THE VARIATION OF INTEGRIN-ß1, FAK, ERK ON POST-BURN HYPERTROPHIC SCARS: AN IMPLICATION FOR PRESSURE THERAPY

YUTING ZHANG

MPhil

The Hong Kong Polytechnic University

2019

The Hong Kong Polytechnic University

Department of Rehabilitation Sciences

The Variation of Integrin-ß1, FAK, ERK on Post-Burn Hypertrophic Scars: An Implication for Pressure Therapy

Yuting Zhang

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Philosophy

August 2018

CERTIFICATE OF ORIGINALITY

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it reproduces no material previously published or written, nor material that has been accepted for the award of any other degree or diploma, except where due acknowledgement has been made in the text.

_____ (Signed)

Yuting Zhang _____ (Name of student)

ABSTRACT

Hypertrophic scar (HTS) is an abnormal yet common complication of severe burns. One key component that affects HTS the formation of HTS during wound healing is mechanical force, which can be converted into chemical signals and thereby later cell proliferation, migration and adhesion. The mechanisms of force-regulating cell behaviors via interactions between mechanosensitive molecules and intracellular signaling like integrin-β1, focal adhesion kinase (FAK) and extracellular (ERK) are known as mechanotransduction. Pressure therapy is a frontline treatment for HTS in which compression force is used to intervene in the scarring process. However, controversy persists among specialists due to its ambiguous mechanism. To date, attempts have been made to explain pressure therapy from a mechanotransduction perspective. Unlike the abundant evidence regarding the interaction between mechanotransduction and stretch-induced scarring during early wound healing, few studies have explored the involvement of mechanotransduction on growing or matured HTS under compression.

Therefore, the aim of the phase I study was to find the relationship between post injury days and the immunoreactivity of integrin-β1, FAK and ERK on human HTS to establish a reference of mechanosensitive molecules on growing or matured HTS. The aim of phase II study was to compare the immunoreactivity of integrin-β1, FAK and ERK on human HTS before and after pressure therapy as a preliminarily exploration of the effect of pressure therapy on mechanotransduction. Finally, the clinical effect of pressure therapy on convex area considering the compression from three dimensions is reported in the phase III study in an attempt to optimize the clinical application of pressure therapy.

This thesis begins with an overview of the epidemiology and pathophysiology of HTS and a review of mechanotransduction in fibrosis, with a focus on predominant molecules in force-regulating scarring such as integrin-β1, FAK and ERK. The history and mechanisms of pressure therapy are then introduced. To assist with the design of the main study, the confounding factors of pressure therapy were summarized in a systematic review.

In phase I of the study, an observational study was conducted to explore the relationship between post injury days and the immunoreactivity of integrin-β1, FAK and ERK on human HTS; 31 subjects with 43 HTS specimens were obtained. An increase in the immunoreactivity of integrin-β1, FAK and ERK was revealed between 2 and 3 months after injury, suggesting a critical time for intervention using external force such as pressure therapy.

A pretest-posttest study was designed in phase II to explore the effects of pressure therapy on mechanotransduction. Forty-three HTS specimens were obtained before and after pressure therapy. The immunoreactivity of dermal integrin-β1, FAK and ERK was found to decrease after pressure therapy, and an earlier treatment showed a greater benefit. The study preliminarily demonstrated an effect of pressure therapy in the down-regulation of mechanosensitive molecules in the dermis.

Based on findings of the phase II study, and the high expression of FAK detected by other researchers over the perilesional region of aggressive scars, phase III of the study describes a case report in which modified pressure therapy generated compression force on convex hypertrophic scars from three dimensions (3D) was described. The objective scar thickness

was found to be controlled after the application of 3D pressure therapy. This case report shows a translation from the preclinical findings to effective clinical practice.

In conclusion, the immunoreactivity of integrin-β1, FAK, and ERK was found to be elevated between 2 and 3 months after injury, and an earlier application of pressure therapy could contribute to the down-regulation of dermal integrin-β1 and FAK expression. From a clincial aspect, the application of adequate compression on areas in which greater activation of mechanosensitive molecules was found, can control the progression of scarring. Further research is needed for a deeper comprehension of the signalling pathway of compression force on HTS in humans.

Keywords: Mechanotransduction, Focal Adhesion Kinase, Integrins, Pressure therapy, Hypertrophic Scar

PUBLICATIONS ARISING FROM THE THESIS

Journal articles

Zhang, Y. T., Li-Tsang, C. W., & Au, R. K. A Systematic Review on the Effect of Mechanical Stretch on Hypertrophic Scars after Burn Injuries [J]. Hong Kong Journal of Occupational Therapy, 2017, 29: 1-9. http://dx.doi.org/10.1016/j.hkjot.2016.11.001.

张玉婷，李攀，罗伦，李曾慧平. 人体增生性瘢痕在瘢痕重塑期间机械信号传导分子的变化 [J]. 中华烧伤杂志，2018.(Accepted)

Zhang, Y. T., Li-Tsang, C. W., Li, P. (2018). The effect of pressure therapy on post-burn hypertrophic scar: from a mechanotransduction perspective. (In preparation)

Conference presentation

Y. Zhang., C.W.P. Li-Tsang, P. Li. (2018) An exploration of mechanosensitive molecules on hypertrophic scars upon pressure therapy: A preliminary study. Annals of Physical and Rehabilitation Medicine, 2018, 61 (supplement): e423. ISPR8-0549.

Zhang, Y.T., Li-Tsang, C.W.P. (2017) 一种新型瘢痕疙瘩压力治疗项目：个案随访及报告. 中华医学会烧伤外科学分会 2017 年学术年会 2017 Annual Meeting of Chinese Burn Association, Wuxi, China.

Zhang, Y.T., Li-Tsang, C.W.P. (2017) The effect of pressure therapy on hypertrophic scars -- from a mechanotransduction perspective. (Oral presentation). Department of Rehabilitation Sciences and Physiotherapy, University of Antwerp, Antwerp, Belgium.

Li-Tsang, C.W.P., Li P., **Zhang, Y.T**., Wei YT, Zhang, W.L. (2017): Investigation in Histological Evidence of Pressure Therapy and its implications. (Oral presentation). British Burn Association Annual Meeting 2017, British Burn Association, London, United Kingdom

Li-Tsang C.W.P., **Zhang, Y.T.** (2016). An innovative pressure therapy for keloid scar: a case report. (Poster presentation). ISBI, ISBI 2016, Miami, U.S.A.

Zhang, Y.T., Li-Tsang, C.W.P. (2016). The role of focal adhesions in compression therapy for scars, a review and hypothesis. (Poster presentation). ISBI, ISBI 2016, Miami, U.S.A.

ACKNOWLEDGEMENTS

First of all, I would like to express my deepest gratitude to my chief supervisor, Professor Cecilia W.P. Li-Tsang, Department of Rehabilitation Sciences, for her generous support and guidance throughout my study, research and life as a MPhil candidate in Hong Kong Polytechnic University.

I would also like to extend my sincere thanks to Dr. Alex Cheung, Scientific Officer, Department of Rehabilitation Sciences, for his kindness and patience in teaching me the fundamental laboratory experimental techniques, and sharing his logistic thinking in the field of basic science. My gratitude is also to Dr. Raymond Chung, Scientific Officer, for his statistical advice.

I would also express my deep gratitude to my team members, Ms. Pan Li, Ms. Yating Wei, Ms. Walei Zhang, Ms. Huan Deng, PhD candidates of Prof. Cecilia Li, in helping recruiting subjects and providing insightful discussions for my MPhil study. Many thanks also go to colleagues Mr. Tim Li, Mr. Ricky Au for their sincere support.

I am grateful to Prof. Chengqi He, Dr. Yonghong Yang, Mr. Shaxin Liu, Department of Rehabilitation Medicine, West China Hospital, for their encouragement for me to enroll into the MPhil program.

Last but not the least, I would like to thank all subjects and colleagues from Chengdu Second People's Hospital and Southwest Hospital, as well as my families for their support throughout my study.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

BSHS-B——Burn Specific Health Scale-Brief questionnaire

CCT——controlled clinical trial

DALYs——disability-adjusted life-years

ECM——extracellular matrix

ERK——extracellular signal-regulated kinase

FAK——focal adhesion kinase

FEM——Finite element model

GST—— Grocery Shelving Task

HDF ——human dermal fibroblasts

HSE——human skin equivalents

HTS——hypertrophic scar

IL——interleukins

MMPs——matrix metalloproteinases

pFAK——phosphorylated focal adhesion kinase

pERK——phosphorylated extracellular signal-regulated kinase

POSAS——patient and observer scar assessment scale

RCT——randominzed contolled trial

ROI——region of interest

ROM——range of motion

SMA——smooth muscle actin

TGF——transforming growth factor

TNF——Tumor necrosis factor-alpha

TIMPs ——tissue inhibitors of metalloproteinases

TEWL—— transepidermal water loss;

UEFI——Upper Extremity Functional Index;

vWF——Von Willebrand factor

VAS—— Visual analogue scale

VEGF ——vascular endothelial growth factor

VSS——Vancouver Scar Scale

WHO——World Health Organization

Chapter 1 Introduction

1.1 Background

In recent years, thriving development of fluid resuscitation, skin grafting, antibiotics and nutritional support contributes to the decreased mortality rate after severe burn injuries (Hart et al., 2000). For most of the burn survivors, the leading cause of morbidity is hypertrophic scar (HTS), which results in joint contracture, disfigurement, disability, and increased disability-adjusted life-years (DALYs) (World Health Organization, 2017). However, because of complex pathophysiology and prolonged fibrosis of human HTS, the effect of HTS treatments is still unsatisfactory (Lawrence, Mason, Schomer, & Klein, 2012). Animal and in-vitro models are normally used for experimental research on mechanism of scar managements. However, physiology of scar formation on these models is different from human being, which results in scar-less healing or atypical HTS (Bouffard et al., 2008; Ogawa et al., 2012; Wong, et al., 2012). There are few preclinical studies focus on scar maturation phase, which imposes difficulty of understanding HTS progression and mechanism of managements on growing or matured HTS.

