of myofibroblasts generates the force of wound contraction (S. E. Mutsaers, Bishop, et al., 1997). As connective tissue cells adjust their ECM to alterations in mechanical load, there has to be a feedback mechanism by which cells sense mechanical stress via their substrate and respond with a modified pattern of protein expression in order to remodel the ECM to encounter the altering mechanical requirements. Focal adhesions (FA), which are formed by integrins, linking the ECM proteins to the cytoskeleton, establish a mechanical connection and enable transduction of mechanical signals. Integrins and associated proteins evidently trigger signals that redound in adaptive cell reactions, such as matrix remodeling to modify its mechanical properties to the shifting conditions (M. Chiquet, 1999; Galbraith & Sheetz, 1998; Shyy & Chien, 1997). The level of tension that is produced by the cells' cytoskeleton correlates with matrix stiffness and in turn, the potency of this applied tension influences the migratory speed of cells (Brüggmann et al., 2010; Steven E Mutsaers, 2004).

Myofibroblasts' behavior and structure resemble smooth muscle cells, as they have substantial endoplasmatic reticulum and microfilaments and compact bodies. The ECM's nature and feedback have a strong influence on cell's structure and behavior and therefore influence

fibroblasts or myofibroblasts as well. The myofibroblast is a key cell for the connective tissue remodeling that takes place during wound healing and fibrosis development. Myofibroblasts are linked to the ECM and react to mechanical stress (mechanosensing) via a modification in gene expression. Increased ECM stiffness triggers stronger myofibroblast contraction and further ECM synthesis, which in turn results in higher ECM stiffness again (Hinz, 2010; S. E. Mutsaers, Bishop, et al., 1997; Swanson, 2013). The generated force seems therefore to be as well decisive for the dimension of wound contraction, as for the regulation of cell's phenotype and function and growth factor and collagen synthesis (Butt et al., 1995; S. E. Mutsaers, Bishop, et al., 1997; D. L. Wang et al., 1995). After tissue reparation MFBs normally undergo apoptosis. Their deactivation or termination can result, if the ECM is re-established and takes over the mechanical stress. Under pathological conditions, as in adhesion or scar formation, this does not happen and they continue their activity, resulting in tissue hypertrophy and exceeding wound contraction. Mechanical strain might hence be as well an essential component in the process of adhesion and scar development (S. E. Mutsaers, Bishop, et al., 1997). Though the principal process of scarring after abdominal and pelvic surgical incisions is of course also vital and indispensable to ensure wound closure and strength (Bouffard et al., 2008).

5.3 Fibrinolysis

The exudation and deposition of fibrin is a crucial and necessary process of tissue repair. If the fibrinolytic system fails to clear the deposition, a re-establishment of pre-operative peritoneal conditions and thereby functional restoration of the peritoneum cannot be achieved.

Mesothelial cells are essential for local fibrin deposition and clearance within the peritoneal cavity, as they have both procoagulant and fibrinolytic capacity. The plasminogen system coordinates the degradation of fibrin. The glycoprotein plasminogen is produced by the liver and exists plentifully in nearly every tissue. The fibrinolytic activity is facilitated through tissue plasminogen activators (PA), mainly the serine protease tissue-type PA (tPA) and to a more limited extent the serine protease urokinase-type PA (uPA). The PAs transform inactive proenzyme plasminogen into active plasmin, which enzymatically breaks down fibrin. TPA and uPA are mainly produced by mesothelial cells and macrophages. Their levels are modulated by inflammatory factors including lipopolysaccharide, tumor necrosis factor-α (TNF-α) and IL-1 and fibrogenic mediators such as TGF-β and thrombin (Holmdahl, 1997; Steven E Mutsaers, 2004). Plasmin, a serine protease, is very active in fibrin degradation, which then plays an important role for ECM degradation, activation of metalloproteinase enzymes (MMP) and growth factors. Aside from growth factors and metalloproteinase enzymes and their inhibitors,

various agents, cytokines and chemoattractants are involved in the process of tissue healing and adhesion formation at the level of the extracellular matrix.

The deposition of fibrin is sustained by secretion of plasminogen activator inhibitors (PAI), PAI-1 and PAI-2. The stronger inhibitor PAI-1 is expressed by mesothelial cells, endothelial cells, fibroblasts, platelets and macrophages. The weaker inhibitor PAI-2 is produced by mesothelial cells, epithelial cells and macrophages. The abrasion and consecutive inflammation of the mesothelium through surgery leads to the diminution of the local t-PA resources and to the potential exposure of PAI-1 contained in the submesothelium, which supports the fibrin's continuance and therefore adhesion formation (Holmdahl, Falkenberg, Ivarsson, & Risberg, 1997; Molinas et al., 2010).

5.4 Adhesion formation

Intraperitoneal adhesions result from an aberrant peritoneal wound healing after any kind of mesothelial damage with an incomplete degradation of fibrin, which is followed by fibroblastic and capillary growth and extracellular matrix deposition (Steven E Mutsaers, 2004).

5.4.1 Influencing factors

The fibrotic response of the tissue after injury is assumed to be dependent on three factors:

- exceeding synthesis of collagen and other ECM components

- a persisting stimulus which triggers the fibrotic processes

- the reduction of the fibrinolytic enzymes degrading adhesion tissue

What a particular stimulus is that either leads to an ongoing fibrotic process or to a self-limiting physiological healing process remains unclear. The involved mediators seem to be the same both in wound healing and fibrosis or adhesion formation. What can be determined is, that the physiological time-dependent control of cell function during the tissue healing process is deranged, accompanied by an over-production of the mediators, which are engaged in the wound healing process (S. E. Mutsaers, Bishop, et al., 1997). Mechanical stress leading to a high-tension matrix, as well as the additional presence of TGF-β are presumed to be the main factors in adhesion formation (Martínez Rodríguez & Galán del Río, 2013; S. E. Mutsaers, Bishop, et al., 1997).

Currently it is not much known about genetic predisposing factors, that raise the risk of adhesion formation and excessive scarring (S. E. Mutsaers, Bishop, et al., 1997). The process of peritoneal wound healing, either resulting in normal physiological reparation or in adhesion formation is shown in figure 1.
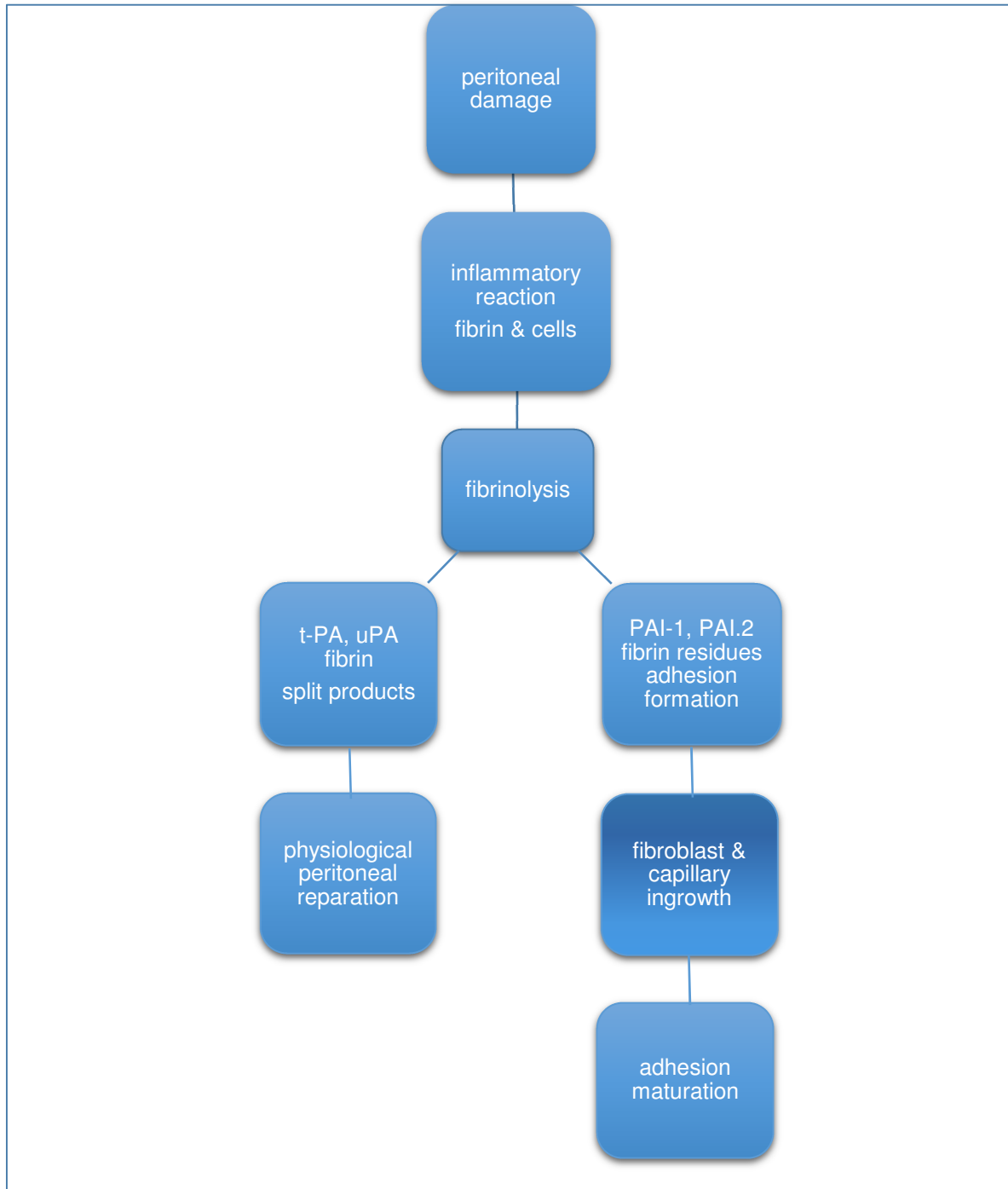

Figure 2 process of peritoneal wound healing (modified from Brüggmann et al., 2010; J.-J. Duron, 2007)

5.4.2 Symptoms and consequences

If the fibrinolytic system fails to degrade the fibrin matrix, the injured side of peritoneum, which is denuded from mesothelial cells, can produce fibrotic connective tissue making up adhesions and adhering to the intact opposite side of peritoneum (van Baal et al., 2016). The formation of fibrous serosal adhesions between organs and the abdominal wall, hampers and diminishes the vital intrathoracic and abdominal movement (Steven E Mutsaers, 2004). Weibel and Manjo for example found out that the omentum was involved in 92% of the post-operative adhesions and Pittaway et al. found more than 90% of ovarian adhesions after ovarian surgery (S. E. Mutsaers, Bishop, et al., 1997; Pittaway, Daniell, & Maxson, 1985; Weibel & Majno, 1973). In the United States of America each year over 1.3 million Caesarean-sections are conducted and 7% to 18% of these are affected by chronic scar pain (Wasserman, Steele-Thornborrow, Yuen, Halkiotis, & Riggins, 2016).

