The function of the myofibroblast during human dermal wound repair

The effect of externally applied physical stimuli on the activity of myofibroblasts during dermal wound repair

Victoria Weps
Klausstrasse 50
8008 Zürich

Bachelor Thesis
Zürcher Hochschule für angewandte Wissenschaften, Winterthur (ZHAW)
2006, PT06e, S01-725-019
Supervisor: Judith Tobler-Harzenmoser
Submission Date: 19.06.2009

Table of Contents

Abstract

Topic

Myofibroblasts are a vital cellular component of the wound healing process. Much interest and research has already been shown in dermatology and biology. To date, relative little research has been conducted in this field from a physiotherapy aspect.

Objective

This paper summarises multiple studies conducted on myofibroblastic involvement in wound healing and tissue repair. Particular attention is given to processes relevant to effective physiotherapy treatment planning with regards to the effects of externally applied forces on myofibroblast and their activity during dermal wound healing.

Results

Research has shown that myofibroblasts communicate with each other as well as the extra cellular matrix (ECM). The ECM shields myofibroblasts from stress. As a result of injury, the damaged ECM is no longer able to provide this function resulting in increased myofibroblastic activity. In conclusion, myofibroblasts are shown to respond to external stimuli with increased activity.

Introduction

Physiotherapists are confronted with patients suffering injury to the musculoskeletal system either due to accident or post-operation. Despite the broad spectrum of injuries, patients must receive the best possible therapy for their rehabilitation which must pay due attention to the physiological wound healing process of the body. Effective rehabilitation, according to van Wingerden (1998), is achieved through regaining homeostasis. For this, physiotherapists must have in-depth knowledge on the physiological processes associated with wound healing. This will allow the construction of a customized rehabilitation plan to suit physiological wound healing stages. Despite this due care, the body can react in unforeseen ways, for example the range of motion can become compromised. The ideal healing process requires a symbiosis of many different processes, all of which a well qualified physiotherapist should be informed and should apply to his or her therapy planning.

In literature aimed at physiotherapists, e.g. the book by Frans van den Berg, the wound healing process is described in four phases. The most important phases are described, in great detail. The cell type, to which the greatest role is attributed, is called the fibroblast.

Van den Berg mentions that the fibroblasts carry a vital role in the healing process, in particular the myofibroblasts, a subtype of fibroblasts. Myofibroblasts, so van den Berg, display a contractile ability and are responsible for the stabilization of the new growth of tissue. This gives the myofibroblasts an elementary role in wound healing. Unfortunately, literature for physiotherapists tends to be rather rudimentary in explanation and discussion of myofibroblasts. This leaves many important questions to their function and structure open. If myofibroblasts have a contractile element, how exactly does this translate to the surrounding tissue? What communication mechanisms do they employ? How do they respond to external stimuli? At which point, during the healing process are they at their peak activity?

The myofibroblasts are of great interest to the medical profession as they are associated not only with functional problems, illnesses but also with aesthetic issues such as hypertrophic scarring.

Since Gabbiani 1973 first coined the term – myofibroblast – many studies have been conducted into this central cell type. Dermatologists researched how the activity of myofibroblasts can be influenced. Which aspect of all this research is relevant for physiotherapy? This is the starting point for the questions that this paper will try to answer. In particular, what is the function of myofibroblasts during wound healing and what is the effect of externally applied mechanical stimuli on the activity of myofibroblasts during dermal wound repair.

1. Tissue repair

A major component of physiotherapy is managing injuries of the musculoskeletal system and therefore the accompanying wound healing plays a vital role which makes in-depth knowledge of the physiological processes involved in wound healing imperative (van den Berg, 2003). Van den Berg (2003, p.48) presents the argument that a physiotherapist should know each of the physiological phases of wound healing in order to construct the most effective therapy plan for the patient . Effectiveness, so van den Berg (2003), means that the therapy is adapted to the different phases of wound healing.

Van den Berg (2003) and van Wingerden (1998) classified the process of wound healing into four phases: the inflammatory, proliferative, consolidation and remodelling phase. Van Wiegerden (1998, p. 61) states that it is not possible to clearly separate and define these phases as they can occur almost concurrently and will overlap.

This is a short overview of the essential processes during wound repair, paying particular attention to the function of myofibroblasts. Other cellular process will not be described in detail.

1.1 The Inflammatory Phase (day 0-5)

The inflammatory phase is separated into a vascular and a cellular phase (van den Berg, 2003). During the vascular phase, the blood coagulates and repair of the vascular system of the injured tissue begins (van den Berg, 2003, p. 47). This stimulates the macrophages that in turn send an impulse to the fibroblasts. Fibroblasts begin to proliferate and differentiate into myofibroblasts (van den Berg, 2003). Furthermore, the production of collagen type I and III is also part of the vascular phase according to van den Berg (2003).

The cellular phase begins on the second day post-trauma. It is characterised by the fibroblast proliferation and the accumulation of myofibroblasts (van den Berg, 2003). This part of the healing process continues up to day five post- trauma.

1.2 The Proliferative Phase (day 5-21)

By day five post-trauma, the inflammatory phase should be terminated (Hüter Becker and Dölken, 2005). The number of leucocytes, monocytes and lymphocytes decrease during the proliferative phase and the collagen synthesis is pronounced at this point (van den Berg, 2003).

According to van den Berg (2003), after 14 days, the only cells in the regenerated tissue are fibroblasts and myofibroblasts. However, there may be some mastcells present around the wound area. Hüter Becker et al. (2005) explain that the myofibroblasts have a stabilizing function around the wound area.

1.3 The Consolidation Phase (day 21-60)

During this phase, the newly produced collagen needs to be stabilized (van den Berg, 2003). The fibroblasts begin to secret the matrix during this phase. It is the increased density of the matrix which increases the tensegrity of new tissue (van den Berg, 2003). According to Hüter Becker et al. (2005) the wound no longer requires the level of protection that the myofibroblasts provide, therefore their number decreases and the number of fibroblasts increases.

The collagen fibres need to become stronger and the production of the matrix is still high (van den Berg, 2003). The stability of the tissue is further increased through the conversion of collagen type III fibres into collagen type I (van den Berg, 2003).

1.4 The Remodelling Phase

The transition from the consolidation phase to the remodelling occurs smoothly with no clear boundaries (van den Berg, 2003). Collagen synthesis remains at a high rate up to day 120 post-trauma. By day 150 post-trauma, approximately 85% of the collagen type III fibres have been replaced by the more stable collagen type I fibre, furthermore, during this phase, the number of fibroblasts will decrease (van den Berg 2003).

2. The myofibroblast

Myofibroblasts were initially discovered in granules tissue sites in healing wounds and described by Desmoulière, Chaponnier and Gabbiani in 2005 as “modulated fibroblast with features of smooth muscle (SM) cells and bundles of microfilaments”.