Recently, more and more researchers find close relationship between HTS and the mechanical force. Mechanotransduction refers to a series of mechanisms that converting mechanical stimulus into chemical signals, and has been proved to participate in tension-regulated HTS formation (Chiquet, Renedo, Huber, &Flück, 2003). Preclinical studies have demonstrated that mechanical force can affect critical process in wound healing such as cell proliferation, differentiation and apoptosis though mechanotransduction. Stretching

on newly healed wound is found to stimulate HTS formation through the activation of mechanosensitive molecules like integrin-β1, focal adhesion kinase (FAK) and extracellular signal-regulated kinase (ERK) (Peters et al., 2015; Huang et al., 2013; Wang et al., 2011; Chin et al., 2009; Junkder et al., 2008). On the contrary, tension reduction during wound healing is found to attenuate the increasing in mechanosensitive molecules and prevent HTS formation (Akaishi, Akimoto, Hyakusoku, &Ogawa, 2010; Gurtner et al., 2011). Although wound healing and scar formation are within the same consecutive process, there are currently few studies explored the involvement of mechanotransduction on growing or matured HTS, and few investigated the effect of mechanical force on formed HTS.

Pressure therapy has long been applied as a standardized conservative treatment for HTS after burn injuries. Improved pliability and thickness on human HTS is found after pressure therapy in clinical trials (Kim et al., 2015; Lai, Li-Tsang, & Zheng, 2010; Lynam et al., 2015). There is also a study reported that pressure therapy can control the inflammation process on human HTS (Li-Tsang et al., 2015). However, inconsistent results of pressure therapy were found across different studies (Ai et al., 2017; Anzarut, et al., 2009). There is still controversy among specialists regarding the effectiveness and mechanism of pressure therapy, and how is compression force translated to influence cell behaviors and scarring process remains unknown. Recently, more and more researchers believe that mechanotransduction might also be involved in the mechanisms of pressure therapy. Further research is needed to verify the presumption.

Previous studies reveal a gap of understanding the mechanotransduction on growing or matured HTS, and how compression force be transformed to affect HTS growing and maturation process. There is a need to further explore the mechanism of pressure therapy from mechanotransduction perspective and improve the treatment technique.

1.2 Aims of the study

In light of the limitations of previous studies, the study intended to investigate the mechanism of pressure therapy from mechanotransduction perspective, through examining the immunohistochemical characteristics of mechanosensitive molecules on growing and matured HTS, and comparing the clinical and immunohistochemical characteristic of HTS before and after pressure therapy. In the pre-post comparison, the influence of confounding factors was taken into account. Considering the influence of compression on different cell types might be different, epidermis and dermis were analyzed separately in immunohistochemical study.

The study aims (1) to find the relationship between post injury days and immunoreactivity of integrin-β1, FAK and ERK on human HTS, establishing a reference of mechanosensitive molecules on growing or matured HTS; (2) to compare the immunoreactivity of integrin-β1, FAK and ERK on human HTS before and after pressure therapy, aiming to preliminarily explore the effect of pressure therapy on mechanotransduction; (3) to examine the clinical effect of pressure therapy on convex area considering the compression from three dimensions, aiming to translate preclinical findings into clinical practice.

1.3 Outlines of the thesis

Chapter 1 Introduction

Chapter 2 Literature review: This chapter starts with an overview of epidemiology and pathophysiology of HTS, a review of mechanotransduction in fibrosis, highlighting predominant molecules in force-regulating scarring as integrin-β1, FAK and ERK. Then, history and mechanisms of pressure therapy were introduced, stating the necessity of investigating and refining pressure therapy. To assist the design of main study, a systematic review was conducted to summarize confounding factors during pressure therapy.

Chapter 3 Phase I of the study: An observational study was conducted to explore the relationship between post injury days and immunoreactivity of integrin-β1, FAK and ERK on human HTS, establishing a reference of mechanosensitive molecules on growing or matured HTS. Thirty-one subjects with forty-three HTS specimens were obtained. An increasing immunoreactivity of integrin-β1, FAK and ERK was revealed from two to three months after injury, suggesting a critical time for intervention using external force like pressure therapy.

Chapter 4 Phase II of the study: A pretest-posttest study was designed in phase II to explore the effect of pressure therapy on mechanotransduction. Thirty-one subjects with forty-three HTS specimens were obtained before and after pressure therapy. Immunoreactivity of integrin-β1, FAK and ERK was compared before and after standardized pressure therapy. The influence of post injury days and scar thickness on the

change of immunoreactivity was also explored. Immunoreactivity of dermal integrin-β1, FAK and ERK was found decreased after pressure therapy, and an earlier treatment was beneficial to a larger effect. The study preliminarily demonstrated an effect of pressure therapy in down-regulating mechanosensitive molecules in dermis.

Chapter 5 Phase III of the study: Based on findings from phase II study, and the high expression of FAK detected over perilesional region of aggressive scars from other researchers, in phase III of the study, a case report of modified pressure therapy generating compression force on convex hypertrophic scars from three dimensions (3D) was described. The objective scar thickness was found being controlled after the application of 3D pressure therapy. This case report showed a translation from preclinical findings to effective clinical practice.

Chapter 6 Discussion and conclusion: Aims and findings of the thesis were summarized and restated. A thorough discussion on the main results was presented. Immunohistochemical findings were interpreted in terms of clinical application. The possible mechanism of pressure therapy in mechanotransduction was suggested. In addition, significance, limitation, and prospects for future research were stated.

Chapter 2 Literature Review

2.1 Epidemiology of burn injury and hypertrophic scars

It is estimated that 265,000 deaths are caused by burns annually. The incidence of burn injuries that requiring medical care is almost 20 times higher in the World Health Organization (WHO) Western Pacific Region than in the United States. In 2008, nearly 11 million people worldwide were reported to suffer from burn injuries that required medical attention. Burns are the eleventh leading cause of death among children aged 1 to 9 years of age and the fifth most common cause of nonfatal childhood injuries (World Health Organization, 2017). Community surveys in Bangladesh and Ethiopia showed that 80% to 90% of burns occur in the home. Children and women are usually burned in domestic kitchens, from upsetting receptacles that containing hot liquids, or from cookstove explosions (World Health Organization, 2004). Men are most likely to be burned in the workplace due to fire, scalding, and chemical and electrical burns (World Health Organization, 2017). A survey in China demonstrated that most inpatients with burn injuries were aged between 20 and 49 years old, with men predominating. Flame (37.04%) and electricity (25.40%) were the most common modes of injury, and hand burns were more commonly in the workplace (60.85%) (Wang et al., 2015).

Hypertrophic scar (HTS) following burn injuries has been regarded as a common problematic concern with a prevalence of 62% in whites and 80% in the nonwhite races (Bombaro et al., 2003). In Chinese population, this prevalence exceeds 70%, much higher than in the Caucasian population (Li-Tsang et al., 2005). Common complications of scarring, such as soft tissue contracture, joint stiffness, chronic pain, impaired body image

and perceived stigma, greatly affect social participation and quality of life in burn survivors (Gabriel, 2011; Lawrence et al., 2012).

2.2 HTS

2.2.1 Characteristics of HTS

HTS is characterized by raised, hypervascular, painful and rigid scar within the boundary of the original wound, accompanied by a loss of rete pegs, adnexa, and hair follicles in the injured area. Disturbance of extracellular matrix (ECM) balance in the wound healing process, as well as inherent characteristics of skin structure, determine the HTS formation. Normal skin has a network of distinct bundles that forms a parallel array, whereas in HTS, myofibroblasts, small vessels, collagen fibers and extracellular collagen filaments are randomly organized with (especially type III collagen), forming a nodular structure (Aarabi et al., 2007; Jumper, Paus, & Bayat, 2015). Compared to normal skin, HTS results in hyper-proliferation of the resident fibroblasts, excessive deposition of ECM proteins, and persistence of myofibroblasts and their biomarker, α-smooth muscle actin (Ehrlich et al., 1994; Song, et al., 2011; Zhu, et al., 2013).

2.2.2 Physiology of wound healing process and HTS formation

2.2.2.1 Wound healing process

The wound healing process is composed of four phases: hemostasis, inflammation, proliferation and remodeling. Upon skin injury, blood clots are formed under the activation of von Willebrand factor (vWF) to prevent hemorrhage and to provide a scaffold for

wound repair. Platelets attract the migration of leukocytes by releasing several growth factors, including transforming growth factor (TGF-ß). The dilation of blood vessels and increased vascular permeability allow the migration of leukocytes into the damaged tissue to defend against microbes (Ham et al., 2010; Reinke & Sorg, 2012).