Abdominopelvic adhesions can cause complications such as meteorism, irregular bowel movements, chronic abdominal, pelvic or vertebral pain, digestive disorders, infertility, and intestinal obstruction, all of which may come up even decades later (Brüggmann et al., 2010). In the worst cases the conditions might even become life-threatening and require adhesiolytic surgery. The symptoms and the associated pain mostly pose a severe impairment in daily activities and a reduction of life quality (Arung et al., 2011; Bove & Chapelle, 2012; Brüggmann et al., 2010; Rice et al., 2013).

In general adhesions are not easy to diagnose. They can be detected by sonography or diagnostic ultrasound, often they are found during re-operations, such as laparoscopy. Differential diagnosis is essential to exclude organ diseases or other severe pathological conditions (A. Kobesova & Lewit, 2000). Many authors recommend the usage of sonography not only for diagnosis, but also for the measurement of treatment outcomes as it seems to be the most reliable, non-invasive tool that can be used for the detection of superficial and deep tissue changes (Chamorro Comesaña et al., 2017; Luomala, Pihlman, Heiskanen, & Stecco, 2014; Martínez Rodríguez & Galán del Río, 2013; Pohl, 2010).

5.4.3 Cellular components

If the bridge between two surfaces is only made of fibrin, the adhesion will be susceptible for fibrinolysis, but if it implies cellular components, it will possibly be structured into a persisting adhesion. This adhesion tissue becomes subsequently organized by wound repair cells and the persisting extracellular fibrin matrix gives rise to a mesothelialized structure that is stabilized by connective tissue, often containing blood vessels (angiogenesis), clusters of

smooth muscle cells and myelinated and non-myelinated nerve fibers. These nerve fibers might be responsible for pain related events, that often occur with adhesions (Brüggmann et al., 2010; diZerega & Campeau, 2001; Herrick et al., 2000; Sulaiman et al., 2000). Herrick et al. establish in their study that adhesions are not just passive scar tissue, but highly cellular, containing dynamic regenerating structures. The examined cellularity is variable, showing numerous fibroblastic cells or a dense collagenized matrix with few fibroblastic cells. However, it can be determined that the prevalent cellular elements are leukocytes, including neutrophils and macrophages, mast cells, mesothelial cells and fibrin (diZerega, 1997; diZerega & Campeau, 2001; Herrick et al., 2000). With adhesion maturation the quantity of fibroblasts diminishes and fibrous bands of different collagen types, scarce cells and occasionally cartilage like tissue or calcifications evolve (Cheong et al., 2001; J. J. Duron et al., 1993; J.-J. Duron, 2007). Normally a sheet of mesothelial cells covers mature adhesions (diZerega & Campeau, 2001; Herrick et al., 2000). In contrast to endometriosis or other adhesion forms the postoperative formation is not progressive (Cheong et al., 2001).

5.4.4 Cellular processes in adhesion formation

The mesothelial cells (MCs), which line the abdominal cavity, undergo a mesothelial-to-mesenchymal transition (MMT), which is a similar process to epithelial-to-mesenchymal transition (EMT). During the wound healing process or under pathological conditions MCs get transformed into myofibroblasts, which are abundant in peritoneal fibrotic tissue, with submesothelial localization and α-smooth muscle actin (α-SMA) expression. Sandoval et al. detected in their study a mesothelial-to-mesenchymal transition (MMT) and plentiful myofibroblasts within all human samples of peritoneal adhesions. The collagen-secreting fibroblastic population seems to derive from the submesothelium and the myofibroblasts derive from the mesothelial cells (MCs). These converted myofibroblasts have a high capacity to synthesize extracellular matrix components such as fibronectin and collagen I, and angiogenic factors, which are decisive for the adhesion formation. These changes in the cellular phenotype are a result of a profound genetic reprogramming, with an up-regulation of mesenchymal markers, such as the α-smooth muscle actin (α-SMA) (Sandoval et al., 2016). Gómez-Gil et al. constitute that mesenchymal cells, dependent on their environment, are at least capable of two different cell phenotypes rendering two types of adhesions with clearly differentiated characteristics. One type has a highly vascularized adipose morphology containing cells differentiating into a vascular lineage and the other type is fibrous with high quantities of collagen and myofibroblasts conferring less compliance to this tissue (Gómez-Gil et al., 2009). Therefore myofibroblasts are key cells in the remodeling of connective tissue during wound healing and fibrosis development (Desmoulière, Chaponnier, & Gabbiani, 2005).

The plasminogen activating action, mainly the PAI release, is the prime cause for the formation of adhesions (Chegini et al., 2001; J.-J. Duron, 2007). The other crucial molecule in this process apart from the plasminogen activators (PAI), is transforming growth factor-beta (TGF-β), which operates on metalloproteinases (MMP), and its tissue inhibitor (TIMP). This action leads to a modified ECM remodeling and may potentially promote adhesion development (Cheong et al., 2001; J.-J. Duron, 2007).

Impaired mesothelial regeneration results in the reduction of the peritoneal fibrinolysis capacity and might therefore be a main factor in the development of post-operative adhesion formation. The destruction of mesothelia, insufficient blood supply, increased synthesis of fibrinolysis antagonists, hypoxia, oxidative stress or bacterial infection are some of the listed reasons that occur in and after surgery and may cause this reduced capacity (Brüggmann et al., 2010; Steven E Mutsaers, 2004).

6 Osteopathic manipulative treatment (OMT)

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, Glossary of Osteopathic Terminology, p.28).

The general aim of all osteopathic manipulative treatments is to improve the physiologic function of tissues and to restore homeostasis in order to encourage the body's self-healing capacity. The treatment can therefore comprise, amongst others, an increase in the functional range of motion on several tissue levels, the reduction of pain or pain-related symptoms and a stimulation of the fluid flow.

This thesis seeks to depict an explanatory model if and how osteopathy can influence the complex pathology of peritoneal adhesions in the light of scientific facts and practical empirical studies or case reports. The author will therefore first display the events that take place on the cellular level and try to give an overview over mechanical effects and processes involving OMTs.

6.1 Mechanical stimulation

The tissues have certain cellular mechanisms to sense and react to changes in their physical environment. The mechanical properties of the cell environment therefore influences the cell's structure and function (Engler, Sen, Sweeney, & Discher, 2006; Martínez Rodríguez & Galán del Río, 2013).

6.1.1 Mechanotransduction and mechanosensing

It is well established that mechanical forces, both internally generated or externally applied, are elemental for the homeostasis of tissues and that ECM is the main conductor of these forces. Mechanical stimuli are the trigger for the expression of certain proteins that are engaged in matrix turnover. Such matrix proteins comprise collagens, tenascin-C and metalloproteinases (Sarasa-Renedo & Chiquet, 2005). The production of tenascin-C and collagen XII, which are typical ECM proteins in tendons or ligaments, is increased in fibroblasts within a strained collagen matrix, but reduced in a relaxed environment. These reactions to variations in tissue tension are fast, invertible and mirrored on the level of mRNA (messenger ribonucleic acid). The gene promoters of tenascin-C and collagen XII comprise stretch responsive enhancer areas with resemblance to shear stress response elements in other genes (M. Chiquet, 1999).

The process of mechanotransduction is inducted on the submembrane cell level of focal adhesions (FA) (Sarasa-Renedo & Chiquet, 2005; Zusman, 2010). In focal adhesion sites the cells' actin cytoskeleton is firmly linked to the underlying ECM, which therefore makes them the area of signal transduction. The guanosine triphosphate-binding (GTP) protein Rho controls the assembly of FAs and incites contractility, which causes isometric tension. As in the process of wound healing fundamental elements for this mechanism are once again cell adhesion molecules (CAMs) and their associated proteins (Zusman, 2010). Integrins and cadherins are two representatives of CAMs engaged in the action of mechanotransduction (Alahari, Reddig, & Juliano, 2002; Schwartz & DeSimone, 2008). As the integrins are exposed to mechanical load, their outer surface hooks mainly attach to the ECM's collagen elements and their inner surface connects to the cytoskeleton's actin filaments, the stress fibers. The cell's capacity to withstand structural deformation is due to its cytoskeletal filaments, which are capable both of resisting and creating mechanical forces (Donald E. Ingber, 2003). The mechanical interactions are sensed at each end and respectively influence the other. The mechanical stimulation leads to the signaling of integrins and the cell react by tightening the contact with ECM collagens. Integrins thereby start polymerization and establish bundling of actomyosin stress fibers, which first communicate via the mechanical pull with the ECM and afterwards via chemical signaling. The strengthening of adhesion by the stimulation of mechanical load might be the prime mechanism for mechanotransduction. Moreover, these mechanisms possibly supply to tissue reactions to tension or compression and are therefore decisive to the cells' morphogenetic fate (Schwartz & DeSimone, 2008).

In the next step these mechanical stimuli are transformed into a chemical reaction (Ingber, 1997). Chemical signaling pathways comprise focal adhesion kinase (FAK), mitogen activated kinase (MAPK) and tyrosine phosphatase (Vogel & Sheetz, 2009; Zusman, 2010). The successional intracellular chemical activation of gene transcription factors seems to be a main factor causative for gene regulation by mechanical stimuli (Matthias Chiquet, Tunç-Civelek, & Sarasa-Renedo, 2007; Sarasa-Renedo & Chiquet, 2005; Zusman, 2010).

The secretion of TGF-β_1 is assumed to be another factor responsible for gene transcription. The growth factor mechanism is seen to be accountable for long-term transcription mediated connective tissue alterations through mechanical load (Matthias Chiquet et al., 2007; Zusman, 2010). Wipff et al. asserted that latent TGF-β_1 is activated instantly when myofibroblast-derived ECM is stretched in presence of mechanically opposing stress fibers. Further myofibroblast differentiation and α-SMA expression are initiated by a signaling molecule, which is produced through the release of TGF-β_1 from its latent form by tension (stretch, shear or glide). As the

integration of contractile protein α-SMA into existing stress fibers that are under substantial load entails an appropriate level of ECM tension, α-SMA is considered to be mechanosensitive (Hinz et al., 2007; Wipff, Rifkin, Meister, & Hinz, 2007; Zusman, 2010).

Summing up, investigations of cultured cells have revealed that integrin- and cadherin-mediated adhesions are mechanosensitive (Schwartz & DeSimone, 2008). It can be determined that matrix adhesion contacts are the main sites of mechanical and chemical information transduction and therefore are also crucial for mechanotransduction and mechanosensing, which is both a determining factor for cell growth and differentiation (Matthias Chiquet, Gelman, Lutz, & Maier, 2009). This mechanism enables fibroblasts, myofibroblasts and other adherent cells to sense variations in their surrounding ECM and to translate physical stimuli into chemical reaction information (D. E. Ingber, 1997a; Martínez Rodríguez & Galán del Río, 2013). The integration of these signals with growth factor derived stimuli redounds to a change of gene expression. A change of mechanical force typically triggers ECM synthesis in connective tissue cells (Matthias Chiquet, Gelman, Lutz, & Maier, 2009).