In 2008 Wipff and Hinz went on to summarize myofibroblasts as “reparative connective tissue cells that contribute to the reconstruction of injured tissue by secreting new extracellular matrix and by exerting high contractile forces”. Smooth muscle (SM) cells appear in vessel walls and in the wall of viscera and are generally SM cells are able to generate muscular tone without expending too much energy (Schiebler and Schmidt, 2003 p.72).

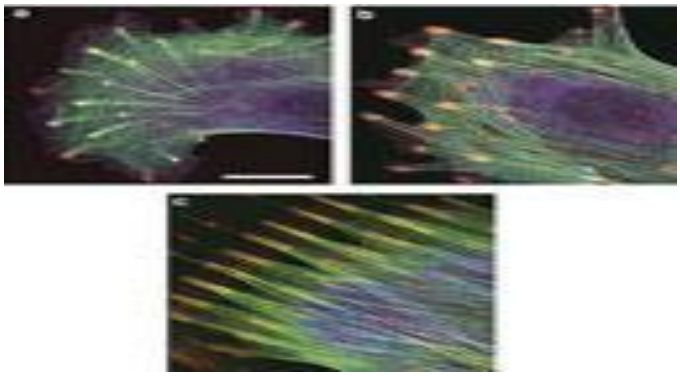

Fig. 1. Myofibroblast morphology. Thick stress fibre bundles that incorporate α-SM-actin (green). Hinz (2007)

According to Desmoulière et al. (2005) myofibroblasts are present in “practically all fibrotic situations characterized by tissue retraction and remodelling”. The myofibroblast cytoplasm contains the contractile elements α-SM-actin and myosin (Tomasek, Vaughan, Kropp, Gabbiani, Martin, Haaksma and Hinz, 2006). The actin and myosin filaments are arranged in bundles and these bundles are called stress fibres (van Wingerden, 1998). Actin in general is a structural protein and by polymerisation it builds microfilaments and can therefore develop properties of stiffness (Schiebler, 2005, p. 18)

2.1 Myofibroblast origin

According to Hinz, Sem, Phan, Thannickal, Galli Bochaton-Piallat and Gabbiani (2007) myofibroblasts have a very heterogeneous origin. However, Hinz et al. (2007) go on to

postulate that “their development follows a sequence of events”. Desmoulière et al. (2005) go on to propose that fibroblasts are recruited from the intact dermis situated next to the wound. Hinz (2007) suggests that another source could be the pericytes from vascular structure. Further sources, summarized by McAnulty (2007) include: Epithelial cells, bone marrow and tissue derived from mesenchymal stem cells. The level of contributions from these myofibroblast sources are “currently a topic of intense debate due to the potential implications for therapy in wound healing, cancer and fibrosis” (McAnulty, 2007). The epithelial source may appear predominantly during cancer progression, however “its role in tissue response to epithelial stress or injury, at least in vivo, is more controversial” (McAnulty, 2007).

2.2. Differentiation of fibroblast into myofibroblasts

The fibroblast-into-myofibroblast differentiation represents a key event during wound repair (Hinz, 2007). After tissue injury, myofibroblasts become activated and migrate into the damaged tissue to synthesize the extra-cellular matrix (ECM), so Hinz et al. (2007). The ECM consists of proteoglycanes and collagen fibres, which are produced by the fibroblasts (Junqueira, Carneiro, & Kelley, 2002). During wound healing and tissue repair, “fibroblasts acquire smooth muscle cell characteristics and differentiate into contractile myofibroblasts” (Desmoulière et al., 2005).

Fibroblasts in intact tissue are stress-shielded by a functional ECM and they do not develop contractile features or cell matrix adhesions (Hinz, 2006). After an injury, the composition, organization and mechanical property of the ECM change (Hinz, 2007). With increasing stress in the ECM, which is a result from their own remodelling activity, protomyofibroblasts develop into “differentiated myofibroblasts” (Hinz, 2007).

Desmoulière et al. (2005) say that the modulation of fibroblastic cells begins with the appearance of the protomyofibroblast. The stress fibres of protomyofibroblasts contain only β- and γ-actins (Desmoulière et al., 2005). Protomyofibroblasts develop into differentiated myofibroblast with stress fibres containing the contractile protein α-SM actin (Desmoulière et al., 2005). The presence of α-SM actin is the most reliable marker of the myofibroblastic phenotype (Desmoulière et al., 2005).

According to Hinz (2006), the differentiation of fibroblast into myofibroblast can be understood as a two-step process. The first step is the modulation of fibroblast, which contain no stress fibres but cytoplasmic actin, into protomyofibroblasts (Tomasek, Gabbiani, Hinz, Chaponnier and Brown, 2002). This modulation process is at present “not well explored” (Desmoulière et al., 2005).

The second step is described by Desmoulière et al. (2005) as a “switch from the protomyofibroblast to the differentiated myofibroblast”. This step has been related “to the production by inflammatory cells, and possibly by fibroblastic cells, of transforming growth factor-β1 (TGF-β1)” (Desmoulière et al. 2005). Protomyofibroblasts are, according to Tomasek et al. (2002), poorly differentiated myofibroblasts, that contain stress fibres and cytoplasmic β- and γ-actin.

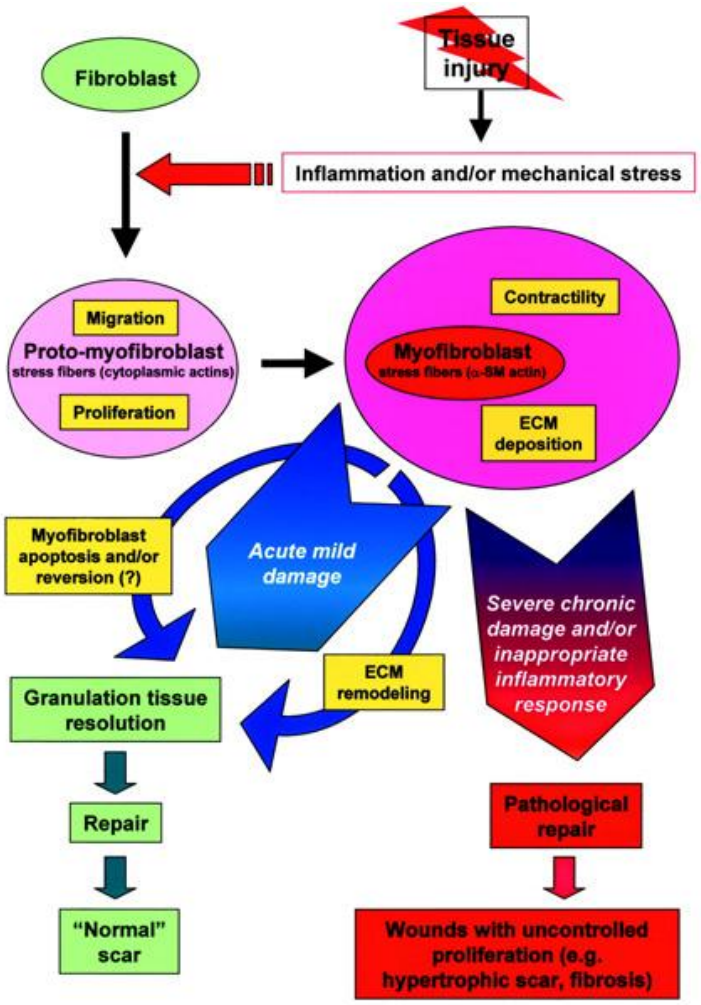

Fig.2 The differentiation of fibroblasts into myofibroblasts
Desmoulière, Darby & Gabbiani (2003).