Afterwards, mediators released by neutrophils such as interleukins (IL)-1, IL-1ß, IL-6) and Tumor necrosis factor (TNF) α amplify the inflammatory response and stimulate vascular endothelial growth factor (VEGF) and IL-8 to initiate the repair process. TNF-α secreted by macrophages promotes synthesis of inflammatory serum proteins (Wang et al., 2011). Profibrotic cytokines secreted by leukocytes, such as IL-13 and TGF-ß, accumulate and are responsible for the recruitment and activation of fibroblasts, endothelial cells, and keratinocytes (Penn, Grobbelaar, &Rolfe, 2012).

In the proliferation phase, TGF-ß stimulates the adjacent capillaries to grow into the wound. Vasodilation and angiogenesis increase the perfusion to the wound to transmit oxygen and nutrition. Fibroblasts are recruited by pro-fibrotic cytokines and produce collagen and other components in the ECM and migrate to the myofibroblasts. Finally, in parallel with connective tissue repairing, epithelial and endothelial cells migrate over the newly forming granulation tissues (Czubryt, 2012; Martins, Caley, & O'Toole, 2013; Zhu et al., 2013).

During remodeling, proteolytic degradation functions to remove excess ECM at the scar sites. Loss of vascularity and degradation of collagen occurs to achieve the scar-less wound healing. The balanced collagen synthesis and catabolism is achieved by regulation of matrix metalloproteinases (MMPs) and their inhibitors, tissue inhibitors of

metalloproteinases (TIMPs). The prolonged the inflammation process, which is characterized by increased pro-fibrotic mediators, and the subsequent excessive deposition of ECM components, will initiate the formation of HTS (Eto et al., 2012; Zhu et al., 2013).

2.2.2.2 Pathophysiology of HTS

HTS is the consequence of imbalance among the overproliferation of fibroblasts and keratinocytes, the excessive deposition of substrates in the ECM, and suppressed cellular apoptosis and depositions degradation (Zhu, Ding, & Tredget, 2016). Different responses of aberrant fibroblast phenotype in HTS to various molecules defected cell proliferation and apoptosis balance in ECM. Increased TGF-ß isoforms were found to be highly correlated to HTS after severe burn injury (Penn et al., 2012). MMP and TIMPs play roles in the regulation of collagen cleavage and degradation. Moreover, the abovementioned features can be stimulated by mechanical tension and persist for more than one year in HTS and lead to prolonged fibrosis, which causes difficulty in effective long-term effective management (Zhu, Ding, & Tredget, 2016).

2.3 Mechanotransduction

In the early 1860s, Australian anatomist Karl Langer discovered topological lines distributed on human body (Langer 1861). The Langer's lines are regarded as the natural orientation of collagen fibers in the dermis and reflects skin tension distribution (Elsner et al., 2002). Clinicians later found that skin tension due to congenital anatomical distribution or mechanical stretching from the environment is associated with the development of HTS (Wong et al., 2010). The concept of skin tension has long been applied in the plastic

surgery to prevent pathologic scarring (Bethke, 2005; Wong et al., 2010). More and more studies have explored the effects of mechanical force in scar formation.

2.3.1 Effect of mechanical force in scar formation

(1) External stretching can induce scar formation during wound healing

At the tissue level, the shape and incidence of aberrant scars are correlated with skin tension distribution (Figure 2-1) (Bouffard et al., 2008; Ogawa et al., 2012; Wong, Levi, Akaishi, Schultz, & Dauskardt, 2012). In animal models, stretching of wounds induces HTS formation, and the severity of scars is correlated with the post-operation time, the intensity and frequency of stretch application (Cheng, et al., 2015, Gurtner et al, 2011).

At the cellular level, stretching on skin surface can induce fibroblasts the migration and differentiation of fibroblasts. Inflammatory cytokines and growth factors, such as TGF-β 1, IL-6, IL-8, were found to ameliorate after stretching (Peters et al., 2015; Huang et al., 2013; Wang et al., 2011; Chin et al., 2009; Junkder et al., 2008). Meanwhile, stretching can control protein cleavage by regulating MMPs (Zhang et al., 2015; Edna et al., 2014). In addition, stretching can initiate HTS formation by decreasing cellular apoptosis via the Akt dependent pathway in a murine model of HTS (Aarabi et al., 2007).

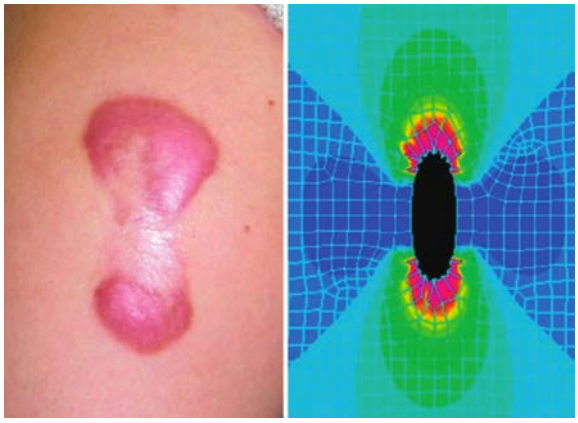


Figure 2-1 HTS (left) are surrounded by increased stretching tension, as indicated by computer modeling (right). Computer simulation (finite element analysis) of a stretched HTS revealed that the edges of the HTS are associated with a high degree of tension. Picture from (Ogawa, et al., 2011)

(2) Tension reduction and scar formation

In contrast, tension reduction strategies have been found to prevent HTS formation in early wound healing. In an animal model with application of 20% compressive strain on wounds, decreased cell density, epithelial thickening and improvement in scarless healing were observed (Gurtner et al., 2011). Finite element modeling (FEM) and biomechanical analysis were applied to simulate and visualize the tension distribution around scars under polymer materials. In a standard scar and a model that simulated the application of a silicone gel sheet, high tension over the boundary of the elevated scar was observed to transmitted to normal skin after silicone sheet application (Figure 1) (Akaishi et al., 2010; Anthonissen et al., 2016; Gurtner et al., 2011; Yagmur et al., 2010).

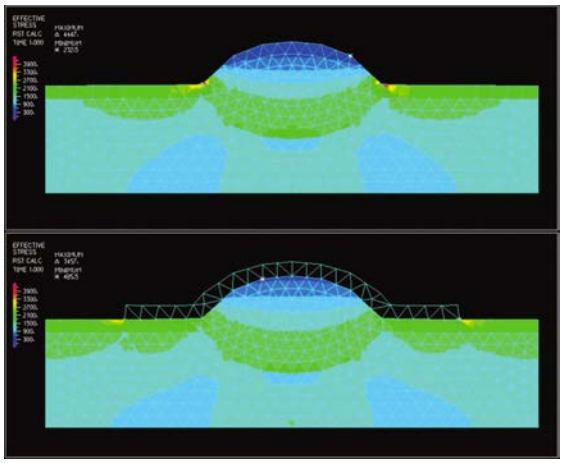


Figure 2-2. The standard scar model and the silicone gel sheet applied model. The normal model (above) indicates high stretching tension, as indicated by the red and yellow coloring, around the border between the scar and normal skin. However, in the silicone gel sheet model (below), there are no red or yellow triangular elements around the border. In addition, stress changes in the triangular element of normal skin under the edge of the silicone gel sheet were observed (skin stress) Picture from (Akaishi et al., 2010).

(3) Effect of force on growing or matured scar need to be investigated

Evidence shows that mechanical force can modulate mechanosensitive cells like fibroblasts and keratinocytes and thus affect HTS formation (Bouffard et al., 2008; Ogawa et al., 2012; Wong et al., 2012). The wound healing and HTS formation processes are consecutive and interrelated. However, accounting for the variation in molecular process in wound healing, the effect of mechanical force on growing or matured scars might be different on healing wounds (Chiquet, Gelman, Lutz, & Maier, 2009; Duscher et al., 2014). To enlighten the study design of exploring the effect of pressure therapy on formed scars, there is a need to summarize the available experiments.

2.3.2 Theory of mechanotransduction

Mechanotransduction refers to the process of converting mechanical signals, such as tension, compression, gravity, and hydrostatic pressure, into chemical signals and

modulating subsequent cascade of cellular response (Chiquet, Renedo, Huber, &Flück, 2003). The regulation is believed to be achieved through cell-cell, ECM-cell, and macromolecular-cell interactions (Silver, Siperko, &Seehra, 2003).

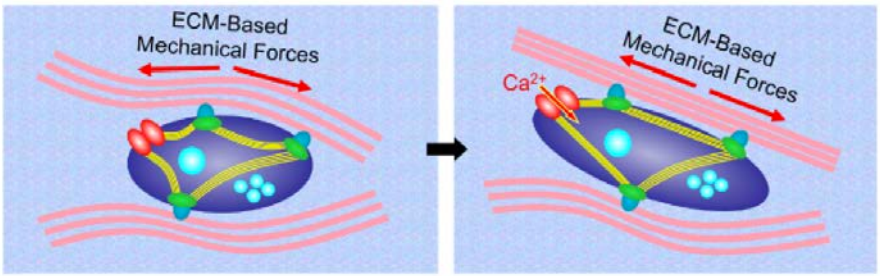


Figure 2-3 Cellular mechanoreceptors. At the cellular level, ECM-based mechanical forces such as stretching tension, shear force, can be perceived by cellular mechanoreceptors including the cytoskeleton, cell adhesion molecules (e.g., integrins), and mechanosensitive ion channels. (Ogawa, 2011)

In mechanotransduction, transmembrane integrins are important for cell migration and contribute to attachment between the epidermis to the basement membrane (Monsuur et al., 2016). Focal adhesion complexes, one of the principal contact sites to transmit force from extracellular matrix to intracellular cytoskeleton, has been detected on the membrane of keratinocytes and fibroblasts (Paterno et al., 2011; Yano et al., 2004). Studies have shown that, mechanical force is transmitted into cytoskeleton through exposing the biding sites of integrin-β1. Conformational change in the integrin allows for binding and activating of focal adhesion kinase (FAK). Afterwards, the initiation of subsequent mitogen-protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathway leads to gene transcription, and finally alters the cell dynamics (Chiquet et al., 2009; Geiger, Bershadsky, Pankov, & Yamada, 2001; Ingber, 1993; Koivisto, Heino, Häkkinen, & Larjava, 2014; Wang et al., 2015; Wong, Longaker & Gurtner, 2012). The spatial relationship of FAK(Tyr397), Integrin-β1 and ERK (1/2) are illustrated in Figure 2-4.