6.1.2 The role of connective tissue

Langevin refers to connective tissue as a body-wide mechanosensitive signaling network, which is closely allied to every other sort of tissue in the body. Its signaling might affect and be affected by any physiological or pathological condition of a wide range of other structures and systems. As CT is omnipresent in all organ systems, it presumably also plays an important role in pathological processes of these systems and has to be included in the treatment course (Helene M. Langevin et al., 2011).

The connective tissue matrix and the extracellular interstitium are essential for the integration of the different cell types' functions within the tissues (Helene M. Langevin, 2006). This and the fact that its structure enables cells to sense and process mechanical stimuli makes CT a main actor in the process of mechanotransduction (M. Chiquet, 1999; Helene M. Langevin, 2006). The long-term shaping of the connective tissue matrix is regulated by the constant interaction of cells, ECM and mechanical stimuli. Information stability and the "memory" of tissues might be due to connective tissue proteins (Brand, 1992; Helene M. Langevin, 2006).

Connective tissue represents a functional network, which contains a multitude of mechanoreceptors and nociceptors. Regarding adhesions and related pain, either locally or distantly, further investigation of the inner communication within this system and the external

dialogue with other tissues, organs and the central nervous system would be useful for comprehending the mechanisms of pathological changes affecting other distant organs or systems (Helene M. Langevin, 2006).

6.2 Tensegrity

The model of tensegrity originates in architecture and is a building principle that was first described by R. Buckminster Fuller (1961). Fuller delineated tensegrity systems as structures that maintain their form by continuous tension, so to say tension integrity, rather than by continuous compression. A main element of the tensegrity model is the tensile prestress (pretension) of continuous tension elements, which provides continuous structural stability against compression and traction forces. The pretension can be alterable, and it varies, subject to the changing mechanical requirements, like absorption and force management (Donald E. Ingber, 2008).

6.2.1 Cellular tensegrity and mechanical load

In the concept of biotensegrity, bones represent the compression-resistant element, the surrounding muscles are the tension-generating element and the fasciae are the tension-resisting element (Donald E. Ingber, 2008). The model of biotensegrity allows the description of the correlation between mechanics and biochemistry at the molecular level. The cytoskeleton, which adjusts much of cell metabolism and transduction, is highly susceptible to mechanical distorsion through cell surface integrin receptors. The gradual deviation of physical parameters, such as the distorsion of cell shape, are decisive for different gene programs, such as proliferation, differentiation or apoptosis. Ingber suggests a combination of cell tensegrity and mechanochemistry may intermediate mechanotransduction and enable integration of physical and chemical signals, which regulate the cell behavior (Donald E. Ingber, 2003). The pre-stressed cellular elements in the biotensegrity model are fibroblasts and myofibroblasts (Bouffard et al., 2008; Swanson, 2013). They are linked to the ECM and through changed gene expression they are able to react to mechanical stimuli. The generated pre-stress is transmitted and amplified in reaction to increased mechanical load (Matthews, 2006; Swanson, 2013; James J. Tomasek et al., 2002). Langevin et al. found out that fibroblasts in areolar connective tissue or fasciae are not only capable of sensing and responding towards varying mechanical forces, but of reducing the entire tissue's tension by modifying their cellular and cytoskeletal shape (Helene M. Langevin et al., 2011). These results, especially their ability to evoke a global release of tissue tension, suggest that fibroblasts and myofibroblasts should be a prime target for any kind of mechanical manipulation, such as myofascial release, unwinding and any kind of direct or indirect technique.

6.3 The effects of mechanical load on the cellular level of fibroblasts

Fibroblasts that are exposed to mechanical load modify their outer shape and their nucleus by flattening and spreading from a dendritic form to a laminar form. The fibroblasts' nuclei have deep invaginations containing α-SMA protein. When they are exposed to tissue stretch, cytoskeletal remodeling and redistribution of α-SMA from the whole cell to the nucleus will be the consequence. The nucleus' reversible deformation takes place within a few seconds. Rheological models propose that nuclear stiffness induced by mechanical stimuli possibly endure over a long period because of cytoskeletal remodeling. In every case the actin cytoskeleton evidently plays a key role linking ECM compression and nuclear deformation (Helene M. Langevin et al., 2010). This has principal effects on the cell chromatin structure and therefore on cell functions, by activating a proliferative phenotype with a high capacity to synthetize collagen (Matthias Chiquet et al., 2009; Gabbiani, 2003; Grinnell, 2003; Hinz, 2007). Mechanical stress and TGF-β_1 are the main activators that lead to the conversion from quiescent fibroblasts into active contractile myofibroblasts. In order to achieve tissue reparation, they secrete new ECM. In contrast in a low tension environment fibroblasts adopt a dendritic form, which allows physical communication with the other fibroblasts. Moreover, the biosynthetic action is subdued and a quiescent status of these cells is achieved. The connection of fibroblasts and ECM through integrin receptor-mediated focal adhesions is a bidirectional relation that is specifically accustomed by pretension (Martínez Rodríguez & Galán del Río, 2013).

As already mentioned it has long been known that differentiated myofibroblasts are capable of exerting traction on tissues during wound healing and in fibrosis states (Gabbiani, Chaponnier, & Hüttner, 1978; H. Langevin et al., 2004). Apart from their supportive function in connective tissues and their ability to produce ECM components, fibroblasts are evidently also able to react to mechanical forces even in the absence of a wound. Cultured fibroblasts respond within minutes to physical stimuli, such as compression, stretch and tension or shear force, with cellular changes. Mechanical forces exerted on loose connective tissue directly affect the mechanotransduction process of fibroblasts. These changes comprised intracellular calcium influx, adenosine triphosphate (ATP) release, activation of signaling pathways, actin polymerization and gene expression (Banes et al., 1995; H. Langevin et al., 2004; Stoltz et al., 2000). It has been shown that these changes in gene expression and matrix quality are the answer to mechanical signals. The alterations of ECM composition seem to be a central way of communication between the various types of cells and the connective tissue. The model of a body-wide communicating and signaling cellular system discloses the option that fibroblasts' response to mechanical stimuli might have strong effects on other structures and systems. This

dynamic response of the fibroblasts has potential implications for the understanding of CT responses towards mechanical changes, such as posture or therapeutical manipulation, and therefore reveals its body-wide integrative importance (H. Langevin et al., 2004).

6.4 The effects of mechanical load on the level of CT

In contrast to actively contracting or relaxing muscle tissue, connective tissue has been regarded as a passive viscoelastic tissue, whose characteristics are determined by the condition of its ECM. In vivo and in vitro fibroblasts in loose CT have been shown to react to tissue stretching with cytoskeletal remodeling within minutes. In their study of mice Langevin et al. examined loose connective tissue and whether this remodeling would account for the viscoelastic behavior of the entire tissue. Tissue stretch of the subcutaneous and deep back muscles of mice was performed uniaxially in the transverse direction, relative to the tissue's orientation in vivo. Their results showed that the cytoskeletal remodeling of fibroblasts enabled tissue relaxation with a decreased tensional level. This submits to another matrix remodeling mechanism through expression of fibroblast mechanosensitive genes within hours (Matthias Chiquet, Renedo, Huber, & Flück, 2003; Helene M. Langevin et al., 2011). These findings are coherent with the models of mechanotransduction anticipated by Ingber (D. E. Ingber, 1997b). As a reaction to an extent of tensile prestress, loose connective fibroblasts strengthen their cytoskeletal structure, which is followed by a tension modification of the entire tissue. The study's data indicate that if a cellular incapability of this remodeling response occurs, the tensional homeostasis cannot be maintained, resulting in tissue stiffness. This active cell-mediated connective tissue reaction might be essential for the protection of blood vessels or nerve fibers from prolonged loading, as in the diverse body positions. Connective tissue tension might therefore affect all the neuronal, vascular and lymphatic structures, immune cells and adjacent organ-specific cells. In pathological cases such as tissue fibrosis or adhesions the raised collagen density impacts fibroblastic reactions and hence the capacity to modulate the tension in the tissue (Helene M. Langevin et al., 2011). On the other hand the long-term effects of reduced or lacking mechanical stress and prolonged or continuing inflammation are a more irregular organization of the connective tissue with more random cross-links between the fibers and the adjacent tissues and a lower tensile stiffness with lower content of water (Threlkeld, 1992).

6.4.1 The cellular effects of direction, duration and magnitude of mechanical strain in CT

The direction, duration and magnitude with which cells and tissues are stretched seem to be decisive for the fibroblasts' remodeling of ECM (Balestrini & Billiar, 2006; Zein-Hammoud &

Standley, 2015). The author already described before that TGF-β_1 is a crucial factor in the process of wound healing, scarring and fibrosis. Bouffard et al. investigated the effects of 10 minutes static tissue stretching on TGF-β_1 levels in mice in vivo and ex vivo. For the in vivo model they performed unilateral subcutaneous microsurgical injury on the back of 22 mice, which then underwent either 10 minutes of 20% to 30% static stretch two times a day for seven days, or no stretch. For the ex vivo group they excised the same tissue of 44 mice, which was either stretched in a culture one time a day with 20% for four days or not stretched. It could be shown that in the ex vivo group the TGF-β_1 level was lower in the stretched than in the non-stretched samples. On the fourth day there was an increase of TGF-β1 protein level in stretched and non-stretched samples, though less increase was measurable in the stretched group. This progressive upturn of TGF-β_1 is coherent with the reaction to the tissue injury of excision. In the in vivo group the injury led to an upturn of procollagen type-1 in the non-stretched tissue, but not in the stretched ones. The short stretch resulted in a diminished increase of TGF-β_1 ex vivo and procollagen type-1 in vivo. These results stand in clear opposition to other studies, which observed an increase of TGF-β_1 and collagen production and deposition under prolonged (hours to days) low amplitude (15% strain) cyclical or static stretch (Balestrini & Billiar, 2006; T. V. Cao, Hicks, & Standley, 2013; Grinnell & Ho, 2002; Lee, Delhaas, McCulloch, & Villarreal, 1999). Bouffard et al. could therefore demonstrate that the extent, duration and timing of mechanical load on injured tissue is decisive for its effectiveness and for a constructive and anti-fibrotic therapeutic treatment. The findings of their study indicate that stretch-initiated reduction of TGF-β_1-mediated new collagen deposition could be an effective mechanism and could be easily used by therapists to prevent and treat excrescent scar and adhesion formation. The local effect of this kind of tissue stretch hampers collagen formation for some days after stretching and could therefore inhibit adhesion formation from the beginning. This seems to be of even higher importance, where intraperitoneal injuries or wounds are concerned, including organs and their fascial structures. This study therefore supports the approach of manual therapy to prevent and treat fibrotic or contract tissue adhesions and scars locally with short-time tissue stretch and mobilization over the usual range of motion to encourage beneficial tissue remodeling (Bouffard et al., 2008; Hardy, 1989). A limitation of this study clearly is that the validity for the human body is lacking and that treatment would have to be daily, which can be difficult to manage in clinical practice.