The differentiation of protomyofibroblasts into myofibroblasts is induced by TGF-β and the local presence of fibronectin, a specialized ECM protein. (Tomasek et al., 2002). The study goes on to claim that it is remarkable, that the myofibroblast differentiation is regulated by both, cell products (e.g. TGF-β1) and ECM components such as fibronectin. Furthermore, it is becoming more accepted that mechanical factors play an important role in the differentiation of the myofibroblast (Desmoulière et al., 2005).

2.3 Deregulation/ Apoptosis

For the treatment of diseases involving myofibroblasts, the question of the reversibility of the myofibroblast differentiation is very important (Desmoulière et al., 2005). The publication assumes that fibroblasts remaining in granulated tissue after reepithelialisation have reverted to a more quiescent, non-contractile phenotype that lacks the microfilament bundles, present during the contractile phase of healing. However, this modulation has not been shown clearly in vivo (Desmoulière et al., 2005).

In physiological remodelling such as during dermal wound healing, the contractile activity of myofibroblasts is terminated as soon as the tissue is repaired (Hinz, 2007). When the continuity of the epithelia has been renewed, in normal tissue healing myofibroblasts disappear through an apoptotic process (Hinz and Gabbiani, 2003). In pathological wound healing, myofibroblast activity persists and leads to tissue deformation (Hinz, 2007), due to the lack of apoptosis (Hinz et al., 2003). This is evident in hypertrophic scars, developing after burn injury and in the fibrotic phase of scleroderma according to Hinz (2007) where the persistence of cell contractility leads to continuous matrix remodelling and retraction.

Contractures, generated by myofibroblasts are also a characteristic of fibrosis affecting vital organs such as the liver (Desmoulière et al., 2005). In cancer, it is the progression of myofibroblasts which plays an important role (Hinz, 2007). They participate in a process, called “stroma reaction” by creating a stimulating microenvironment for epithelial tumour cells (Hinz, 2007). The study postulates that the myofibroblasts may promote the progression of cancer invasions.

3. Contractility

The closure of cutaneous wounds involves three processes: epithelialisation, connective tissue deposition, and contraction (Grinell, 1999). “Epithelialisation results in resurfacing of the wound, connective tissue deposition results in replacement of damage dermis and contraction brings the margins of open wound together” (Grinell, 1999).

The contribution of the myofibroblast contraction to the physiological wound closure is a topic of current discussion. Hinz (2006) mentions in his article an experiment that has been conducted on rats. Rat wounds were kept open for 10 days with a plastic frame and once released, the wound contracted 50% within five hours. Hinz (2006) concluded that this contraction of 50% within five hours cannot be explained by “enhanced proliferation of fibroblasts“. The investigations assumes that there is a mechanism that has “not been yet elucidated”.

3.1 Contractile Apparatus

Myofibroblasts are able to synthesize components of the ECM, such as collagen, and they can develop tensile force through the formation of α-SM-actin, forming cytoplasm stress fibres (Hinz et al., 2003). The tension generated by the myofibroblasts, has been shown to be a regulator of connective tissue remodelling (Hinz et al., 2003).

3.2 Force generation

Myofibroblasts have a specialised cytoskeleton which allows them not only to contract, but to use these to generate forces (Gabbiani, 2003). These forces affect their surroundings, which is an important aspect for wound closure. As myofibroblasts are differentiated fibroblasts, the question arises whether fibroblasts also have this ability. A multitude of studies has been conducted into the question which of these cell types generate the greatest force and under what conditions they do so.

3.2.1 Fibroblast versus myofibroblast

Fibroblasts can be distinguished from myofibroblasts by their ultra structural features (Wrobel, Fray, Molloy, Adams, Armitage, and Sparrow, 2002). Compared to fibroblasts, myofibroblasts contain α-SM-actin stress fibres (Wrobel et al., 2002). A study was designed by Wrobel et al. (2002) to demonstrate the contractile proprieties of fibroblasts and myofibroblasts. They found out, that fibroblasts, in substrates with low elastomeric stiffness produce no significant different force from the force, generated by the myofibroblasts.

In substrates with higher elastomeric stiffness, the forces produced by fibroblasts where unaffected. But the forces, produced by the myofibroblasts where significantly higher. Wrobel et al. (2002) concluded that a higher proportion of myofibroblasts is able to produce wrinkles on elastomers of high stiffness, compared to fibroblasts.

The ECM stiffness can influence the cytoskeleton assembly and the ECM protein organisation (Wrobel et al., 2002). The cytoskeleton of the myofibroblasts changes when they are cultured on stressed substrates. They develop bundles of actin filaments and fibronectin fibrils (Wrobel et al., 2002). Thus, myofibroblasts use the rigidity of the substrate “as an environmental cue to produce more force” (Wrobel et al., 2002).

Wrobel et al. (2002) further claim that α-SM-actin negative cells, namely fibroblasts, can also produce contractile forces. They suggest that the wound contraction could be initiated in the absence of myofibroblasts. As the tension, in the granulose tissue increases during wound repair, it is possible, that the weak forces produced by fibroblasts may be lost in later phases of wound healing (Wrobel et al., 2002).

Wrobel et al.’s (2002) research has shown that mechanical tension has to be important for the development and maintenance of the myofibroblast. It predicts that “increases in the substrate stiffness later on in wound healing will induce the generation of higher forces from myofibroblasts.” Fibroblasts can produce sufficient force to close wound in the absence of myofibroblasts (Wrobel et al., 2002).

3.2.2 Wound closure

In study (Shin and Minn, 2003) it was demonstrated that the mechanism of wound closure from cultured myofibroblast and fibroblasts in collagen gel. They found that the myofibroblast and fibroblast groups showed no significant difference on the first day, but from the third day until the thirteenth day, the myofibroblast group showed a significant increased contraction of the collagen gel. Another observation was that the fibroblasts, when cultured in the collagen gel lattice, gathered in the centre of the gel lattice, whereas the myofibroblasts were localized on the periphery (Shin et al., 2003).