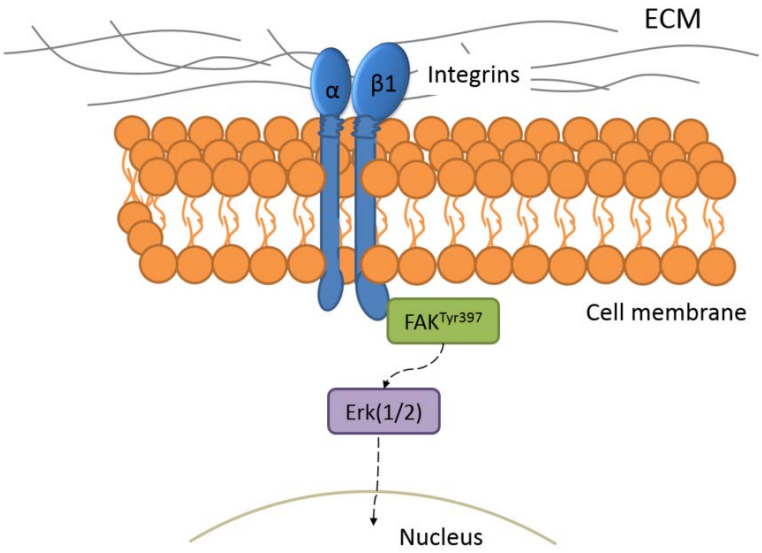


Figure 2-4 Illustration of spatial relationship of Integrin-β1, FAK(Tyr397) and ERK (1/2).

2.3.3 Mechanosensitive molecules and scarring

In vivo animal studies have found that, FAK-ERK- monocyte chemoattractant protein-1 (MCP-1) pathway is predominant in stretch-induced TGF-β1 secretion in inflammatory response (Chiquet et al., 2009; Wong et al., 2012). As for the protein degradation process regulated mainly by metalloproteinases (MMPs) and their inhibitions (TIMPs), some researchers suggested that stretch-mediated MMPs was controlled through activation of α2β1 integrin and subsequent FAK(Tyr397) (Zhang et al., 2015; Edna et al., 2014). Meanwhile, mechanically activated FAK was found to be a candidate for upstream cell apoptosis signaling pathway in human fibroblasts, and was predicted to be involved in compression for hypertrophic scars (Aarabi et al. 2007; Niland et al., 2001; Reno et al., 2003; Tian et al., 2002). And the inhibition of FAK or integrins through fibroblast-specific knockout or pharmacologic blockade is sufficient to interrupt mechanotransduction,

prevent augmentation of inflammation and promote ECM homeostasis, resulting in markedly less scarring (Leask, 2013; Wong et al., 2012; Zhang et al., 2015). These studies demonstrated that the activation of force-regulated FAK(Tyr397) and Integrin-β1 can affect HTS formation through regulating inflammatory, degradation and apoptosis process in scarring. In addition, previous research analyzing the known downstream mediators of FAK, found only ERK (1/2) was activated by mechanical stretching and regulated by FAK. Overall, these studies indicated that FAK(Tyr397), Integrin-β1 and ERK (1/2) can be used as a functional group to investigate the force-regulated scarring process.

Meanwhile, pressure therapy is found to control scarring through regulating similar processes such as inflammatory, degradation and apoptosis. TGF-β1, MMP-2, MMP-9 were found attenuated after pressure therapy, and apoptosis process examined through tunnel assay was reported to be activated (Feng, 2012; Kim et al., 2015; Li-Tsang et al., 2015). There are opinions that compression may alter the rigidity of ECM, control the activity level of mechanoreceptors, and control scar development (Silver, Siperko, & Seehra, 2003; Yagmur, Akaishi, Ogawa, & Guneren, 2010). Further research is needed to verify the presumption. As mentioned earlier, functional group of FAK(Tyr397) together with integrin-β1 and ERK (1/2) are force-sensitive molecules that can affect HTS formation through inflammatory, degradation and apoptosis. The similar scarring processes controlled by stretch and compression indicate that the FAK(Tyr397) together with integrin-β1 and ERK (1/2) can also be a candidate functional group to narrow the knowledge gap between compression and force-induced scarring process.

Therefore, the following molecules were critical for both mechanotransduction and scarring, they were selected in phase I and phase II of the study:

(1) Integrin-β1: a transmembrane receptor that bridges for cell-cell and cell-ECM interactions. Integrin-β1 can be triggered by mechanical force and in turn trigger the interior pathways, and results in an activation of transcription to regulate cell function, thus affect HTS formation (Jean, Gravelle, Fournie, & Laurent, 2011; Zhang, Truskey, & Kraus, 2007)

(2) Phosphorylated FAK(Tyr397) (focal adhesion kinase): a protein tyrosine kinase. It has been shown that on integrin binding to ligand and subsequent clustering of integrins, FAK becomes autophosphorylated, mainly at the site of Tyr397, to regulate cell adhesion and spreading process (Zebda, Dubrovskyi, & Birukov, 2012). It is a key mediator of TGF-β signaling in fibroblasts (Leask, 2013).

(3) Phosphorylated ERK (1/2) (extracellular signal-regulated kinases): a chain of proteins in fibroblasts that communicates a signal from a receptor on the cell membrane to the DNA in the nucleus of the cell. It could initiate a series of transcription after activated by FAK. Previous research analyzing the known downstream mediators of FAK, found only ERK was activated by mechanical loading and regulated by FAK (Hong et al., 2010; K. Zhang et al., 2015; Wong et al., 2011).

2.4 Pressure therapy

2.4.1 History and clinical application pressure therapy

There is a wide spectrum of treatments applied to prevent or treat HTS. Non-invasive conservative treatments are recommended as frontline managements. Their cost-effectiveness and painless caring are favored in clinic comparing to invasive treatments like surgical excision and intralesional corticosteroid injection (Monstrey et al 2014). Pressure therapy have long been applied as frontline conservative treatments for HTS after severe burns (Larson et al., 1974; Ward, 1991)). It refers to the process of applying compression force on HTS to control the scar progression. Various modalities of material have been applied to exert compression for scars. Easily available materials like elastic wraps and tubular bandages are used for temporary pressurization in early times (Ward, 1991; Todd, 2011). Nowadays, customized pressure garments fabricated using spandex, neoprene are developed for consistent and effective management (Li-Tsang, 2009; Yelvington et al., 2013).

Although more and more non-invasive treatments are developing, such as silicone gel sheets and vacuum massage, pressure therapy is still prioritized for managing widespread scars (Li-Tsang & Zheng, 2010; Saxena et al., 2004; Steinstraesser et al., 2011; Van denKerckhove et al., 2005). As there is a high prevalence of burn injuries occurred in low-income countries and in pediatric populations, cost-effective and comfortable scar managements are required (World Health Organization, 2017). Compare to other treatments, the wide ranges of applicable materials make pressure therapy available to diverse circumstances.

Thriving to improve the clinical outcomes during pressure intervention, more and more clinical trials provided the evidence to support the application protocol. A pressure dosage of 15mmHg is widely accepted as a minimal ‘‘dose’’ necessary for an effect, and an interface pressures over than 40 mmHg could lead to discomfort and complications (Engrav et al., 2010; Van den Kerckhov et al., 2005; Giele et al., 1998; Naismith et al., 1980). Lai’s group compared the difference between the five-month pressure intervention with high pressure (20– 25 mmHg) and low pressure (10–15 mmHg). They found a statistically difference between the improvement in high pressure group and low pressure group (Lai et al., 2010). However, there is still controversy among specialists. Previous Meta-analysis summarized the subjective outcomes of HTS upon pressure treatments, and resulted in inconsistent conclusion in terms of thickness (Ai et al., 2017; Anzarut, Olson, Singh, Rowe, & Tredget, 2009). The studies with objective measurements also failed to reach the consensus on the clinical effectiveness of pressure therapy (Atiyeh, El Khatib, & Dibo, 2013). In addition, despite the world-wide clinical application, the mechanism pressure therapy is still unknown. Therefore, there is a need to objectively assess the HTS upon pressure therapy and explore the underlying mechanism.