Cao et al. discovered in their in vitro model of strained fibroblasts that repetitive motion strain (RMS) with a magnitude of 10% over a duration of eight hours (simulating a normal working day) leads to a reduction of wound closure rates and a diminishment of fibroblast proliferation (T. V. Cao, Hicks, & Standley, 2013). In contrast to the previous study of Bouffard et al., Cao et

al. detected an increase of apoptosis and an upturn of inflammatory cytokines accompanied by morphologic alterations in actin architecture (T. V. Cao, Hicks, & Standley, 2013; Dodd et al., 2006; Eagan, Meltzer, & Standley, 2007; Meltzer et al., 2010).

Physiologically seen, a varying extent of static prestress tension exists in the different tissues and prolonged cyclic stretch is exerted on all tissues through diaphragm contractions, arterial pulsation or during cyclic movement such as walking or running (Bouffard et al., 2008; Donald E. Ingber, 2006; James J. Tomasek et al., 2002). In the process of wound healing and under pathological conditions, as is the case in fibrosis and adhesion formation, tissue tension is gradually elevated over days and weeks by contractile myofibroblast force (Bouffard et al., 2008; Grinnell & Ho, 2002; Hinz, Mastrangelo, Iselin, Chaponnier, & Gabbiani, 2001; J. J. Tomasek, Haaksma, Eddy, & Vaughan, 1992).

Eagan et al. demonstrated in their in vitro study of equibiaxial (strained equally along two axes) and heterobiaxial (strained unequally in two axes) strained fibroblasts that the direction of strain does stimulate fibroblastic functions differently (Eagan et al., 2007). Equibiaxially strain causes a lower level of proinflammatory IL-6 and macrophage-derived chemokine (MDC) and less fibroblastic proliferation than heterobiaxially strain. Heterobiaxial straining, on the other hand, produces higher inflammatory levels, fibroblast proliferation and hypertrophic responses (T. V. Cao, Hicks, Campbell, & Standley, 2013; Eagan et al., 2007). The fibroblasts' morphology is only affected by heterobiaxial strain, which might be because the actin-mediated calcium release is dependent on the strain direction. This release affects cell contractility, which then further affects tissue stiffness. These data imply that human fibroblasts are receptive to different strain directions and respond with altered gene expression and growth functions. The authors assume that immobilization might result in modified fibroblastic proliferation and cytokine production (Eagan et al., 2007). Transferring these data to the pathology of peritoneal adhesions and scars, it seems likely that a balanced and continuous stimulus provides more beneficial input.

In a previous study Dodd et al. (Dodd et al., 2006) showed that human fibroblasts undergo hyperplasia and modify their shape and alignment, when acyclically strained. Moreover, Dodd et al. detected raised secretion of nitric oxide secretion and IL-6. A strain magnitude of 10% stimulated cell proliferation and a slight loss of cell viability. Strain magnitudes of 30% led to prevailing cell destruction (Dodd et al., 2006). Langevin et al. assume that tissue stretching over 25% might disrupt fibroblast processes or cell-to-cell contacts (H. M. Langevin, 2004). Cao et al. state that strain magnitudes over 10% lead to a thinning of collagen fiber diameters in bioengineered tendons, whereas strain magnitudes between 3% and 9% did not lead to

measurable alterations (T. Cao, Hicks, Zein-Hammoud, & Standley, 2015). In other studies, it could be shown that this strain-induced alignment and cell migration occur three hours after injury, enduring a little longer than strain cessation in vitro. These cell responses indicate their aim to form the energetically most efficient architecture, which is lost, with strain cessation. Misalignment of fibroblasts and their associated matrix proteins during acyclic straining shows parallels to the pathological genesis of fibrosis and adhesion formation (Dodd et al., 2006; H. Wang, Ip, Boissy, & Grood, 1995).

6.4.2 Effects of mechanical stretch on tissue swelling and interstitial flow

The response of CT to static stretching depends on the content and organization of its ECM and the fibroblastic activity. Within minutes of the stretch, dynamic expansion of fibroblastic shape results and contributes to a decline of tissue tension during the viscoelastic relaxation. Langevin et al. suggest that this fibroblastic reaction during matrix stretching is a mechanism to adjust the influx of extracellular fluid into the tissue, therefore actively preventing against swelling. As loose connective tissue matrix has the tendency to bind water and swell, the interstitial fluid flow is restrained by the restrictive tension on the matrix collagen network created by fibroblasts (Helene M. Langevin, Nedergaard, & Howe, 2013; Reed, Lidén, & Rubin, 2010). Under physiological conditions the fibroblasts' regulation activity prevents the connective tissue matrix from maximum hydration, whereas in the pathological state of inflammation these regulatory mechanisms fail and a decline of interstitial fluid pressure is measureable. Apart from the raised vascular permeability during inflammation stages, the tissue's tendency to absorb fluid like a sponge and the inability of fibroblasts to control the influx, might be another mechanism causal for the emergence of tissue edema. Inflammatory mediators distract the integrin-mediated cell-matrix connections and so lower the matrix tension, which enables the interstitial fluid to inflow in the tissue and cause matrix swelling. This raised turgor leads to enhanced matrix stiffness. Langevin et al. hypothesize that stretching for longer than a few minutes would have negative effects on the tissue, as the stretched matrix pores would then suck in even more fluid and therefore enhance fluid stasis and edema. On the other hand compression, for example through body movements such as walking, is well-known to support interstitial flow and tissue drainage (Helene M. Langevin et al., 2013). These facts suggest that tissue stretching in inflammation states should be avoided to prevent increased matrix stiffness and edema or in the worst case tissue destruction.

6.5 Different OMTs and their potential effects on postoperative peritoneal adhesions

The common objective of all OMTs is to set a biomechanical stimulus to affected or restricted

tissues and their associated areas, which potentially leads to a change of the cellular functions (Zein-Hammoud & Standley, 2015). Only little literature could be retrieved on specific treatment and its effects on postoperative abdominal or pelvic adhesions. Some of the studies found seem to contain poor scientific evidence, as they are often case reports or animal research studies. One study was found that explicitly treated postoperative adhesion with visceral mobilization and investigated the effects on adhesion development and visceral mobility. This study of Bove and Chapelle (Bove & Chapelle, 2012) was performed on rats, but as the investigated adhesion development and quality does not seem to differ significantly from that in humans, the results might be applicable for human osteopathic treatment as well. The main amount of the found literature concerning osteopathic manipulative treatment was on connective tissue treatment. Nevertheless, many of the gained results could be implemented to the treatment on peritoneal adhesions, as their cellular composition consists mainly of fibroblasts and myofibroblasts or a dense collagenized matrix, which are all elements susceptible for applied mechanical stimuli.

6.5.1   Direct OMTs (DOMT)

There are different ways to approach and treat somatic dysfunctions, either by direct, indirect or combined methods. A direct method (D/DIR) is defined by Treffer et al. as "…an osteopathic treatment strategy by which the restrictive barrier is engaged and a final activating force is applied to correct somatic dysfunction." (Treffer et al., 2011, p. 28).

Both the superficial scar and profound adhesion are mainly located in the soft tissues and lead to restriction and dysfunction of the surrounding tissue (A. Kobesova & Lewit, 2000). It is therefore reasonable and necessary to treat all the afflicted tissue layers. The majority of the reviewed clinical studies implicated direct techniques for the treatment of connective tissue restrictions, superficial or deep scars or adhesions and their related symptoms.

One of the studies that seems to be most relevant for the manual treatment of peritoneal adhesions is the experimental study of Bove and Chapelle on the potential of visceral treatment to prevent and lyse postoperative peritoneal adhesions (Bove & Chapelle, 2012). To substantiate their hypothesis that anatomically-based manual treatment that supports normal mobility of the visceral contents is able to limit postsurgical adhesion formation, they conducted a surgical cecal and abdominal wall abrasion to induce adhesion formation on thirty rats. The authors separated the animals in three groups, consisting of a lysis, preventive and control group. The rats of the lysis group were treated on postoperative day seven using a palpation, followed by a direct manual technique to mobilize the viscera

against each other and against the abdominal wall for five to ten minutes, till a relief of stiffness and a reduction of restriction were perceived by the practitioner. The preventive group was treated daily from the first postoperative day in the same way. The control group received no treatment. All rats were killed on the seventh postoperative day to examine the adhesion's quality. The results of the preventive group showed a significantly lower number and severity of adhesions in comparison to the other groups. The four adhesions that could be determined in this group appeared to be disrupted and healed, the superficial peritoneal defects did not seem inflamed compared to the lysis group. In the lysis group Bove and Chapelle found six evidently disrupted adhesions. The mobility of the lysis and preventive group was increased compared to the control group. These findings suggest that adhesion formation can be reduced, when manual mobilization of the viscera and the abdominal wall is performed starting on the first postoperative day. As the first few postoperative days obviously are decisive for adhesion genesis, timely preventive action is essential to accomplish the best results (Bove & Chapelle, 2012; diZerega & Campeau, 2001). Furthermore, there is evidence that the repeated disruption of fibrin bridges inhibited fibroblastic invasion resulting in adhesion formation (Bove & Chapelle, 2012; Raftery, 1981). Facilitating visceral mobility supports fibrinolysis through enhanced fluid flow and thus increased metabolite transition within the peritoneum. Moreover, the authors suggest a continuing mobilization treatment after the lysis of early adhesion, to avoid a re-formation (Bove & Chapelle, 2012).

This study was designed to evaluate the early stages of postoperative abdominal adhesions and therefore gives no account for more established adhesions and tissue fibrosis. Unfortunately, data are missing in this study concerning the strength and exact technique of the applied forces, as well as the number and grade of adhesions that were found in the control group. Nevertheless, it holds very interesting evidence for the early postoperative manual treatment potentially influencing the formation process of adhesions positively.

In a following experimental study with forty rats Chapelle and Bove examined the effects of visceral massage on postoperative ileus (Chapelle & Bove, 2013). The authors divided the animals in a surgery, surgery treatment and control group. The group with included treatment showed enhanced gastrointestinal transit and decreased inflammation parameters compared to the surgery group. The findings indicate that visceral treatment is able to reduce postoperative ileus and inflammation. It was anticipated that postoperative adhesions are promoted by ileus (Chapelle & Bove, 2013; Fu, Hou, Jiang, Wang, & Liu, 2005; Springall & Spitz, 1989). Chapelle and Bove therefore reason that by promoting physiological peristaltic motions, the diminution of postoperative ileus might likely reduce the development of

adhesions. The authors suggest that the treatment should be performed within twelve hours after surgery to minimize adhesion development and inflammation proteins (Chapelle & Bove, 2013).