However, the myofibroblast group brought about significantly more contraction to the collagen gel then the fibroblast group (Shin et al., 2003). The high contraction force of myofibroblast is possible, because they possess the morphological and biochemical characteristics of both, fibroblasts and smooth muscle cells (Shin et al., 2003). By reducing their own cell length, they contract the ECM to which they are attached and thereby they can contribute to the mechanism of wound closure (Shin et al., 2003).

In their paper, Moulin, Auger, Garrel & Germain (2000), declare, that two phenomena occur in human wound surface during healing: Neodermal formation and re-epithelialisation. The study says that a contraction phenomenon occurs too, but compared to other mammalians, this contributes only to a small percentage of the closure process in human wounds.

In early wound healing, so Moulin et al. (2000), fibroblasts infiltrate into the damaged area, where they proliferate and differentiate into myofibroblasts. However, another cell type, namely keratinocytes, is important for the formation of a complete basal membrane (Moulin et al., (2000). Keratinocytes are cells of the epidermis and guarantee the structural and mechanical stability of the derma-epidermal junction (Moulin et al., 2000). Numerous studies, so Moulin et al. (2000) report the role of interactions between keratinocytes and myofibroblasts in process of basal membrane formation and wound closure. However, the action of these two coexisting cells is not clear yet (Moulin et al., 2000).

One hypothesis is that fibroblasts interact with keratinocytes. This hypothesis is based on a histological experiment which found that a continuous epidermis was formed in seven to ten days in a dermis populated with fibroblasts, but not with myofibroblasts (Moulin et al., 2000). In contrast to the function of myofibroblasts in wound healing the complete reepithelialisation never occurred over the ten day period (Moulin et al., 2000). In the light of used data, the study concludes that myofibroblasts could be involved in the process of neodermis formation and contraction. Moreover, fibroblast could be involved in stimulation of keratinocyt growth factor and in neodermis formation. It further suggests that myofibroblasts are not the inducers of reepithelialisation during wound healing.

3.3 Force Transmission

Mechanical forces induce a large number of biological processes as cell shape, mobility, cell differentiation and survival (Wang, Zohar & McCulloch, 2006). The force transmission is a process by which cells convert mechanical forces into biochemical signals, these signals then have to be integrated into appropriate cellular responses that mediate, for example, tissue remodelling (Wang et al., 2006).

3.3.1 Cell to matrix contacts

In contrast to normal dermal fibroblasts, myofibroblasts in granulated tissue and fibro contractive diseases develop complex adhesion structures with the ECM (Hinz et al., 2003). In vitro these contacts are called supermature focal adhesions (FA) and in vivo fibronexus (Hinz et al., 2004). These contacts of the myofibroblasts with the ECM are thought to be important because they transmit the contractile force from the myofibroblast to the ECM (Hinz et al., 2003). However, little is known about the development of the fibronexus during myofibroblast differentiation in vivo (Hinz et al., 2003). The study mentions, that most studies have been performed on cultured fibroblasts allowing the function and proprieties of these FAs to be elaborately described and explained.

Another function of the cell-matrix interaction is that myofibroblasts can regulate the tissue interstitial fluid volume and pressure by using integrin receptors and anchoring them onto ECM proteins (McAnulty, 2007).

3.3.2 Cell to cell contacts

The communication of myofibroblasts among themselves is controlled by an intercellular mechanical coupling (Follonier, Schaub, Meister and Hinz, 2008). The differentiation of myofibroblasts is accompanied by the formation of cell-cell adherence junctions that couple intercellular bundles of actin, so called contractile stress fibres (Follonier et al., 2008). The adherence junctions transmit contractile forces between myofibroblasts (Follonier et al., 2008).

In addition to these adherence junctions, myofibroblasts have the ability to communicate electromechanically via gap junctions (Follonier et al., 2008). The formation of gap was shown in 1978 by Gabbiani between wound granulated tissue and myofibroblasts and, has also been reported between dermal fibroblasts in vivo by Salomon in 1988 Follonier et al., 2008).

Gap junctions are channels composed of transmembrane connexion in the cytoplasm membrane that allows the intercellular passage of small molecules and ions, such as Ca^{2+} (Follonier et al., 2008). Electrochemical and mechanical cell coupling improve the remodelling of the tissue (Follonier et al., 2008). They also coordinate spontaneous and periodic transient increase in the intercellular Ca^{2+} concentration; this is called oscillation (Follonier et al., 2008).

Fibroblasts exhibit mechanical coupling via gap junctions, whereas mechanical adherence junctions coordinate the Ca^{2+} oscillations between myofibroblasts (Follonier et al., 2008). Therefore adherence junctions, but not gap junctions synchronise the activity of the myofibroblasts (Follonier et al., 2008). This was demonstrated by Follonier et al. (2008) on an experiment conducted on cultured myofibroblasts. Follonier et al. (2008) suggest, that local contractile events, following single Ca^{2+} transients, “are transmitted via adherens junctions to adjacent myofibroblasts”.

The α-SM-actin stress fibres of contacting myofibroblasts are connected to the ECM at sites of focal adhesions contacts and intercellularly at sites of adherence junctions

(Follonier et al., 2008). The mechanosensitive ion channels are closed in relaxed myofibroblasts, thus the Ca^{2+} ions cannot enter (Follonier et al., 2008). By extracellular events the Ca^{2+} transient is triggered and this rise of Ca^{2+} results in stress fibre contraction (Follonier et al., 2008). The resulting cell contraction will establish a mechanical feedback loop by recruiting other connected cells and through the opening of mechanosensitive ion channels (Follonier et al., 2008). Two neighbouring myofibroblasts have a feedback loop relationship to each other (Follonier et al., 2008).