2.4.2 The mechanism of pressure therapy at the molecular level

Extensive clinical trials have verified the effectiveness of pressure therapy on HTS in decreasing hardness, thickness, and increasing elasticity (Kim et al., 2015; Lai, Li-Tsang, & Zheng, 2010; Lynam et al., 2015). Biophysiological effect of pressure therapy has also been explored. In vitro studies, decreased growth of keratinocyte, fibroblasts,

myofibroblasts and collagen were observed under compression both in human and murine models (Li-Tsang et al., 2015; Feng, 2012; Chang, Deng, & Yeong, 2008). Moreover, several cytokines and pathways mentioned in 2.2.2, such as cell proliferation (TGF-β1) and substrate deposition (MMPs) in proliferative scars, were regulated through applying pressure therapy (Li-Tsang et al., 2015; Huang et al., 2014; Reno, 2002; Chang, Deng, & Yeong, 2008). Apart from biophysiological response exert by pressure therapy, it is also important to elaborate how does this external force been "translated" into molecular signals.

Many theories have been proposed to explain how pressure therapy works on hypertrophic scar. Previous researchers proposed that pressure therapy could reduce scar hydration, thus stabilizing the mast cells, decrease neovasculaization and inhibit ECM substrates deposition (Baur et al., 1976). Besides, Lynam et al. found that pressure can reduce capillary perfusion and induce hypoxic effects. Severe hypoxia and malnutrition suppress cell viability, resulting in fibroblast degeneration and collagen degradation (Lynam et al., 2015). There is also speculation that pressure therapy can decrease the nociceptor activities, thus to reduce the release of neuropeptide and control excessive scarring (Chin et al., 2009; Wong, Longaker, & Gurtner, 2012). Up to now, there is still no solid evidence and general agreement on the mechanism of pressure therapy.

There are opinions that the effect of pressure therapy on HTS may act through mechanotransduction (Carver & Goldsmith, 2013; Haga, Li, & Chien, 2007). Researchers believe that compression can change the properties of ECM, regulate the activity level of mechanoreceptors, and control scar development (Silver, Siperko, & Seehra, 2003;

Yagmur, Akaishi, Ogawa, & Guneren, 2010). Previous research showed that, the inhibition of integrin-β1 and FAK can suppress inflammatory cytokines (TGF-β1, IL-4, and IL-13), activated molecules for protein degradation (MMP-2, 9), and augment apoptosis process, thus to prevent scar formation (Chiquet et al., 2009; Wong et al., 2007). The above-mentioned cytokines and processes activated by mechanical stretch was found to be controlled after pressure therapy (Feng, 2012; Kim et al., 2015; Li-Tsang et al., 2015). Therefore, we hypothesized that, pressure therapy can control HTS progression through affect the activation of mechanosensitive molecules.

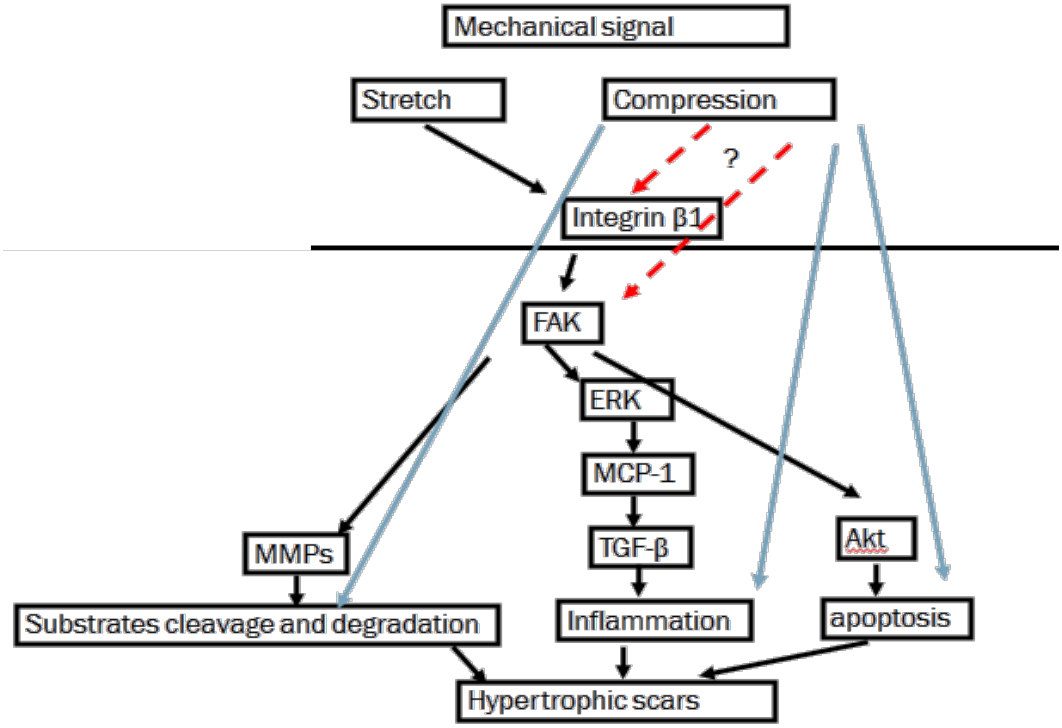


Figure 2-5 Proposed theoretical framework on the mechanisms of compression (pressure therapy) using the mechanotransduction model.

2.5 Systematic review on the effect of mechanical force on HTS: from clinical and preclinical aspects.

To explore the effect of mechanical force on scar tissues or cells in terms of the mode, intensity and frequency, a systematic review was conducted. Articles fulfilled the pre-defined criteria were extracted from the Cochrane Library, MEDLINE, CINAHL, Science direct, SPORTDiscus, Embase and PEDro between 1995 and 2016. The studies were summarized from clinical and preclinical aspect (Zhang et al., 2016).

2.5.1 General information of recruited studies exploring the confounding factors during force application

For clinical trials, 10 studies were included in the summarization and three of them examined stretching, six on massage, and one study on splinting. As for preclinical trials, a total of 19 studies were summarized. There were 5 in-vivo animal studies, conducting on C57/BL6 mice, Sprague-Dawley female rats and Red Duroc swine; 11 in-vitro studies were reviewed and their experiments utilized human dermal fibroblasts (HDF), human skin equivalents (HSE), human keloid fibroblast, murine dermal fibroblasts, TRPC3 fibroblasts, and 3 ex-vivo studies used ex-vivo Human HTS tissue and healthy human skin tissue.

For outcome measures, Vancouver Scar Scale (VSS), modified VSS, and patient and observer scar assessment scale (POSAS), ultrasound and Doppler were used as for scar assessments. In preclinical studies, dermal thickness, epidermal thickness and cellular density were used to describe the histological difference after force application. To observe the effect on inflammation, the change of isoforms of TGF-β, especially TGF-β1, were assessed in most of the studies. Subtypes of MMP and their inhibitors TIMP, were measured mainly in in-vitro experiments as monitor of degradation process. In addition, the molecules critical in mechanotransduction, such as integrins, FAK, vinculin, ERK were examined together with proteins in their intracellular subsequence (Table 2.1-2.7).

2.5.2 The force application time is critical for scar management

2.5.2.1 Clinical trials

The post-injury days for stretch on scars varied from 48 hours to 16 years after the injury. Among 3 articles examined the effect of stretching on scars, two randominzed contolled trials (RCTs) addressed the early stretching within one-week (Okhovatian & Zoubine, 2007; Perera et al., 2015). One of the studies found that, stretching initiated within 48 hours after 2nd degree burns, or 5 days after grafting, resulted in significant improvement in VSS of axillary scars (Perera et al., 2015). Okhovatian had similar emphasis on early stretch. He compared the training group of an stretch prescibed on the first day of admission or the third day after grafting, with the control group which started training 2 weeks later. Early stretched group had significant less number of contrancture than the control group (Okhovatian & Zoubine, 2007). Another controlled clinical trial (CCT) explored the effect of stretch on scars one to three months after injuries. Through a weekly comparison of the change, the largest effect of increased range of motion (ROM) was found in the first week (Godleski et al., 2013).

2.5.2.2 Preclinical studies

The time of force application was proved to be an important factor in animal studies. In Aarabi's study, continuous static tension applied 1~3 days after incision resulted in wound dehiscence. If the force applied 3~6 days after incision, typical histology of HTS can be observed, while applied 6 days later could not trigger the HTS growth (Aarabi et al., 2007). The importance of time was also addressed in Cheng's team (Cheng et al., 2015). Whereas

the results of wound after stretch were different across the animal studies. Aarabi (Aarabi et al., 2007) found no significant difference in scar proliferation when a static tension is applied 6 days post-incision, while Cheng's team observed an HTS-like red, hard scar when adding tension 7 days after the operation (Cheng et al., 2015).

2.5.3 The importance of force quantification

2.5.3.1 Clinical trials

Two controlled studies conducted by Roh in 2007 and 2010 took scar properties as outcome measures (Roh et al., 2007; Roh et al., 2010), shared the similar post-injury time, treatment regime and predefined intervention duration (3 months). In the study conducted in 2007 (Roh et al., 2007), VSS scores showed significant improvement. Whereas no significant difference was found in 2010 with a similar massage protocol.

2.5.3.2 Preclinical studies

As for the magnitude of the applied skin tension, there were studies quantified a tension of 2.7 N/mm^2 on mice wound to be equivalent with tension from human resting wound (Aarabi et al., 2007; J. et al., 2011; Wong et al., 2012). Different magnitude of mechanical tension on red Duroc swine's' early wounds showed dependent severity of scar formation (Gurtner et al., 2011). Attempts have also been made in in-vitro studies to compare the influence of cyclic stretch with high (1Hz) or low (0.1Hz) frequency. There is a consensus that stretch with high frequency augment the inflammatory signaling and mechanotransduction pathway, while contradictory results are obtained with low frequency stretch (Kanazawa et al., 2009; Kuang, Wang, Xu, Cai, & Liu, 2015; Rolin et al., 2014).