Partial or total small bowel obstruction (SBO) is one of the severe complications of abdominal adhesion formation (Menzies & Ellis, 1990; Rice et al., 2013). Adhesions that form between bowel loops and the peritoneum or other organs can impede the passage of nutrients through the digestive tract. That may cause the acute and life-threatening condition of obstruction, which instantly demands surgery and adhesiolysis, causing in turn new adhesion formation. SBO symptoms comprise bloating, vomiting, nausea, constipation, abdominal distension and pain and the inability to eat and digest normally. In their study, Rice et al. presented two case reports of patients with a history of SBO due to adhesion formation after repeated abdominal surgery (Rice et al., 2013). The patients' symptoms included digestive problems, pain symptoms, restriction in range motion of the abdomen and hip or lumbar spine and a clear impairment of their daily activities. The authors treated these patients with site-specific manual therapy with the target on manual decrease of adhesions to re-establish physiological mobility and motility of the adherent organs. The therapy was conducted for four hours on five consecutive days with techniques that focused on the disruption of adhesive crosslinks. An exact description of the performance of these techniques was not given in the report by the examiners. Rice et al. assert that restrictions of the visceral gliding between the organs and the abdominal wall were assessed and then treated aiming to produce micro-failure in the restricted tissue to lyse adhesions.

The outcome in both cases was a significant relief of pain of 90%, an improvement of motion and in posture, enhanced visceral, myofascial and osseous mobility and a return to a normal diet. Both patients were able to participate in activities of daily life promptly. The status was evaluated in a one-year follow-up after these treatment sessions and there were no further incidents of SBO or SBO-related symptoms, so that there was no necessity of any further surgical interventions (Rice et al., 2013). Criticism on the study's method could be expressed, because of the lack of an external control group.

The case reports of Rice et al. give a proposal of what manual visceral therapy is potentially able to accomplish if it comes to adhesion-related symptoms and pathologies. To serve as a scientific verification of any posed hypothesis, however these reports are clearly not sufficient.

Infertility is another momentous consequence of adhesion formation in the pelvic area (Wurn

et al., 2008). Given the anatomical proximity of the ovary and the peritoneum, surgical or inflammatory tissue damage can lead to tubal or ovarian dysfunction and result in mechanical infertility. Obstruction of the access of the fallopian tubes by pelvic adhesion is known to be the main reason for biomechanical infertility. Pelvic adhesions can further cause symptoms such as dyspareunia, dysmenorrhea, incontinence and chronic pelvic pain.

Wurn et al. investigated the efficiency of site-specific manual soft tissue treatment promoting fertility and enhancing in vitro fertilization (IVF) rates in women with existing abdominopelvic adhesions (Wurn et al., 2004). In their two pilot studies 53 infertile, premenopausal women with suspected or diagnosed pelvic adhesions were included and underwent ten to twenty hours of site-specific manual treatments. These treatments aimed to improve mobility of the viscera, restrictions of the soft tissues and biomechanical dysfunctions of the pelvic osseous structures that impair the normal reproductive function. The therapists assessed restrictions in the tissues and organs surrounding the fallopian tubes and treated them by manipulating the peritoneum, the ovarian and uterine ligaments and the adherent structures.

The participants were divided in two groups: a natural fertility group and a pre-IVF group. Ten of 14 included women in the natural fertility group became pregnant within one year after treatment. Three of them became pregnant again after the first delivery, which accounts for an enduring therapy success. 22 clinical pregnancies of 25 women were documented in the pre-IVF group. The examiners therefore found evidence in sustaining their hypothesis that site-specific manual treatment promotes fertility in women with abdominopelvic adhesions (Wurn et al., 2004).

In a later study Wurn et al. treated 28 infertile women at the age of 26 to 43 years with fallopian tube occlusion and abdominopelvic adhesions (Wurn et al., 2008). The manual site-specific treatment was coherent to the proceedings of the previous study. The patients were evaluated within a period of three years and the follow-up assessment ended two years after the final treatment. Tubal patency was detected and measured with a hysterosalpingography (HSG) meta-analysis. 17 of the 28 patients showed uni- or bilateral tubal patency, whereof nine of these 17 women reported natural intrauterine pregnancy. Wurn et al. therefore submit that the applied manual soft tissue treatments promoted tubal patency and lysed adhesion tissue.

Further findings of the therapists in this study comprise enhanced flexibility of the ligamentous and soft tissue structures, as well as increased range of motion, accompanied by an improved alignment and biomechanics of the osseous elements (Wurn et al., 2008). The results of these pilot studies indicate that manual treatment of the reproductive organs

and their related structures, which are functionally affected by peritoneal adhesion, might pose an effective and non-invasive and therefore little risk adjuvant treatment option. As in many of the empirical studies, a critical aspect of these studies is again the missing specifications of explicit techniques, but from the rough descriptions it can be assumed that the performances are mainly direct techniques.

Kobesova and Lewit reported on the diagnosis and treatment of a painful active appendectomy scar in their case study (A. Kobesova & Lewit, 2000). The patient suffered from movement restriction and severe lower back pain with radiations. The scar was examined and then treated with a direct technique engaging the barrier of restriction and maintaining tension until a release was palpable. Then the deeper tissue layers including the fascia and the abdominal muscles were treated in the same way till no pathological restriction barrier could be felt. The authors reported a prompt remission of pain symptoms and tissue restrictions. This case report, amongst others, depicts that a global approach to several tissue layers is striking when it comes to the treatment of scars and adhesions.

6.5.2 Indirect OMT (IOMT)

Treffer et al. define an Indirect Method (I/IND) as "…a manipulative technique where the restrictive barrier is disengaged and the dysfunctional body part is moved away from the restrictive barrier until tissue tension is equal in one or all planes and directions." (Treffer et al., 2011, p 30).

In their in vitro experiment Meltzer and Standley studied the varying behavior of fibroblasts, which were either treated with 60-seconds indirect osteopathic manipulative treatment (IOMT) or exposed to eight hours of repetitive motion strain (RMS) and then either treated with IOMT or not (Meltzer & Standley, 2007). The RMS should mimic the conditions of unphysiological or injurious postures or motions and sustained forces. Many motion disorders and pathologies have their origin in biomechanical strain-induced dysfunctions (Zein-Hammoud & Standley, 2015). In the case of abdominal or pelvic adhesions they could be seen as inert contractile forces. The fibroblasts that were exposed to RMS showed significantly raised inflammatory response, including increased secretion of interleukins (IL). 24 hours after strain cessation the IL levels were still increased and in addition fibroblasts showed 15% less proliferation rate. The fibroblasts that were only treated with IOMT did not show raised interleukin levels or proliferation, but a decrease in the IL-3 level of 44%. The fibroblasts that underwent RMS followed by a 60-seconds IOMT exhibited a decrease in proinflammatory IL-6 of 46% and an increase of proliferation of 51% compared to the RMS group. The authors therefore conclude

that IOMT is able to reverse the inflammatory effects in strained fibroblasts and thus underlines the clinical value of IOMT (Meltzer & Standley, 2007).

6.5.3 Combined treatments:

Some authors recommend combined treatment methods, in which certain techniques are applied in sequential combination. One study using a sequence of MFR and direct techniques is the case series description by Wasserman et al. of two female patients with painful, restrictive Caesarean-section scars (Wasserman et al., 2016). The objective of their investigation was to identify whether scar flexibility and related pain and pressure thresholds could be improved by MFR and deep direct techniques. The authors described their interventions briefly in three sequential steps. The first step consisted of five to ten minutes pelvic and abdominal MFR, the second of a direct deep pressure to release scar tension, which was hold for 60 to 120 seconds and in the final step adherent viscera were mobilized. The entire treatment duration was 30 minutes and was performed two times a week, for two weeks. For measurement a Pressure Algometer, an Adheremeter and the numeric pain rating scale were used. The outcomes of all measurements showed clear improvements in scar mobility in all directions, pain and pressure thresholds and pain free activities in both patients. On the evidence of these measurements it can be assumed that a combined treatment including the involved restricted and adherent structures holds great benefits in the pathology of peritoneal adhesions.

6.5.4 Specific OMTs and manual treatment of the fascia

The fascia consists of dense fibrous connective tissue and forms a continuous network around muscles, joints, bones and organs. As fasciae connect, separate and enclose the organs of the abdominal and pelvic cavity, they also pose a relevant objective when it comes to the treatment of adherent tissues and organs through injury or surgery (Minasny, 2009; Zein-Hammoud & Standley, 2015). Fasciae participate actively in tissue repair processes and wound healing, as well as in fibrotic pathologies and adhesion formation (Gabbiani, 2003; Tozzi, 2015).

Fasciae are important elements of force transmission throughout the body (Chaudhry et al., 2007). The fascia's elasticity accounts for its passive resistance to tensile forces. Physiologically the fasciae are in a fluid condition, which enables gliding. Under pathological circumstances such as injury from trauma or surgery, repetitive motion strain and inflammation of the fascia's length and elasticity is reduced, resulting in a fascial restriction and changes of tissue consistency (Meltzer et al., 2010). Schleip et al. report that fibroblast density and the

production of CT proteins such as collagen and myofibroblasts are strongly affected by physical strain (Meltzer et al., 2010; Schleip et al., 2006, p. 2006).

There are several techniques of fascial manipulation, which aim to improve restrictions or pathologies in the fascial system. Myofascial release and fascial unwinding are two techniques used frequently by osteopaths and are reported to improve these abnormalities and restrictions (Meltzer et al., 2010). These techniques are designed to release tension, reduce pain and secretion of inflammatory mediators and to re-establish tissue and joint function (Tozzi, Bongiorno, & Vitturini, 2011). The objectives of a fascial manipulative OMT are the fibroblasts (Minasny, 2009; Zein-Hammoud & Standley, 2015). If the collagen fibers are dismissed from tension, they are able to reorganize themselves and tissue remodeling will be the consequence. As manual treatment of the fascia is apparently able to return the matrix to a low-tension condition, it thereby limits the biosynthetic activity, which is accountable for profuse pathological collagen crossovers. Apart from achieving alterations in the tension of the cell-matrix state, fascial manipulation potentially affects the local release of growth factors, leading to fascial reorganization (Martínez Rodríguez & Galán del Río, 2013). Furthermore, the tissue's gliding capacity will be enhanced by increased fluid viscosity and raised production and distribution of hyaluronic acid (Tozzi et al., 2011). Tozzi suggests that improved gliding between the fascial layers leads to a reduction of collagen cross-links, producing microfailure of the collagen fibrils (Martínez Rodríguez & Galán del Río, 2013; Tozzi, 2012).