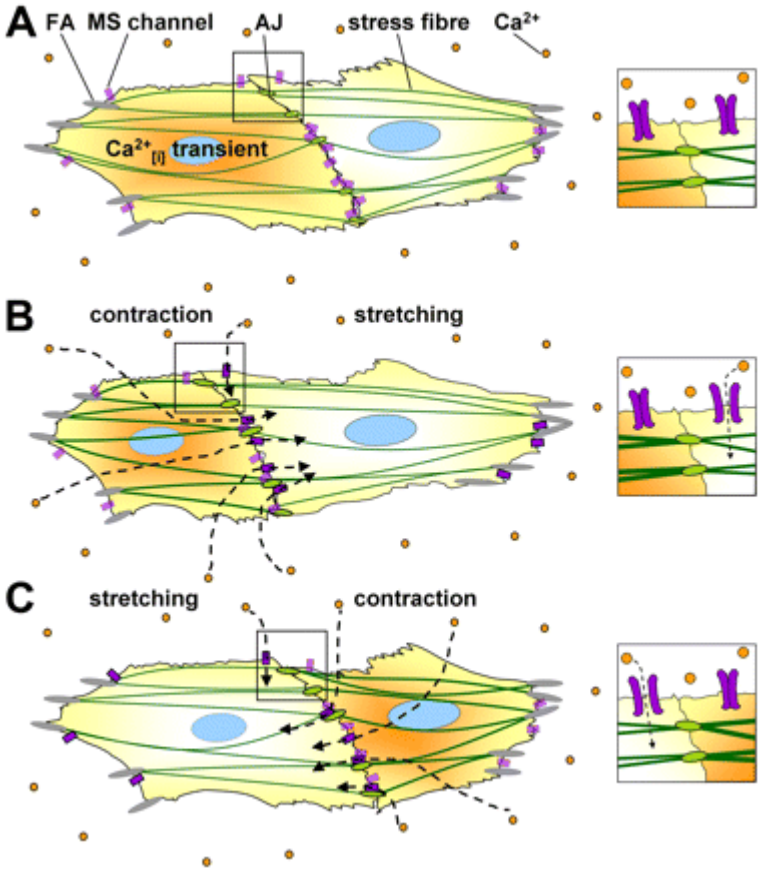

Fig.3 Model of mechanical communication between myofibroblasts.

(A) α-SM-actin stress fibres (green) of contacting myofibroblasts are connected to ECM at sites of focal

Follonier et al. (2008) propose that periodic Ca^{2+} oscillations are accompanied by periodic Ca^{2+} micro-contractile events. The sum of this adds up to overall tissue contraction. This process implies a “lock-step” mechanism in which the locally contracted ECM is stabilised by the addition of new cell material (Follonier et al., 2008). During the relaxation of the myofibroblasts, the ECM remains shortened. The numerous repetitions of these cycles result in tissue contracture (Follonier et al., 2008).

4. Myofibroblast in pathological situations

During physiological wound repair, the myofibroblasts disappear due to the apoptotic process initiated as soon as the continuity of the epithelial structure has been reconstructed (Hinz et al., 2003). It is this process which becomes disrupted in the development of fibro contractive diseases and hypertrophic scars (Hinz et al., 2003).

Interestingly the wound of a human foetus does not form a scar (Shin et al., 2003). Therefore there must be a mechanism that is lost during human development. This has given rise to continual efforts to identify the processes associated with the formation of scarring and to establish methods to prevent this process (Shin et al., 2003).

One hypothesis is that hypertrophic scar formation is a result of disruption between the interactions of multiple factors (Shin et al., 2003). At the molecular and cellular levels, the myofibroblast and fibroblasts are considered to have an important role (Shin et al., 2003). When myofibroblasts do not disappear through apoptosis, they remain in the dermis and continuously contract the regenerating tissue which may result in scar contracture formation (Shin et al., 2003). However, Shin et al. (2003) mention that it is still controversial if the fibroblast or the myofibroblasts have the dominant role in scar contracture. The entire mechanism of wound contraction and scar formation has not been exhaustively elucidated as yet. However, it is known that multiple factors, including ECM, serum-signalling molecules, several types of cells and intercellular cytoskeletal components all contribute to this mechanism (Shin et al., 2003).

5. Discussion

This section it will be discusses the effects external stimuli on myofibroblasts. Of particular interest to physiotherapists is to deduct the ideal load which should be applied to the injured tissue to facilitate wound healing. This raises the question of the effects on the healing process if too much stress is placed on the tissue. Possibly, it would result in an increase of myofibroblastic activity and hence in extra stiffening of the scar, but this has not been proven yet. In particular an investigation into the effects of externally applied stimuli on the myofibroblasts of ligaments and joint capsules would prove to be an invaluable data set for physiotherapy. This is, however, very difficult to conduct in human tissue. Usually animal tissue is used for studies on the wound healing process. This is due to the fact that animal tissue and animal models are easier to access. Studies conducted on animals are easier to standardise than studies conducted with human subjects. However, we must not be forgotten that there are differences between the healing processes in animals and in humans and due consideration needs to be given to the ethical implications of animal testing. To date, studies have only been conducted on in vitro models designed to emulate myofibroblasts.

Dermatology has also shown a great interest in myofibroblastic activity. Myofibroblasts are contributing factors in the development of hypertrophic scarring after a burn injury. These hypertrophic scars carry functional and aesthetic implications for the patient. The hypertrophic scar tissue is easier to remove, allowing the cells to be cultivated in vitro for the use of scientific research into, for example, myofibroblastic activity. A study has been conducted on myofibroblastic activity and the mechanical tension of burn scar tissue.

5.1 Human burn scars

Burn patient rehabilitation focuses on preventing the damaged tissue from scarring. In paper, (Junker, Kratz, C., Tollbäck & Kratz, G., 2008)the effect of mechanical tension on the differentiation of fibroblasts into myofibroblasts in human burn scars is discussed. The problem encountered with scar formation as a result of burn wounds is the contraction of the newly formed granulated tissue and this causes functional impairment (Junker et al., 2008). According to Junker et al. (2008) physiotherapy for these patients include

techniques such as stretching, positioning, splinting and range of motion exercises. However, little is known about the effects of these therapies on scar tissue and there is little knowledge of optimal levels and the duration of the forces applied to the burn scars (Junker et al., 2008). One hypothesis in Junker et al. (2008) is that decreasing the number of myofibroblasts in the scar tissue could prevent the formation of hypertrophic scars. But the critical point, as discussed above, is that mechanical force is an inducer for the transformation of fibroblasts into myofibroblasts. However, the actual required magnitude or duration of such forces to induce the transformation is still unknown (Junker et al., 2008). To demonstrate the effect of mechanical stretching on the number of myofibroblasts, Junker et al. (2008) used samples from five hypertrophic burn scars taken from routine reconstructive surgery. All samples were from hypertrophic scars older than 12 months. The samples were prepared and connected to a stretching device. A sample control group was put in the device, but there was no stretch applied. Presence of the myofibroblasts was shown between the stretched and un-stretched samples after one and six days. Smooth muscle actin (SMA) served as a marker for myofibroblasts. In un-stretched scars only a few myofibroblasts were identified, there was no significant difference regarding the presence of SMA between the samples that had been incubated for one or six days ($P>O.5$, $n=50$). In the stretched samples there was a significant result when testing for the existence of SMA ($P<0.001$, $n=50$). Already after one day of stretching, there were increased numbers of myofibroblasts found in the samples. After six days of incubation, the samples were stained to detect SMA. There was significantly higher staining for SMA in the samples after six days than after one day. During the transformation of myofibroblast from fibroblasts SMA is formed. By this marker, Junker et al. (2008) could demonstrated, that the continuous stretching of human burns scar increases the differentiation of fibroblasts into myofibroblasts. The study suggests that there is a “very sensitive balance between the positive and negative effects regarding physical stimulation of a burn scar”. Furthermore it was concluded that the effects of physical therapy on burn scars should be investigated in vivo. The experiment of Junker et al. (2008) supports the hypothesis, that mechanical force induces the differentiation of fibroblasts into myofibroblasts. Notably these studies were conducted on myofibroblasts from tissue which was older than 12 months. It is therefore not necessarily directly applicable to the myofibroblastic activity found during wound healing processes. The study investigated the effects of static stretching; it would be interesting to conduct a

differentiated study to determine optimal levels and application times of static stretching which would avoid a hyperactivity of myofibroblasts.