2.5.4 Enlightening the design of main study and clinical application

In summary, general agreements were made on the importance of timing and intensity of force in scar formation. However, controversy of early stretch force existed between clinical trials and preclinical studies. Previous force-related clinical trials failed to establish the effective application regime on HTS with high-level evidence. There was evidence that the magnitude and frequency of force can affect the signaling and cell behaviors. This implied the importance of controlling force in an objective way when exploring the effect of force on HTS.

Notwithstanding there are pioneers who investigated the effect of tension reduction in preventing HTS scars early after wound healed, only few studies focused on the therapeutic effect on HTS after burn injury or in the proliferation stages. One reason was the scarcity of established effectiveness in valid preclinical experiments. Preclinical studies provide solid evidence on early-stretch-induced scar formation, whereas the findings from animal or in-vitro studies would be different to interpret in human beings. Variations of prognostic factors and ethical considerations pose difficulties in performing HTS therapeutic studies on human beings to elaborate the true effectiveness of a treatment. This suggested a necessity of research on human HTS.

Overall, this systematic review of previous literature could assist in our main study design. The following factors should be controlled in the experiment:

(1) Controlling for the time post-injury which could affect the effect of treatment.

(2) Monitoring the intensity of force during experiments objectively.

(3) Bridging the gap between clinical observation and molecular signaling.

Table 2-1 Stretching protocols and outcomes.

Stretching									
Author	Population and Patients	Scar type	Experiment group (EG)	Control group (CG)	Time of treatment initiation	Regime	Intervention length	Follow-up length	Outcome measure (+/-/0):+p<0.05
Perera, 2015, Level I RCT	Sri Lanka; n=220; age:15-50; TBSA: 10%-45%	Axillary scar after burn injury	CT + stretch N=110	CT N=110	2nd degree burns: within 48hr; 3rd degree burn + skin graft: after 5 d	Repetitions increased gradually	14 days	3, 6, 12 months	Quick Dash: +; VSS:+
Okhovatian, 2007, Level I RCT	Iran; n=30; age: CG 36±10, EG 39±9	Group match burn injured patients	Burn specific rehabilitation protocol: early initiation, stretching exercise, active exercise, daily activities, N=15	CT N=15	EG: 1st day of admission and 3rd day after grafting CG: 2 weeks after admission, 10-15 d after grafting	CG: 15-20 min/session, 1 session/d EG: 30-45 min/session, 2-3 session/d	CG: 26±15 EG:22±12	Pre-post	No. of contracture (ROM<functional range): +, thrombosis: 0; length of stay 0, skin graft 0;
Godleski, 2013, Level IV CCT	America n=9; age: 39.4±13.5; TBSA: 40.3±21.8	After skin graft after burn injury	Intensive stretch for active area + CT	CT: Strengthening, mobility, self-care activities	61.4±25.5 days	>1hr/d (30min-OT, 30min-PT)	61.4±25.5	0, 4 weeks	Within group comparison: Goniometry + Finger flexion + Kapandji opposition scale + Largest gain in week 1

Note. EG: Experimental group; CG: controlled group; CT: conventional treatment; TBSA: total body surface area; PT: physical therapy; OT: occupational therapy; hr: hour; d: day; min: minute

Table 2-2 Massage protocols and outcomes part A.

Massage									
Author	Population and Patients	Scar type	Experiment group	Control group	Time of treatment initiation	Regime	Intervention length	Follow-up length	Outcome measure (+/-/0):+p<0.05
Cho YS, 2014, Level I RCT	Korean N=146; EG: age: 46.06±8.63 TBSA: 37.25±18.60, CG: age: 47.21±8.22	Hypertrophic scars after acute management of burns, including skin graft	CT + massage (Effleurage, friction, petrissage massage after cream, oil and lotion; n=80	CT (ROM + silicone gel + pressure therapy + intralesional corticosteroid injection + cream/oil; n=80	CG:156.47±56.48 d EG: 148.77±56.85 d	3 sessions/week, 30 min/session for each area	CG: 35.85±11.80 d EG: 34.69±22.53 d	Pre-post	-pruritus (VAS): +; Itching scale +; scar thickness +, melanin and erythema +, TEWL +, scar sebum 0, scar elasticity 0
Morien, 2008, Level III CCT	America n=8; age 13.5±2.6(10-17y)	Well-healed skin grafts >2 years after third degree burns	5 min effleurage, 5 min stretching and rolling strokes, 2-5 min friction, 5 min lengthening and rolling	Contra-lateral scar site without massage	2-16 years after burn injury	1session/d, 20-25min/session	5 children for 4-5days, 3 for 3days	Pre-post	-Subjective reported mood: 0, -ROM of scar adjacent joints: EG:+, CG: 0
Roh, 2010, Level IIII, CCT	Korean N=26, age>18, EG: age 37.7±13.67, TBSA: 29.54±16.44	partial or full-thickness burn on forearm or hand	skin rehabilitation nursing program: light palm stroking, acupressure and occlusive dressing n=13	CT without massage n=13, ,	EG: 3.46±2.40 month; CG: 3.38±2.26 month	30min/session, 3 session/wk	3months	Pre-post	-scar thickness (ultrasound) 0, -blood perfusion (Laser Doppler Imager) 0, -POSAS: 0, -depression (CESD): 0, - BSHS-B-K: 0

Note. VAS: Visual analogue scale; TEWL: transepidermal water loss; POSAS: patient and observer scar assessment scale; CES-D: Korean Center for Epidemiologic Studies Depression Scale; BSHS-B-K: Korean Burn Specific Health Scale-Brief

Table 2-3 Massage protocols and outcomes part B

Massage									
Author	Population and Patients	Scar type	Experiment group	Control group	Time of treatment initiation	Regime	Intervention length	Follow-up length	Outcome measure (+/-/0):+p<0.05
Roh, 2007, Level III, CCT	Korean; n=34, age>18, EG: age: 33.3±8.3, CG: age: 39.1±8.2;	post burn scar at hand or forearm, partial or full-thickness	massage, light stroking of palm, acupressure on unscarred areas on forearm and hand n=18	conventional without massage n=17	EG: 127±171.1; CG: 95.3±83.7	Care giver massage 10min/d, skin rehabilitation massage therapy 30min/week	3months	0, 3 months, subjective skin status at 3 month	total VSS and subscores: skin status (subjective): + depression (CES-D) +, ithicness (Itch Man Scale)
Patiño, 1999, Level I RCT	Argentina, children, n=30, EG: age: 59.4±5.3 months; CG: age: 51.3±4.1 months	pediatric patients, HTS>30% TBSA. Worst 10cm2 area of HTS identified by VSS	pressure garment and friction massage with plain cream	pressure garments only	unknown	massage 10min/d, daily	3months	0, 3 months, subjective skin status at 3 month	modified VSS 0
Silverberg, 1999, Level II RCT	USA n=10, (white:3, black:4, hispanic:3; mean age=51, TBSA=25.5%;	post burn scar at wrist; EG: 2 dorsal wrist burn, 3 volar wrist burn, CG:5 dorsal wrist burn	CT + soft tissue mobilization, (direct oscillation, friction massage) n=5, mean age=51,	CT (active assisted ROM) n=5,	1-11 months after burn injury	10-15min	10-15min	Pre-post	CG ROM: wrist extension +; radial deviation: +. Tot ROM: 0; VSS 0 EG ROM: wrist extension +, ulnar deviation: +; Tota ROM: 0; VSS 0

Table 2-4 Splint protocol and outcomes.

Splint									
Author	Population and Patients	Scar type	Experiment group	Control group	Time of treatment initiation	Regime	Intervention length	Follow-up length	Outcome measure (+/0):+p<0.05
Kolmus, 2012, Level II RCT	Melbourne n=52, age>18 EG: age: 43.5±18.0(3-50y), TBSA:19.1±14.2 CG: age 49.4±19.0, TBSA: 18.6±10.6 (3-40)	Axillary burn require surgery;	Splint: shoulder splint (immobilization abduction 90°) + CT n=27	CT: stretching, strengthening and functional retraining N=25	Usually 5 days after grafting	first 6 wk all day + 6 wk: overnight	12 weeks	6, 12 weeks	ROM (Plurimeter-V Inclinometer) -shoulder abduction: -shoulder flexion: 0; BSHS-B: 0; UEFI: 0; GST: 0

Note. BSHS-B: Burn Specific Health Scale-Brief questionnaire; UEFI: Upper Extremity Functional Index; GST: Grocery Shelving Task

Table 2-5 In vivo animal study of mechanical stretch.