Chaundhry et al. explored the viscoelastic deformation of human fascia under extension in manual therapy. They suggest that viscoelastic fascial deformation without tissue damage can be achieved by applying continuous force, which should be equally sustained for 60 seconds. Slow increasing deformation with time should be avoided, in order to permit a plastic stress relaxation response of the tissues (Chaudhry et al., 2007).

Roman et al. investigated the effects of three different types of manual therapy motions - constant sliding, perpendicular vibration and tangential oscillation - on the flow characteristics of hyaluronic acid (HA) below the fascial layer. HA is present throughout the extracellular space in loose connective tissue and in the skeletal muscles of the lower extremity in the human body (Laurent & Fraser, 1992; Piehl-Aulin, Laurent, Engström-Laurent, Hellström, & Henriksson, 1991; Roman, Chaudhry, Bukiet, Stecco, & Findley, 2013). HA provides a gliding interface between the deep fascia, which consists of several layers of loose and dense connective tissue and the epimysium of the muscles (Roman et al., 2013; Stecco et al., 2011). Fasciae and HA are crucial for the transmission of forces and therefore enable the tissues' mobility during breathing or global motions. Roman et al. found out that the fluid pressure of

HA is significantly elevated when the fascia is deformed under manual treatment and that the fluid gap between two fascial layers is thickened. This increased fluid gap enables better gliding between the different tissue layers. The authors detected a diminution of adhesion between the tissue layers. Investigated three different motions, Roman et al. state that perpendicular vibration and tangential oscillation exerted an increased pressure rate compared to constant sliding. This higher pressure led to a better lubrication. The authors' conclusion therefore is that fascial osteopathic manipulation improves the sliding abilities between tissues and that therapists should consider implying the motions of perpendicular vibration and tangential oscillation to achieve an optimal treatment outcome (Roman et al., 2013).

Another recent pilot study of Chamorro Comesañy et al. investigated the effects of a myofascial induction therapy on ten women with Caesarean-section scars. The therapy was performed weekly for eight weeks on scars more than 1.5 years old. This manual treatment method intends to induce CT remodeling through the performance of several, mainly direct techniques, including longitudinal and transversal sliding movements. The measurements of deep tissue changes were conducted with ultrasound and revealed an alteration of aponeurosis thickness after the treatment. The superficial scar changes were performed by scar fold measurement and showed a reduction of scar fold thickness. Chamorro Comesañy et al. could therefore demonstrate that manual therapy leads to CT and collagen remodeling, even in mature scar and adhesion tissue (Chamorro Comesaña et al., 2017). These results indicate that osteopaths and therapists should also include fascial treatment, seeking to improve superficial and deep mobility, when dealing with pelvic or abdominal adhesive tissue (Meltzer & Standley, 2007).

6.5.4.1 Myofascial Release (MFR)

A myofascial release is defined as "A system of diagnosis and treatment first described by Andrew Taylor Still and his early students, which engages continual palpatory feedback to achieve release of myofascial tissues" (Treffer et al., 2011, p.31). The MFR is a specific technique of manual manipulative therapy to treat various tissue restrictions, pain, edema or inflammation states (Bron et al., 2011; T. V. Cao, Hicks, Campbell, et al., 2013; G. D. Deyle et al., 2000; Gail D. Deyle et al., 2005; Walker et al., 2008). These indications are main symptoms of peritoneal adhesions and abdominal scars and therefore make the MFR a likely prime technique to be applied in that sort of pathology. Moreover, as fibroblasts are the main elements of the host response to mechanical forces and are both the predominant cells in adhesion tissue and in fascial tissue, techniques addressing these tissues seem to be striking.

In the MFR treatment a slow-loaded stretch is performed to the long and transvers axis of the affected tissue, which will be held for 90 -120 seconds until a tissue release is felt (Barnes M.F., 1997). A MFR can be either performed as a direct MFR, in which "…, a myofascial tissue restrictive barrier is engaged for the myofascial tissues and the tissue is loaded with a constant force until tissue release occurs" (Treffer et al.,2011, p.31) or as an indirect MFR, in which "…, the dysfunctional tissues are guided along the path of least resistance until free movement is achieved" (Treffer et al., 2011, p. 31).

Practitioners report a change in tissue quality, accompanied by an improvement of mobility and fluid flow and a decrease of inflammation or pain perception in the treated area (T. V. Cao, Hicks, Campbell, et al., 2013; Cubick, Quezada, Schumer, & Davis, 2011; Eisenhart, Gaeta, & Yens, 2003; LeBauer, Brtalik, & Stowe, 2008). This could be due to the viscoelastic and piezoelectric characteristics of connective tissues, which are stimulated by the applied manual load. Scientific evidence from valid clinical studies would, however, be needed to confirm these practical experiences (T. V. Cao, Hicks, Campbell, et al., 2013).

In their study Meltzer et al. explored the potential cellular and molecular mechanisms of RMS and MFR to evaluate their proximate effects (Meltzer et al., 2010). They used cultured human fibroblasts, which were either strained for eight hours with RMS or a 60-second MFR, or received a combined treatment. The RMS mimicked the conditions of unphysiological or injurious postures or motions and sustained forces (Zein-Hammoud & Standley, 2015). In the case of abdominal or pelvic adhesions it could be seen as inert contractile forces. The cells of the RMS group showed a transformation to an elongated fibrotic cell type with actin-containing lamellopodia, decentralization and enlarged intercellular distances. If these responses are applicable for the tissues' reaction to RMS in vivo, these results may display properties of abnormal tissue texture changes, similar to fascial constraint. The results showed that a 60 second 6% applied equibiaxially load is not sufficient to provoke a proliferative or hypertrophic response in human fibroblasts. These findings complement the study results of Dodd et al. and Eagan et al. that acyclic strain induces morphological changes in fibroblasts (Dodd et al., 2006; Eagan et al., 2007). After the MFR treatment the increased apoptotic activity, resulting from the strain, was set to a normal level again. The changes in fibroblasts' morphology might be coherent with fascial restrictions occurring through repetitive motion injuries (Meltzer et al., 2010).

In their 2013 study Cao et al. found out that in bioengineered tendons a raised magnitude and duration of strain leads to progressive upturns of inflammatory cytokines and growth factors. Apart from that Cao et al. found a higher tissue dry weight of the bioengineered tendons after

higher magnitude of MFR treatment (from 9% to 12% elongation), which they explained with an increased fibroblastic ECM production (T. V. Cao, Hicks, Campbell, et al., 2013). They referred to the findings of Breen and Xu et al. that mechanical strain potentially influences the ECM protein production of fibroblasts (Breen, 2000; T. V. Cao, Hicks, Campbell, et al., 2013; Xu, Liu, & Post, 1999). In another study Cao et al. investigated the effects of different duration and magnitude of MFR on the wound size and wound closure rates. The results show that a lower magnitude (3% to 6%) and longer duration (beyond 5 minutes) of MFR resulted in accelerated wound healing, whereas higher magnitudes led to an augmentation of wound size and therefore impaired wound closure (T. Cao et al., 2015). Zein-Hammoud and Standley support these findings with their study and emphasize the impact that direction, duration and magnitude of strain have on the cells' shape and proliferation (Zein-Hammoud & Standley, 2015). The upregulation of ECM production and the inflammatory reaction might be a required event during the proliferative and remodeling phase of wound healing, but it remains to be clarified wether in the later states, such as during adhesion formation, this higher magnitude and duration of strain might have a negative impact potentially enhancing hypertrophic fibrotic events (T. V. Cao, Hicks, & Standley, 2013; Hübner et al., 1996; Tettamanti et al., 2004).

The data of these studies therefore suggest that MFR might both potentially serve as a prophylaxis, dampening inflammation and optimizing wound healing processes. Opposing it could be an activator of fibroblast-mediated inflammation and ECM production.

6.5.4.2 Fascial unwinding (FU):

Treffer et al. define fascial unwinding as

> …a manual technique involving constant feedback to the osteopathic practitioner who is passively moving a portion of the patient's body in response to the sensation of movement. Its forces are localized using the sensations of ease and bind over wider regions. (Treffer et al., 2011, p.29)

This technique is an indirect manual approach to release fascial constraints and re-establish the tissue's mobility. The fascial unwinding aims to stimulate the fascial mechanoreceptors through moderate stretching and so provoke tissue relaxation and activation of the central nervous system until a release is achieved. (Minasny, 2009; Zein-Hammoud & Standley, 2015). The therapist uses shearing, torsional or rotational components to reinforce and unwind the dysfunctional pattern to bring the restricted structure into a position where it is held, till a release takes place (Tozzi, 2012). As in MFR, fascial unwinding intends to release fascial adhesions and to re-establish tissue gliding and mobility (Tozzi et al., 2011). In their study

Tozzi et al. investigated the effects of MFR and FU on pain perception and on sliding mobility of the fascial layers in patients with neck pain and low back pain (Tozzi et al., 2011). The gliding capacity of the fascial layers was measured with dynamic ultrasound (US) before and after treatment or with no treatment. The results showed an improvement in fascial sliding characteristics and a short-term pain relief. Whether these conditions can be established in a long-term state needs to be clarified in further investigations.

There were no articles found examining the effects of FU on the pathology of peritoneal adhesions, but Tozzi and many other authors underline the intense connection between organs and the fascial system (Tozzi, 2012; Tozzi et al., 2011). As it is the aim of these fascial techniques to re-establish tissue mobility and eliminate restrictions, FU seems to be another interesting technique, particularly for early stages of adhesion formation or for balancing the entire restriction-affected body environment.

6.5.4.3 Strain & counterstrain (CS)

Strain & counterstrain 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 response 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 can be reduced by indirect OMTs, such as 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 improves wound healing, whereas the inflammatory interleukins can be decreased, which is beneficial for the termination of a repair process. A minimum and maximum threshold, which affects cellular viability and physiologic change, can 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). As these techniques are known to have beneficial impact on inflammatory processes and interleukin secretion, they seem to pose another remarkable facility of affecting adhesions.

7   Discussion

Peritoneal adhesions are a frequent pathology that osteopaths encounter in their daily practice. For many patients adhesions pose a severe impairment and a considerable reduction of their daily life quality and in some cases they can even result in life-threatening conditions. However, there is a restricted amount of studies investigating the non-invasive manual treatment options. Therefore, the intention of this thesis is to review and discuss existing literature regarding peritoneal adhesions and their manual treatment, under consideration of the wound healing process and influencing factors, such as the mechanical environmental conditions. The author gives an overview of possible manual treatment options, in particular the osteopathic manipulative treatment, and refers to their cellular effects, thereby providing a theoretical basis on the treatment of peritoneal adhesions. Before returning to answer the research question and the hypotheses, the most relevant results of literature are summarized.