5.2 The effect of external applied stretch

During wound healing, the remodelling process of the ECM causes an increase in the tensile strength and stiffness of the scar (Balestrini & Billiar, 2006). Tissue stiffness combined with contracture often results in reduced range of motion (Balestrini et al., 2006). Clinicians recognize that the mechanical state of a wound during the wound healing process affects the proprieties of the resulting scar (Balestrini et al., 2006). Massage, range of motion exercises and stretching techniques, according to Balestrini et al. (2006), are utilized to influence the appearance and proprieties of scars. However, the study concludes the observation “that both positive and negative outcomes can result from altering the mechanical environment during healing is a troubling clinical dilemma.”

It also investigated the effects of cyclic stretching on the mechanical, morphological and biomechanical properties of fibroblast-populated fibrin gels in vitro, comparing a stretched to a static group. The stretch was applied for eight days. It found out, that the cyclic stretching did not modify the number of myofibroblast in the fibrin gel. However, Balestrini et al. (2006) cannot say with certainty, that stretch did not affect cell proliferation, because a change in cell proliferation may have been balanced with change in apoptosis. The study was able to demonstrate that cyclic stretching stimulates fibroblasts to produce a stronger matrix by dramatically increasing the compaction and matrix fibre reorganization. Zheng, Song, Li, Fan, Zhao, Chen, Deng & Hu (2008) explore in their cytomechanical study the effects of cyclic strain loading on myofibroblast. It was found that almost no visible morphological changes were observed in myofibroblast during the early stages of cyclic strain loading (< 1h). However after 6 h to 12 h post loading, the myofibroblast change their direction to align with the direction of strain. Zheng et al. (2008) suggest that cyclic strain has two ways in which it influences the adaption of myofibroblasts: “By directly effecting actin cytoskeleton and by later chemical signals transmitted from the extracellular side to intracellular side to initiate re-polymerization of actin”. In study, (Balestrini et al., 2006) the effect of the cyclic applied stretch on the activity of myofibroblasts was not mentioned. But this could be due to more efficient stress shielding of the myofibroblasts by the newly compacted ECM. This would result in a reduction of

fibroblastic differentiation to form myofibroblasts. Hence, another interesting target to influence the myofibroblastic differentiation is the ECM.

5.3 The myofibroblast and the extracellular matrix

Myofibroblasts communicate, as discussed above, with the ECM. An interesting consideration is that it may not be the myofibroblast causing the stiffness of the ECM, but rather that the stiffness of the ECM affects the activity of the myofibroblast. “Myofibroblasts spend most of their lives shielded by a protective ECM”, explain Wipff and Hinz (2009). When tissue injury occurs, it is an enormous stress for the myofibroblast, as soon as they lose the protective structure of the ECM. It is interesting to know, that myofibroblast develop tension on their own, to develop a contractile stress fibre apparatus (Wipff et al., 2009). This apparatus is used by the myofibroblasts to stiffen newly secreted ECM (Wipff et al., 2009). Myofibroblasts have the ability to feel stress in their surrounding tissue (Wipff et al., 2009). They can feel mechanical changes in the microenvironment through proteins called integrins (Wipff et al. 2009).

A possible form of physiotherapeutic intervention may be that physiotherapist could impair the activity of the myofibroblast by manipulating the stiffness of the ECM. The stiffness of newly polymerized collagen is 10-100 Pa, and this is comparable to the stiffness of the ECM of early wounds. (Wipff et al., 2009). In such gels, fibroblasts organize actin filaments (Wipff et al., 2009). In mechanically restrained gels, the tension is gradually increasing and this induces the formation of α-SM-actin stress fibres (Wipff et al., 2009). In vitro, the formation of α-SM-actin into stress fibres begins after 2-3 days and after 8-9 days in experimental rats wounds (Wipff et al., 2009). After Mori, Bellini, Stacey, Schmidt & Mattoli (2005), myofibroblasts begin with α-SM-actin between days four and seven. The stiffness of the ECM in these models then rises up to 20`000 Pa (Wipff et al., 2009).

It may be a worthwhile consideration to find a method applicable during physiotherapy which could counteract this extreme rise of ECM stiffness. For example, manual techniques, to soften the ECM and to prevent the rise in pressure in the ECM augments, could be applied during the inflammatory phase of wound healing. Thus, from day two on the α-SM-actin formation into stress fibres takes place. In this situation, interactive signals give myofibroblasts the information that the ECM has enough stability on its own. Thus,

the level of stress hast to be decreased. In other words, physiotherapeutic intervention should begin by day 2 post-trauma, during the formation of the new fibroblasts.
As discussed above, fibroblasts have contractile features, too. As another possible intervention a method could be developed to prevent the cell differentiation from fibroblast to myofibroblast. Or at least to prevent the excessive new building of myofibroblasts.

In the medical treatment of burns, pressure is used to prevent hypertrophic scarring. This could as well be used in physiotherapy as an alternative through the application of manually applied pressure techniques after an injury has occurred. It may be possible that applying external pressure could interrupt the mechanical feedback loop. The mechanical feedback loop is described by Hinz (2006) and it describes the interaction between myofibroblasts and the ECM. As elaborated before, myofibroblasts in an intact tissue are stress shielded and they do not develop contractile features or matrix adhesions. It should therefore be part of the goal of an effective physiotherapy to induce the exitation of myofibroblast cycle once the original structure of the ECM has been reconstituted. Then, the ECM is able to once again take over the mechanical load. Stress released myofibroblasts will eventually undergo apoptosis (Hinz, 2006).

Physiotherapeutic interventions to prevent contractures and hypertrophic scars should be applied during the early stages of wound healing. Furthermore, physiotherapist should be aware of the interaction of the myofibroblasts and the ECM: An injured ECM activates myofibroblasts. Ultra structural analysis of myofibroblasts in fibrotic and wound tissue has revealed the existence of numerous cell-matrix contacts, namely fibronexus in vivo and FA in vitro. In vitro, myofibroblasts reduce the FA when they are cultured on soft substrates (Wipff et al., 2009).