In-vivo	Equipment	Modality	Intensity	Duration	Post-incision day	Outcomes	Author
C57/BL6 mice	customized loading device: 22-mm expansion screws and Luhr plate supports	continuous static tension	2.7 N/mm^2	10d (2week)	day 1-3	histology: wound dehiscence	(Aarabi et al., 200
					day 3-6 (day 4)	- histology: ⬆ in dermal thickness, cellularity, vessels; ⬇ in hair follicles; - inflammatory cells & cytokines: ⬆ mast cell, macrophage, IL-4, IL-13, MCP-1; - apoptosis: ⬆activated p-Akt proteinI⬇ apoptosis; - ECM: ⬆α-SMA, collagen (collagen per cell remains stable after 2wk). - intracellular process: ⬆p-FAK	(Aarabi et al., 2 Paterno et al., 201 W.Wong et al., 2
					after 6 days	- no effect on HTS	(Aarabi et al., 200
Sprague-Dawley female rats	customized elastic tension driven applier	continuous static tension	2N	7day	day 1	- histology: erythema in wk 1 and disappeared after the tension removal, faint red scar on day 9; - inflammatory cells & cytokines: ⬆ TGF-β	(Cheng et al., 201
			day 7:3N; day 14: 4N; day 21: 5N	21day	day 7	- histology: wounds healed in week 1, turned into red and hard scar from day 7; white, stiff, and elevated scar from week 5; - inflammatory cells & cytokines: ⬆ TGF- β	
			day 1: 2N; day 7:3N; day 14: 4N; day 21: 5N	28day	day 1	- histology: erythema in week 1, turned into red and hard scar from day 7; white, stiff, and elevated scar from week 5. thickest epidermal thickness among all groups; - Inflammatory cells & cytokines: highest TGF-β,⬆ mast cell infiltration	
Red Duroc swine	stress shield silicone polymer sheets on the side of long axis of the incision	continuous static tension	20% strain	8 week	day 4	- histology: ⬆cellular density, vessel density, epithelia thickness, ⬇ rete pegs, profibrotic signaling at the epidermal-dermal junction at week 8; - inflammatory cells & cytokines: ⬆ TGF-β, ECM, ⬆α-SMA	(Gurtner et al., 20

Table 2-6 Ex-vivo study of mechanical stretch.

Ex-vivo	Equipment	Modality	Intensity	Duration	Scar/postincision time	Outcome	Author
human HTS tissue	customized spring stretching device	Continuous static stretch	0.66 N/cm2	1d	12 months' scar post-operation	- ECM: ⬆α-SMA:	(Johan P EJunker et al., 2008; Kratz et al., 2001)
				6d		- histology: ⬆ cell density in basal layer of epidermis, ⬇in dermis; - ECM: ⬆α-SMA; ⬇collagen in dermis	
	skin-stretching device with a caliper	Continuous static stretch	55N	30 min	12 months' scar post-operation	- ECM: ⬆COI; × collagen bundle thickness	(Pauline D.Verhaegen et al., 2012)
healthy human skin tissue	skin-stretching device with a caliper	cyclically stretched (4min*6 times, 1 min interval)	55N	30 min	post-operation	- ECM: ⬆COI; ⬆collagen bundle thickness	
			30N	30 min	post-operation	- ECM: ⬆COI; ⬆collagen bundle thickness	

Note. ⬆: increase; ⬇: decrease; ×: no significant difference found

In-vitro	Equipment	Modality	Frequency	Intensity	Duration	Outcome	Author
murine dermal fibroblasts	FX-4000 dermal fibroblasts ERK, p	Cyclic stretch	0.1 Hz (6 cycles/min)	10 % strain	24hr	- inflammatory cells & cytokines: ⬆ TGFβ1, CTGF	(Peters et al., 2015a)
	cell stretcher (STB-140-10, STREX)	Cyclic stretch	0.5 cycle/min	20% strain	4hr	- apoptosis: ⬆ p-Akt 30 min from onset - apoptosis: ⬆ p-Akt at 15 min and peak at 60 min, higher than low frequency group	(Paterno et al., 2011)
HDF	FX-4000	Cyclic stretch	1 Hz	10% strain	96hr	- ECM: ⬆type I procollagen, TIMP-1and α-SMA, ⬇MMP-1 after 48h, 72h, 96h;	(Rolin et al., 2014a)
		static strain	11 cycle/min			- apoptosis: ⬆ p-Akt at 30 min; ⬆ Akt at each 30-minute cycle;	
			0.1 Hz	10% strain	48hr	- ECM: ×: α-SMA - histology: ⬆fibroblasts proliferation;	(Kuang, Wang
TRPC3 fibroblast	cell stretcher (STB-140-10, STREX)	uniaxial Cyclic stretch	10 cycles/min	20% strain	24hr	- ECM: ⬆TRPC3 regulate ⬆ expression of fibronectin; - inflammatory cells & cytokines: ⬆ TGF-β after 36hr stretch - intracellular process: ⬆TRPC3, NF-κB, calcium influx; - intracellular process: ⬆integrin β1, p130Ca after 36hr stretch	(H.Ishise et al, 2015b)
	Equibiaxial strain device		15 cycles/min	10% strain	24hr	- inflammatory cells & cytokines: ⬆MCP-1; - intracellular process: ⬆p-ERK 10 min after strain	(Wong et al., 2011)
	cell stretcher (STB-140-10, STREX)		10 cycles/min	20% strain	24hr	- histology: ⬆cell migration velocity, - inflammatory cells & cytokines: ×: TGF-β1/2/3, CTGF, IL6/CXC18, TNF; - ECM: ⬆Fibronectin, MMP-1, MMP-3; ×: TIMP-2; - intracellular process: ×: smad 2/3/4/7, RhoA, Rac1, ROCK, STAT 3, MAPK/G protein	(C.Huang, Miyazaki, et al 2013b; H.Ishis et al., 2015)
	apparatus (NS-500)		0.16 Hz	20% strain	24hr	- inflammatory cells & cytokines: ⬇CTGF and its mRNA expression	(Kanazawa et al., 2009a)
HSEs	cell stretcher (STB-140-10, STREX)	Cyclic stretch	10%/sec for 30 sec	10% strain	5 d	- histology: ⬆ thickness of epidermal layer, basal cells, volucurin, hemidesmosomes, length of lamina densa of each focal area; - ECM: ⬆ laminin 5, collagen IV/VII in the basal layer	(Tokuyama et al., 2015)
human keloid fibroblast	Equibiaxial strain device	Cyclic stretch	15 cycles/min	10% strain	24hr	- inflammatory cells & cytokines: ⬆ TGF-β1/2,0:TGF-β3; - ECM: ⬆ Collagen Ia and peaks at 6h and is at baseline by 24 h; - intracellular process: ⬆ integrin β1, vinculin, p- ERK, p-FAK, FAK 30 min after stretch	(Z.Wang et al. 2006b)

Table 2-7 In-vitro study of mechanical stretch

Note. HDF: human dermal fibroblasts; HSEs: human skin equivalents; TRPC3 fibroblast: TRPC3 overexpressing fibroblast ⬆: increase; ⬇: decrease; ×: no significant difference found.

2.6 The Main study

2.6.1 Justification of the study

In this chapter, the author firstly described the epidemiology and pathophysiology of HTS formation. The high prevalence of burn victims from low- and middle-income countries addressed the importance of cost-effective scar managements. As a complex and consecutive process followed wound healing, HTS required a sustained management and dynamic observation.

Then, mechanotransduction and fibrosis was introduced. Findings from stretch-induced and pressure-controlled scarring process were reviewed. Mechanosensitive molecules of integrin-β1, FAK, and ERK were selected as target proteins in the main study. The unknown mechanotransduction in the formed HTS was pointed out.

The application and possible mechanisms of pressure therapy were then introduced. Comparing to other non-invasive methods, pressure therapy was outweighed in the continuous management of massive scars, and capability of application via diverse materials. Meanwhile, the controversy on the pressure therapy regarding inconsistent outcomes and unclear mechanisms was also pointed out. This part emphasized the necessity of bridging the understanding between clinical effects and molecular reactions of HTS upon pressure therapy. A theoretical framework explaining the effect of pressure therapy based on the concept of mechanotransduction was proposed.

Finally, critical factors that could influence the effectiveness of force-based scar managements were reviewed from both clinical and preclinical studies. Two factors, treatment initiation time and intensity of force, were extracted from previous literature and controlled in the following experiments.

2.6.2 Study objectives

- Phase I of the study: An observational study was conducted to explore the relationship between post injury days and immunoreactivity of integrin-β1, FAK and ERK on human HTS, establishing a reference of mechanosensitive molecules on growing or matured HTS.

- Phase II of the study: A pretest-posttest study was designed in phase II to explore the effect of pressure therapy on mechanotransduction. The influence of post injury days and scar thickness on the change of immunoreactivity was also explored.

- Phase III of the study: A case report of modified pressure therapy generating compression force on convex scars from three dimensions (3D) was described, aiming to explore the effect of modified 3D pressure therapy on scar thickness.

Chapter 3 Phase I study: The relationship between the immunoreactivity of mechanosensitive signaling molecules on post-burn hypertrophic scars and the post injury days.

3.1 Background of the study

Hypertrophic scar (HTS) is a common pathological consequence of abnormal wound healing (Zhu, Ding, & Tredget, 2016). HTS related functional limitation and disfigurement can lead to increased disability-adjusted life-years (DALYs) (WHO, 2017). In Chapter 2.1 and 2.2, we introduced the need of effective and economical scar managements to prevent and control HTS progression. Recent evidence (Chapter 2.3) shows that either anatomical skin tension or external force can affect HTS formation (Bouffard et al., 2008; Ogawa et al., 2012; Wong et al., 2012). Mechanosensitive molecules such as integrinβ1, FAK^{Tyr397} and Erk (1/2) were found to be critical in mechano-regulated HTS formation. Researchers found that the application of stretch on the newly healed wound on mice or porcine can trigger the formation of HTS through FAK—ERK—MCP-1 pathway. The inhibition of integrin β1 or FAK^{Tyr397} by genetic knockout or pharmacologic blockade, on the other hand, interfere in the mechanotransduction and prevent the formation of HTS under the same wound-stretching model (Huang, Akaishi & Ogawa, 2012; Wong et al., 2012). These studies indicate that the expression of FAK^{Tyr397}, integrin-β1 and Erk (1/2) may reflect the vulnerability of wounds to mechanical stretch in developing HTS.