7.1   Discussion of literature results

A crucial role in the genesis of adhesion formation is TGF-β, whose production is mainly dependent on the mechanical environment (Chegini et al., 2001; J.-J. Duron, 2007; S. E. Mutsaers, McAnulty, et al., 1997; James J. Tomasek et al., 2002). The kind and duration of mechanical stimuli therefore pose an important factor for the secretion of this growth factor and for the course of tissue repair. Moreover, the organization and density of fibers is mainly dependent on mechanical input. The results of the studies investigating the influence of strain magnitude and duration of stretch on CT confirmed that these parameters are decisive in modulating fibroblasts' remodeling of ECM (Balestrini & Billiar, 2006; Bouffard et al., 2008; Zein-Hammoud & Standley, 2015).

Bouffard et al. demonstrated that magnitude, duration and timing of mechanical load on injured tissue are pivotal for a constructive and anti-fibrotic therapeutic treatment effect (Bouffard et al., 2008). They showed that ten minutes of 20% to 30% static stretch two times a day for seven days resulted in a decrease of TGF-β_1-mediated new collagen deposition. The local effect of this tissue stretch inhibited collagen formation for some days after stretching and could therefore possibly impede adhesion formation from the beginning, if regularly applied. Hence this study supports the approach of manual therapy to prevent and treat fibrotic or contract tissue adhesions and scars locally with short-time tissue stretch over the usual range of motion to encourage beneficial tissue remodeling (Bouffard et al., 2008; Hardy, 1989). Thus these results stand in opposition to the findings of Cao et al. and others, who detected that repetitive motion strain with a magnitude of 10% over a duration of eight hours resulted in a decrease of wound closure rates and a diminishment of fibroblast proliferation (T. V. Cao, Hicks, &

Standley, 2013). The authors detected an increase of fibroblast apoptosis and production of inflammatory cytokines, accompanied by morphologic alterations in actin architecture (T. V. Cao, Hicks, & Standley, 2013; Dodd et al., 2006; Eagan et al., 2007; Meltzer et al., 2010). Raised pro-inflammatory cytokine secretion and induction of fibroblast proliferation were shown through a heterobiaxial acyclic strain direction in the study of Eagan et al. (Eagan et al., 2007). Furthermore Cao et al. showed that a lower magnitude (3% to 6%) and longer duration (more than five minutes) of MFR accelerated wound healing, whereas higher magnitudes again led to an expansion of wound size and therefore impaired wound closure (T. Cao et al., 2015). This is partly consistent with the findings of Eagan et al. that a short duration, cyclic moderate (< 10%) strain would be most beneficial in the treatment of tissues (Eagan et al., 2007). This would be valuable for the cessation of a repair process. The data suggest that MFR might both potentially serve as prophylaxis, dampening inflammation and optimizing the wound healing process, and also act as an activator of fibroblast-mediated inflammation and ECM production. In addition to this Meltzer and Standley could demonstrate that indirect OMTs, such as CS are able to reduce the level of inflammatory interleukins IL-1β and IL-6 (Meltzer & Standley, 2007).

Langevin et al., and Dodd et al. demonstrated that increased magnitude and longer duration of strain led to a higher collagen density and to deformation of fibroblast cell shape. (Dodd et al., 2006; Helene M. Langevin et al., 2013). A strain magnitude of 10% stimulated cell proliferation and a slight loss of cell viability. Strain magnitudes of 30% led to prevailing cell destruction (Dodd et al., 2006). Langevin et al. assume that tissue stretching over 25% disengages fibroblast processes or cell-to-cell contacts (H. M. Langevin, 2004). Cao et al. state that strain magnitudes over 10% led to a thinning of collagen fiber diameters in bioengineered tendons, whereas strain magnitudes between 3% and 9% did not lead to measurable alterations (T. Cao et al., 2015). Cao et al. and Eagan et al. state that only heterobiaxial strain affects the morphology of fibroblasts (T. V. Cao, Hicks, Campbell, et al., 2013; Eagan et al., 2007). These might be important considerations when it comes to manual treatment of mature adhesions, in which crosslink disruption and a restart of the tissue remodeling process is the target, whereas in new and less established scars or adhesions, a balanced and continuous stimulus might possibly provide a more beneficial input.

Roman et al. and Tozzi et al. assert that manual treatment of the fascial layers can remove restrictions and dysfunctional patterns (Roman et al., 2013; Tozzi et al., 2011). By enhancing the fascial gliding capacity, tissue and joint function are re-established and at the same time pain and inflammatory processes are reduced. Tozzi further states that when the tissues are released from adhesions or tension, they are able to reorganize themselves (Tozzi et al., 2011).

Wasserman et al. assert that a combination of MFR and direct techniques improved scar mobility, associated pain and pressure thresholds in Caesarean section scars (Wasserman et al., 2016). The findings of Chamorro Comesañy et al. are complementary to this, as they showed that manual myofascial therapy can induce both deep and superficial CT remodeling in mature Caesarean section adhesive tissue (Chamorro Comesaña et al., 2017). Wurn et al. could further verify their hypothesis that site-specific manual treatment promoted fertility in women with abdominopelvic adhesions (Wurn et al., 2004) and lead to significantly increasing patency of infertile women with fallopian tube occlusion (Wurn et al., 2008).

On the basis of the different results, it can be hypothesized that especially in the early stages of wound healing the prolonged strain duration over eight hours is responsible for the counterproductive effects. Apparently the kind of technique has to be adapted in its magnitude, duration, direction and timing to the stage of tissue healing or adhesion formation. For example the upregulation of ECM production and inflammatory reaction are mandatory events during the inflammatory and proliferative phase of wound healing, but it remains to be clarified if in the later states, such as during adhesion formation, a higher magnitude and duration of strain could possibly bear negative influence, potentially enhancing hypertrophic fibrotic events (T. V. Cao, Hicks, & Standley, 2013; Hübner et al., 1996; Tettamanti et al., 2004). In contrast, a re-activation of the inflammatory response and induction of tissue remodeling might be necessary in the treatment of mature adhesions.

7.2   Personal interview with Michaela Liedler, Osteopath

In the framework of the discussion the author wants to report a personal conversation with Michaela Lieder, a physiotherapist and osteopath, who passed her final exam at the WSO (Wiener Schule für Osteopathie) in Vienna 2011. Liedler focuses on the treatment of scars and adhesions and recently held a lecture on the topic of scars and the treatment of intraabdominal adhesions at the WSO in April 2017. In the course of her practice she has worked a lot with patients suffering from the long-term effects of scars and abdominopelvic adhesions. Therefore, Liedler gained a lot of experience in their treatment and developed a certain treatment concept including individual techniques and handholds. These developments were initiated, when she treated a patient with temporomandibular joint (TMJ) fibrosis, who reported pain during chewing and decreased mouth opening ability. Additionally, this patient showed an adhesive clotted appendix scar. In their first therapy session Liedler treated only the scar, but not the TMJ. Afterwards the patient immediately perceived a pain relief in her TMJ and was able to open her mouth much more easily. This is

just one descriptive example of many more, which illustrate the extensive consequences that adhesive tissues and scars have on a range of functions in the body.

Liedler's focus is set on the gliding capacity of the different tissue layers and organs, on the patency of respiration movements through the body diaphragms and a physiological range of motions in the surrounding joints. These findings are coherent with those in the case studies of Kobesova and Lewitt (Kobesova & Lewit, 2000). Improvement of the fascial gliding ability through manual therapy motions was also investigated by Roman et al. (Roman et al., 2013). They confirmed that increased pressure on the fascial layer leads to an upsurge of the hyaluronic acid fluid flow and therefore to a better lubrication between the tissue layers (Roman et al., 2013).

Liedler confirms that the therapist has to differentiate between new scar tissue and mature scar or adhesion tissue, as mature adhesion tissue can be collagenous ligament-like established tissue. In this case Liedler emphasizes strong direct techniques, in which she pulls the scar tissue to the restriction barrier and treats it with three-dimensional oscillatory gliding movements beyond the adhesional tissue barrier. The duration of the single techniques is orientated on the individual pain perception of the patient. Starting from VAS (visual analogue scale) 9 or 10 the therapist has to hold on until the patient reports a decline to VAS 2 or 3. This is subject to broad individual fluctuation in time.

Liedler underlines the importance of breaking the collagenous cross-links in adhesions and therefore bringing the tissue to a state of collagenase activity. This is conform with the study results from Langevin et al. that tissue stretching over 25% might disrupt fibroblast processes or cell-to-cell contacts (H. M. Langevin, 2004) and Cao et al., who state that strain magnitudes over 10% lead to a thinning of collagen fiber diameters (T. Cao, Hicks, Zein-Hammoud, & Standley, 2015). Furthermore, it would be necessary to induce an inflammatory reaction to re-start tissue remodeling and collagen organization. The study findings of Cao et al., Eagan et al. and Meltzer et al. that strain magnitudes of 10 % lead to an increase of apoptosis, an upturn of inflammatory cytokines and morphologic changes in actin architecture and therefore subsidize the fact that manual treatment is able to induce such reactions (T. V. Cao, Hicks, & Standley, 2013; Dodd et al., 2006; Eagan, Meltzer, & Standley, 2007; Meltzer et al., 2010).

However, in acute states or if manual treatment of such symptomatic mature ligamentous adhesions cannot relieve the symptoms, an adhesiolytic surgery might be necessary. When dealing with new scars and adhesion tissue the primary aim is to dampen the inflammation to prevent the establishment of adhesions and accelerate and optimize the healing process. As

discussed before, an overreaching inflammatory reaction with enhanced cytokine levels reduces the fibrinolytic capacity and therefore facilitates adhesion formation. Referring to the articles of Cao et al., Eagan et al. and Meltzer & Standley low magnitude and longer duration of equbiaxial strain have shown to be most effective in downregulating the proinflammatory IL levels and in accelerating wound healing (T. Cao et al., 2015; Eagan et al., 2007; Meltzer & Standley, 2007). In addition Bouffard et al. demonstrated that the appropriate extent, duration and timing of mechanical load on injured tissue is decisive for the of TGF-β_1-mediated new collagen deposition (Bouffard et al., 2008). Moreover, Boland and Weigel pointed out that the therapeutic window in adhesion prophylaxis lies within the fifth to seventh postoperative day (Boland & Weigel, 2006). This implies that an early treatment would be most beneficial to avoid adhesion development from the start. On the other hand, it has to be considered that a limitation to an early treatment begin might be the surgeon's concerns regarding the impact on suture and wound strength and recurrent bleeding.

To sum up these findings, the type of mechanical input is decisive for the effect on the tissue and has to be chosen in regard to the tissue's condition and the focus of intervention. The duration of treatment should be varied in regard to the maturation and strength of the existing adhesions. Consistently with the experimental results of Bove and Chapelle and (Martínez Rodríguez & Galán del Río, 2013)Liedler attests that the treatment of mature and profound adhesion tissue requires longer enduring techniques than new and not established ones (Bove & Chapelle, 2012; Martínez Rodríguez & Galán del Río, 2013). Liedler further confirms that no matter how old the adhesion or scar tissue is, manual treatment can always achieve a functional and integrative improvement within the whole system. In regard of the gathered data and reports, it is now possible to answer the posed research question and its hypothesis.