5.4 Physiotherapeutic relevance

For physiotherapist the theoretical background on wound healing is essential. This paper summarized the processes during wound healing in which myofibroblasts are involved and the effect of externally applied stimuli on myofibroblasts. This knowledge serves as a base to develop a deeper understanding of the aforementioned processes in order to develop a physiological rehabilitation plan. This work is an addition to the books on

rehabilitation during wound repair of van Wingerden (1998) and van den Berg (2007), to impart knowledge of myofibroblastic function. Myofibroblasts achieve their full potential in the proliferations phase during wound repair. The theory of van den Berg (2007) that targeted weight bearing exercise, with an ideal intensity, is an important stimulus for connective tissue synthesis, can be supported in view of the previously discussed findings. There was no study found giving any indication of the level of intensity which would be ideal for the synthesis for connective tissue in particular the stabile ECM. In addition to this, a study conducted by Neidlinger-Wilke, Grood, Claes & Brand (2002) has shown that fibroblast orientation to stretch begins within three hours. According to Neidlinger-Wilke et al. (2002) fibroblasts continue to optimize their orientation over the next 24 h, which leads them to conclude that fibroblasts are extremely sensitive to changes in their mechanical environment. The same can be said about myofibroblasts, as they are sensitive and feel stress in their environment as well. Physiotherapists should be aware that myofibroblasts form connections with their surrounding ECM resulting in a feedback loop between the ECM and the myofibroblasts.

In situation in which an increased level of myofibroblastic activity is not desired, due care must be given to the amount of external stimulus applied. For example for the prevention of hypertrophic scars, external stimuli need to be applied cautiously as they will stimulate the transformation of fibroblasts into myofibroblasts. Further studies need to be conducted to elucidate the ideal intensity of physiotherapy without placing the myofibroblasts under stress.

5.5 Open questions / Knowledge gaps

One of the main problems, which have not yet been solved, is a thorough understanding of the biology of myofibroblasts. In particular how myofibroblasts appear and why their existence persists in pathological situations such as hypertrophic scarring and fibrosis. Desmoulière et al. (2005) propose one possible explanation to these questions as a lack of inhibition of the cells characterized in the terminal phase of wound healing. Unfortunately, it is difficult to prove this in a clinical situation and at present there are no “reliable models of hypertrophic scarring in experimental animals” (Desmoulière et al., 2005).

Not yet definitively proven is the actual origin of myofibroblasts. There are various possible models aimed at answering this question. Phan (2008) declares that more coordinated research needs to be carried out to uncover the key mechanism involved in the genesis of myofibroblasts and their various phenotypes. Hinz (2007) suggests that more effort should be made to understand the molecular mechanism of myofibroblast differentiation and function. Furthermore Hinz (2007) proposes that novel strategies and drugs that counteract the myofibroblast functions are needed.

An interesting physiotherapy study would be to develop a model to explain what the effects of externally applied mobilization techniques, or continues passive motion on myofibroblastic activity.

6. Conclusions

As Balestrini et al. (2006) postulate in their paper, the “designing of a treatment regimen that would result in superior mechanical properties without detrimental side effects requires a more thorough understanding of mechanobiology and the mechanism underlying wound remodelling”. If the aim of the therapy is to reduce the activity of myofibroblast, it is essential to reduce stress in the newly built matrix. As Wipff et al. (2009) conclude in their study: “Myofibroblasts work best under stress”. The stress could be created by the activity of the rebuilding and remodelling of the ECM or externally applied stimuli. Myofibroblast communicate among themselves and can thereby adjust their contractile force. Studies have shown that mechanical stretching of myofibroblasts can stimulate their proliferation through force carrying connections that extend from the cell membrane to the nucleus (Glanz, 1997, as quoted by Ghelsen, Gale, Ganion, Larry, Helfset & Robert, 1999). Ghelsen, Gale, Ganion, Larry, Helfset & Robert (1999) summarize that mechanical stimuli have been shown to alter many functions including ion transport, protein synthesis and gene expression. Other studies came to the result that fibroblasts are able to produce contractile force as well, but this depends to the stiffness of the substrate they are cultured on. When the substrate is too stiff, myofibroblasts generate a higher force. Which cells play the key role during wound closure, is not clear at this stage, but it seems, that apart from fibroblasts and myofibroblast, the keratinocytes are important to guarantee wound closure.

Declaration of originality

I hereby declare that this Paper is all my own work and all references contained within it have been correctly cited and the original authors acknowledged.

7. Indices

7.1 Literature List

Books

Desmoulière, A. & Tuchweber, B. (1999*). Tissue Repair and Fibrosis. The Role of the Myofibroblast*. Berlin: Springer.

Hüter Becker A. & Dölken, M. (2005). *Physiotherapie in der Orthopädie.* Stuttgart: Thieme.

Junqueira, L., Carneiro, J. & Kelley, R. (2002). *Histologie*. Berlin: Springer.

Schiebler, T., & Schmidt, W. (2003). *Anatomie.* Berlin: Springer.

Stark, G., Horch, R., Tanczos, E. (1998). *Biological Matrices and Tissue Reconstruction.* Berlin: Springer.

Van den Berg, F. (2003). *Angewandte Physiologie. Das Bindegewebe des Bewegungsapparates verstehen und beeinflussen.* Stuttgart: Thieme.

Van den Berg, F. (2007). *Angewandte Physiologie. Therapie, Training, Tests*. Stuttgart: Thieme.

Van Wingerden, B. (1998). *Bindegewebe in der Rehabilitation*. Schaan: Scipro.

Papers

Balestrini, J. & Billiar, K. (2006). Equibiaxial cyclic stretch stimulates fibroblasts to rapidly remodel fibrin. *Journal of Biochmechanics, 39, 2983-2990.*

Desmoulière, A., Chaponnier, C. & Gabbiani, G. (2005). Tissue repair, contraction and the myofibroblast. *Journal of Wound Repair and Regeneration, 13, 7-12.*

Follonier, L., Schaub, S., Meister, J., Hinz, B. (2008). Myofibroblast communication is controlled by intercellular mechanical coupling. *Journal of Cell Science, 121, 3305-3316.*

Gabbiani, G. (2003). The myofibroblast in wound healing and fibrocontractive diseases. *Journal of Pathology, 200, 500-503.*

Ghelsen, Gale, M., Ganion, Larry, R., Helfset & Robert. (1999). Fibroblasts response to variation in soft tissue mobilization pressure. *Journal of Medicine & Science in Sport & Exercise, Volume 31, 531-535.*

Grinnel, F. (1999). Signal Transduction Pathways Activated During Fibroblast Contraction of Collagen Matrices. In A. Desmoulière & B. Tuchweber (Eds.), *Tissue repair and Fibrosis-The role of the myofibroblast* (pp. 61-73). Springer: Berlin.