7.3 Discussion of the research questions and hypotheses

7.3.1 Research question

Is osteopathic manual treatment (OMT) a suitable option to influence the development of post-operative abdominal adhesions and already existing adhesions and have there been specific methods mentioned that seem effective?

7.3.2 Hypothesis I

OMT is able to influence both the development of and the already existing post-operative abdominal adhesions.

In the case studies and reports discussed before, therapists reported concordantly on a notable release in the restricted tissue during and after their manual treatment, accompanied by a pain relief perceived by the patients. These recordings indicate that the decrease of tension in the adherent tissues and mechanical input on nerves and pain-sensitive structures during manual treatment enhance normal mobility and function (A. Kobesova & Lewit, 2000; Alena Kobesova, Morris, Lewit, & Safarova, 2007; Wasserman et al., 2016; Wurn et al., 2008). It has been reported that adhesion-related symptoms, such as SBO, infertility, movement restrictions and chronic pain could be significantly relieved or even eliminated using visceral OMT techniques (A. Kobesova & Lewit, 2000; Alena Kobesova et al., 2007; Rice et al., 2013; Wasserman et al., 2016; Wurn et al., 2004, 2008). Bove and Chapelle indicated that visceral mobilization can prevent the development of adhesion and lyse existing adhesions in early stages (Bove & Chapelle, 2012; Chapelle & Bove, 2013). In addition to that Wasserman et al. and Chamorro Comesaña et al. demonstrated that even mature adhesion tissue can be treated successfully with myofascial techniques, achieving measurable improvements in pain perception and tissue quality (Chamorro Comesaña et al., 2017; Wasserman et al., 2016). Kobesova emphasizes the importance of the mobilization of all tissue layers by using direct stretch technique, but points out that an exact differential diagnosis is needed to distinguish adhesion tissue from severe organic pathologies (A. Kobesova & Lewit, 2000; Alena Kobesova et al., 2007). For an optimal treatment outcome, the practitioner has to distinguish between the maturity and strength of adhesions, as they have to be addressed in different ways. The consensus of the reviewed literature seems to be that manual treatment, either direct or indirect, of adherent peritoneal or pelvic organs and their related structures poses an effective, non-invasive and therefore low risk adjuvant treatment option. The earlier postoperative treatment can be applied, the better adhesion formation and establishment can be avoided and prognosis can be influenced in an optimal way (Boland & Weigel, 2006; Bove & Chapelle, 2012).

From the personal practical experiences of Michaela Liedler and the author of this thesis, direct techniques have occurred to be more effective in the treatment of mature adhesions. The author therefore regards hypothesis I to be verified.

7.3.3 Hypothesis II

Visceral and fascial techniques, such as myofascial release (MFR) and fascial unwinding (FU) have been mentioned in literature, and seem promising in the treatment of postoperative abdominal adhesions.

Osteopathic techniques addressing peritoneal adhesions can either be direct, indirect or combined. In the reviewed literature, there was no evidence found that one of these techniques is more efficient than the others. Though the author could retrieve more literature examining direct manual treatments, many reports or studies did not explain or describe their used techniques accurately, so it remained to the author to determine to which type they might belong.

The myofascial release and its tissue effects were investigated by several authors. Cao et al. for example suggest that moderate manual treatment, such as a MFR, is beneficial to dampen systemic and local inflammation by downregulating the secretion of several inflammatory cytokines (T. V. Cao, Hicks, Campbell, et al., 2013). Meltzer et al. assert further that MFR regulates fibroblast apoptosis, proliferation and actin architecture (Meltzer & Standley, 2007). Hence it can be assumed that slow-loading MFR and FU are well suited to release tissue tension, which is more beneficial for the early stages of adhesion formation. Fascial manipulations such as FU or MFR have not only been confirmed to achieve a release of tissue tension, but also to have beneficial effects on fluid flow and therefore on circulation. These techniques in particular enhance the gliding capacity of fascial tissues, which reduces potential adherences. It can therefore be assumed that fascial techniques, such as MFR and FU exert beneficial influence on the pathology of peritoneal adhesions. Hypothesis II can therefore be considered to be verified, within the limits of scientific validation of the reviewed experimental and case studies.

7.4 Limitations of this thesis

This thesis is based on existing English or German literature examining or describing the pathophysiology and development of adhesions and their manual treatment, considering cellular effects. Therefore, this thesis can only consider existing data from literature reviewed until April 2017 and summarize and discuss their study results. Only a limited quantity of literature was found examining the manual treatment of adhesions in humans in-vivo. More studies found based on in-vitro experiments investigating the effects of mechanical load on tissues or animal studies, both of which cannot be transferred one-to-one to the patient in-vivo. Many studies examining connective tissue of bioengineered tendons or of loose CT were found, but none investigated mesothelial CT. Some deductions therefore remain speculative. Nevertheless, the author regards some of these study results to be important and applicable for the pathology of human peritoneal adhesions and tried to transfer their essential findings to it. It has to be mentioned that the drawn conclusions thereby underlie personal considerations.

Moreover, only a restricted amount of literature could be found investigating manual treatment of mature adhesions. Many of the studies and reports neither give detailed information of the maturity of scar or adhesion tissue, nor of the severity or tissue quality. Furthermore, many empirical studies lacked a detailed and reproducible description to make their measurements and treatments traceable. The reliability of these studies and their results have therefore to be considered critically for their applicability. A quantifying scale or measurement could provide better confirmability and validity. Ultrasound sonography measurements have been reported to deliver reliable results and therefore could be used as a non-invasive tool to show the abdominopelvic conditions before, during and after the treatment. It would be essential to have reliable study results on this to develop a well-grounded therapy concept.

8   Conclusion

Peritoneal adhesions are a frequent pathology that osteopaths encounter in their daily practice. For the patients adhesions mostly implicate serious confinements and related pain, accompanied by a grave disturbance of their daily life. In many cases the concerned person cannot consciously associate the symptoms with their real origin. Hence it is in the duty of the therapist to trace these dysfunctions to their source and to treat them, aiming to restore unimpaired functions. A variety of osteopathic manipulative techniques potentially holds considerable benefits for patients who are affected by peritoneal adhesions. The reviewed literature comprised direct, indirect or combined osteopathic techniques, which all seem to comprise constructive input regarding the different stages of wound healing and adhesion formation or establishment. A combination of techniques is recommended repeatedly and seems to pose a substantial and efficient treatment approach (referring to the conversation with Michaela Liedler or regarding the articles of (Chamorro Comesaña et al., 2017; Wasserman et al., 2016)). The gliding capacity of tissues and the ability of viscera to move freely during motion and breathing is the essential principle for unimpaired function and vitality of all organs and tissues (A. Kobesova & Lewit, 2000; Alena Kobesova et al., 2007). The prime target of all OMTs is therefore to establish tissue mobility and to remove restrictions, which is affirmed by almost all authors cited before. Therapists such as Tozzi, Kobesova et al. and Liedler emphasize the importance of restoring the tissue's full gliding capacities.

This thesis sought to provide an overview and résumé over the most important treatment options and their potential effects and serve as a basis for new investigations or studies on this issue. A reliable empirical study using ultrasound to investigate the effects of osteopathic manipulative treatment on peritoneal adhesions would be needed to provide new, valid and objectified   results.

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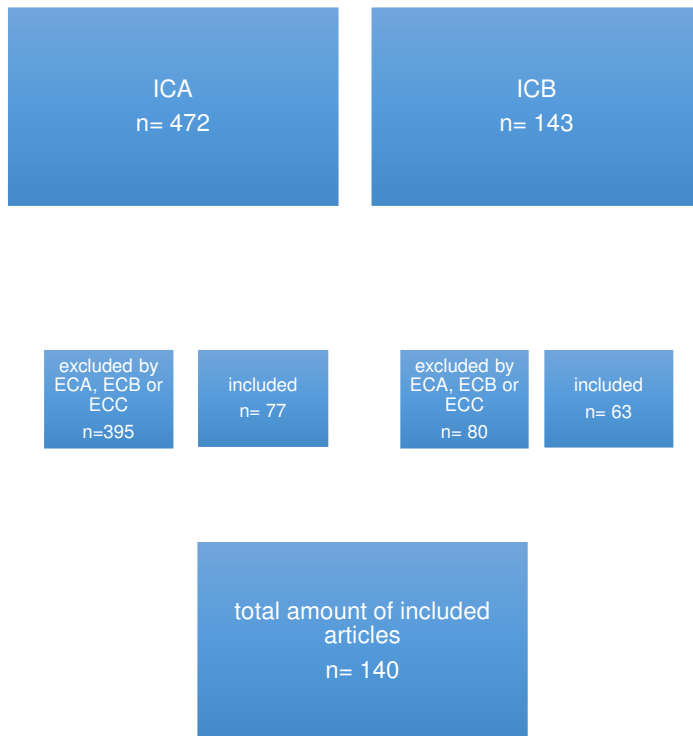
FIGURES

ABBREVIATIONS

α-SMA   alpha-smooth muscle actin

ATP   adenosine triphosphate

CAM   cell adhesion molecule

CT   connective tissue

CS   strain & counterstrain

(D/DIR)   direct method

DOMT   direct osteopathic manipulative treatment

ECM   extracellular matrix

EGF   epidermal growth factor

EMT   epithelial-to-mesenchymal transition

FA   focal adhesion

FAK   focal adhesion kinase

FB   fibroblast

FGF   fibroblast growth factor

FN   glycoprotein fibronectin

FU   fascial unwinding

GTP   guanosine triphosphate

HSG   hysterosalpingography

ICA   inclusion criteria A

ICAM   intercellular adhesion molecule

ICB   inclusion criteria B

ICC   inclusion criteria C

IL   interleukin

ILc   interferon-c

I/INDIR   indirect method

IOMT	indirect osteopathic manipulative treatment
IVF	in vitro fertilization
MC	mesothelial cell
MFB	myofibroblast
MFR	myofascial release
MAPK	mitogen activated kinase
MDC	macrophage-derived chemokine
MMP	metalloproteinase
MMT	mesothelial-to-mesenchymal transition
mRNA	messenger ribonucleic acid
OMT	osteopathic manipulative treatment
PA	plasminogen activator
PAI	plasminogen activator inhibitor
PAI-1	plasminogen activator inhibitor type 1
PAI-2	plasminogen activator inhibitor type 2
PDGF	platelet-derived growth factor
RMS	repetitive motion strain
SBO	small bowel obstruction
TGF-β	transforming growth factor beta
TIMP	tissue inhibitor of metalloproteinase
TMJ	temporomandibular joint
TNF-α	tumor necrosis factor alpha
tPA	tissue plasminogen activator
uPA	urokinase plasminogen activator
US	ultrasound
VAS	visual analogue scale

VCAM   vascular cell adhesion molecule

VEGF   vascular endothelial growth factor