Hinz, B. (2007). Formation and Function of the Myofibroblast during Tissue Repair. *Journal of Investigative Dermatology, 127, 526-537.*

Hinz, B. (2006). Masters and servants of the force: The role of matrix adhesions in myofibroblast force perception and transmission. *European Journal of Cell Biology, 85, 175-181.*

Hinz, B. & Gabbiani, G. (2003). Cell-matrix and cell-cell contacts of myofibroblasts: role in connective tissue remodelling. *Journal of Thrombosis and Haemostasis, 90, 993-1002.*

Hinz, B., Pittet, J., Smith-Clerc, J., Chaponnier, C. & Meister, J.-J. (2004). Myofibroblast *Development Is Characterized by Specific Cell-Cell Adherens Junctions* [On-Line]. Available:http://www.molbiocell.org/cgi/doi/10.1091/mbc.E04-05-0380. (02.02.2009)

Hinz, B. Phan, S., Thannickal, V., Galli, A., Bochat-Piallat, M. & Gabbiani, G. (2007). The Myofibroblast. One Function, Multiple Origins*. The American Journal of Pathology, Volume 170, No. 6.*

Junker, J., Kratz, C., Tollbäck, A. & Kratz, G. (2008). Mechanical tension stimulates the transdifferentiation of fibroblasts into myofibroblasts in human burn scars. *Journal of Burns, 34, 942-946.*

Mori, L., Bellini, A., Stacey, A., Schmidt, M. & Mattoli, S. (2005). Fibrocytes contribute to the myofibroblast population in wounded skin and originate from the bone marrow. *Journal of Experimental Cell Research, 304, 81-90.*

McAnulty, R. (2007). Fibroblasts and myofibroblasts: Their source, function and role in diseases. *The International Journal of Biochemistry & Cell Biology, 39, 666-671.*

Moulin, V., Auger, F., Garrel, D. & Germain, L. (2000). Role of healing myofibroblasts on re-epithelialisation of human skin. *Journal of Burns, 26, 3-12.*

Neidlinger-Wilke, C., Grood, E., Claes, L., Brand, R. (2002). Fibroblast orientation to stretch begins within three hours. *Journal of Orthopaedic Research, 20, 953-956.*

Phan, S. (2008). Biology of Fibroblasts and Myofibroblasts. *Proceedings of the American Thoracic Society, 5, 334-337.*

Shin, D. and Minn, K. (2003). The Effect of Myofibroblast on Contracture of Hypertrophic Scar. *Journal of the American Society of Plastic Surgeons, 113, 633-640.*

Tomasek, J., Gabbiani, G., Chaponnier, C., Hinz, B. & Brown, R. (2002). Myofibroblasts and mechano-regulation of connective tissue remodelling. Journal of Nature Reviews and molecular cell biology, 3, 349-363.

Tomasek, J., Vaughan, M., Kropp, B., Gabbiani, G., Martin, M., Haasksma, C. & Hinz, B. (2006). Contraction of myofibroblasts in granulation tissue is dependent on Rho/Rho kinase/myosin light chain phosphatise activity. *Journal of Wound Repair and Regeneration, 14, 313-320.*

Wang, J., Zohar, R. & McCulloch, C. (2006). Multiple roles of α-smooth muscle actin in mechanotransduction. *Journal of Experimental Cell Research, 321, 205-214.*

Wipff, P. & Hinz, B. (2009). Myofibroblasts work best under stress. *Journal of Bodywork and Movement Therapies, 13, 121-127.*

Wrobel, L., Fray, T., Molloy J., Adams, J., Armitage, M. & Sparrow J. (2002). Contractility of Single Human Dermal Myofibroblasts and Fibroblasts. *Journal of Cell Motility and Cytoskeleton, 52, 82-90.*

Zheng, L., Song, J., Li, Z., Fan, Y., Zhao, Z., Chen, Y., Deng, F. & Hu, Y. (2008). The mechanism of myoblast deformation in response to cyclic strain – A cytomechanical study. *Journal of Cell Biology International, 32, 754-760.*

Figures:

Fig .1

Hinz, B. (2007). Formation and Function of the Myofibroblast during Tissue Repair. *Journal of Investigative Dermatology, 127, 526-537.*

Fig. 2

Desmoulière, A., Darby, I. & Gabbiani, G. (2003). Normal and Pathological soft tissue Remodelling: The role of the Myofibroblast, with special Emphasis on Liver and Kidney Fibrosis. *Laboratory Investigation, 83, 1689-1707.*

Fig. 3

Follonier, L., Schaub, S., Meister, J., Hinz, B. (2008). Myofibroblast communication is controlled by intercellular mechanical coupling. *Journal of Cell Science, 121, 3305-3316.*

Appendix

Abbreviations

AJ – adherens junctions
ECM – extracellular matrix
FA – focal adhesions
MS channels – mechanosensitive ion channels
TGF – transforming growth factor
α-SMA-actin – α-smooth muscle actin

Diseases of excess extracellular matrix deposition

Lung	Skin	Multiple Systems
Emphysema	Scleroderma	Renal fibrosis
Asthma	Hypertrophic scars	Liver sclerosis
COPD	Dupuytren`s contracture	Diabetes
Obliterative bronchiolitis		Pleura adhesions
Interstinal lung diseases		Rheumatoid arthritis
		Arteriosclerosis
		Cardiac fibrosis
		Tendinitis

(McAnulty, 2007)

Reflection

Due to the nature of this topic it has been a fascinating journey to gain insight in the level of research and type of research conducted on myofibroblasts. It has become clear that little research has been done by physiotherapists on the effects of physiotherapy planning and the activity of myofibroblasts. This is due to the fact that it is very difficult if not impossible to study this in vivo. Most studies on a cellular and hence microscopic level are conducted in vitro. Therefore, a wealth of studies on the myofibroblast activity conducted by medical and biological research teams were found

Due to the in-depth scientific papers, it was interesting to gain an oversight of this fascinating and still open topic of myofibroblast activity. It is hoped that in the future technical developments will allow us to gain a further insight and conduct in vivo studies.

Acknowledgments

I would like to thank Judith Tobler-Harzenmoser for overseeing this work and for her helpful inputs.

Thank you, Lynn Watkins, for spending your time in proof-reading and for our endlessly supportive friendship.

My special thanks go to Matthias Galus: Thank you for being there for me and keeping me focused.

My deepest thank goes to my parents for not only providing me with financial support to follow my dreams but for all their emotional support in allowing me to aim high and reach my full potential.

This work is dedicated to my mother, who will always live on in my heart.