Comparing to abundant evidence related to inflammation and proliferation phases of wound healing, scar maturation, particularly related to the dynamic alterations of mechanosensitive signaling pathways over time, is relatively the least understood process. On one hand, previous studies which examined the interaction between mechanical stimuli and mechanotransduction in wounds or scars were mostly conducted in cell culture or animal models (Chin et al., 2010; Germscheid, Thornton, Hart, & Hildebrand, 2012). These in-vitro and animal models displayed diverse immunological and fibrotic response, and could hardly mimic the prolonged fibrosis in human HTS (Wong et al., 2011). On the other hand, while non-invasive scar managements such as pressure therapy are mostly applied on the formed HTS, little is known about the effect of mechanical stimuli on formed scar without any information of the responsiveness of the mechanosensitive signaling molecules during scar maturation. Therefore, there is a need to investigate the dynamic variation of expression of mechanosensitive molecules along with scar maturation phase using intact HTS or biopsies from human to enlighten further research of mechanism of pressure therapy as well as the optimal timing of treatment.

3.2 Objectives of the study

Observational study was conducted to explore the relationship between post injury days and immunoreactivity of mechanosensitive molecules (integrin-β1, FAK and ERK) on human post-burn HTS.

3.3 Methodology

3.3.1 Study design

An observational study was conducted to examine the relationship between immunoreactivity of mechanosensitive molecules (Integrin-β1, FAK and ERK) and post injury days. Asian male and female subjects aged 18 to 60 years who had developed HTS were recruited from Chengdu Second People's Hospital (Sichuan, China), Southwest Hospital of Third Military Medical University (Chongqing, China) between May 2013 and April 2016 by convenience sampling. Written consent was obtained from subjects. Ethical approval was obtained from the hospitals and the Hong Kong Polytechnic University. Demographic information of subjects, including age, gender, post-injury days and total burn surface area (TBSA) of subjects were obtained.

3.3.2 Inclusion and exclusion criteria

Subjects were recruited according to the following criteria: (1) had second or third degree burns, (2) wounds healed up no less than 21 days after injury or split-thickness skin grafting, (3) scars were scored ≥ 4 in Vancouver Scar Scale, with each item scored ≥ 1, (4) scars were over four extremities, (5) scars were of 4×4 cm2 or above. Subjects were excluded if: (1) the scars were over head, hands, trunk, or face, (2) the scars were having open wounds or infection, (3) the depth of burn was not confined due to the type of injury, such as chemical burns, (4) the patients received other scar treatments before our study, such as corticosteroid injection and laser therapy, (5) the patients were accompanied with

medical conditions that affect wound healing like diabetes mellitus, (6) the patients were accompanied with other severe complications such as multiple organ dysfunction syndrome.

3.3.3 Clinical assessment

Vancouver Scar Scale (Baryza, M. J., & Baryza, G. A., 1995) was used to measure clinical scar appearance in height (scoring 0, 1, 2, 3), vascularity (scoring 0, 1, 2, 3), pigmentation (scoring 0, 1, 2) and pliability (scoring 0, 1, 2, 3, 4, 5). It is a validated scar assessment tool widely used in clinical settings. A higher score indicated a more progressive scar.

3.3.4 Tissue preparation

One or two HTS (different sites) that met the predefined criteria were selected from each subject. A 3mm HTS biopsy (comprising both the epidermis and dermis) was collected by experienced surgeons under standard protocol. Normal skin specimens adjacent to excised scars were also obtained as reference, aiming to demonstrate the potential differential expression of mechanosensitive molecules between normal skin and HTS. Afterwards, specimens were preserved with 4% paraformaldehyde before undergoing routine paraffin embedding procedures as previously mentioned (Li-Tsang et al., 2015).

3.3.5 Immunohistochemical (IHC) staining

Tissues sections of 5 μm thick were deparaffinized, hydrated and immunostained at one time to avoid bias (Yaziji & Barry, 2006). Immunohistochemical staining for

Phosphorylated FAK^{Tyr397}, integrin-β1, and phosphorylated ERK (1/2)$^{Thr202/Tyr204}$ were performed using ImmPRESS™ Excel Amplified HRP Polymer Staining Kit (Anti-Rabbit IgG, MP-7601, Vector Laboratories, USA) according to manufacturer's instruction. Except for phosphorylated FAK, detection of integrin-β1 and phosphorylated ERK (1/2) required the retrieval of antigens, which have been masked during the fixation and paraffin-processing procedures, by boiling the hydrated sections in the respective antigen retrieval solutions in microwave. The duration of antigen retrieval was determined through pilot study (see Table 3-1). Afterwards, the sections were allowed to cool to room temperature and sections were first incubated with 0.3% hydrogen peroxide (in phosphate buffered saline, PBS, pH 7.4) for 15 minutes to inhibit endogenous peroxidase activity, or otherwise false positive signals will be present. Sections were then washed with PBS and subsequently blocked with 2.5% normal horse serum to minimize non-specific binding before incubating with appropriately diluted primary antibodies (see Table 3-1 for working dilutions of primary antibodies) at 4℃ overnight. On the next day, sections were washed with PBS for three times (5 min each) and incubated with amplifier antibody at room temperature for 15 minutes. PBS-washed sections were further incubated with ImmPRESS™ Excel reagent for 30 minutes at room temperature. Finally, color development was conducted by staining the sections with ImmPACT™ DAB EqV working solution. Sections could then be washed, counter-stained with hematoxylin for nuclear visualization and mounted with DPX The air-dried sections were ready for examination under light microscopy for subsequent image capturing and analysis. The

brown color product after DAB staining represented cells or tissues immunopositive for the respective protein molecules.

Table 3-1 Summary of primary antibodies

Antibody	Cat no.	Vendor	Host	Dilution	Antigen retrieval	Antigen retrieval time
Anti-FAK (phospho Y397)	ab39967	Abcam	Rabbit polyclonal	1:1500	-	-
Anti-Integrin beta 1	ab179471	Abcam	Rabbit monoclonal	1:5000	Tris/EDTA, pH 9.0	5 min
Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204)	4370s	Cell Signaling Technology	Rabbit monoclonal	1:800	Citrate acid, pH 6.0	3 min

3.3.6 Image processing

Traditionally, combinative semiquantitative methods were mostly used to quantify the staining intensity on IHC stained specimen (Table 3-2). However, variability in the scoring existed due to visual perception on a hematoxylin counter-stained tissue section among different analyzers (Kraan et al., 2000; Fedchenko et al., 2014; Klein et al., 1999). In addition, dermal fibroblasts have a branched cytoplasm and are morphologically diverse on the stained slides, it would be difficult to count cells and determine its percentage either manually or automatically (Figure 3-1). Therefore, quantitative image analysis employing mean optical density in the region of interest computerized by specialized image processing software was used in our study (Fedchenko et al., 2014).

Table 3-2 Combinative semiquantitative scoring of IHC staining

A=Qualitative scoring of percentage of IHC labeled cells	B=Qualitative scoring of IHC reaction	Final score
0 = 0% 1 = <30%	0 = no reaction 1 = weak	A+B = range from 0 to 6
2 = 30-60% 3 = >60%	2 = mild 3 = strong	A*B = range from 0 to 9

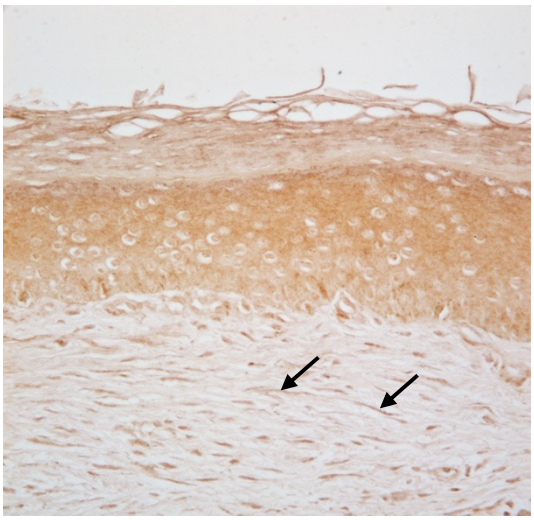


Figure 3-1 Demonstration of shape of dermal fibroblasts (black arrow) in FAK-positive-stained specimen.

Counterstained and positive-stained-only images were captured by microscope (40X magnification, Eclipse 80i, Nikon) using Spot Advance Software (Diagnostic Instruments, Inc. USA) with consistent settings. NIS-Element-Advanced Research program (Version 4.10, Nikon, Japan) was used to analyze images (Fedchenko et al., 2014; Kraan et al., 2000). Mean staining intensity in the outcome was defined as the mean intensity values of pixels in the stained area (NIS-Element AR manual). Since epidermis is predominated by keratinocytes and dermis by fibroblasts, two skin layers were analyzed separately. Two analyzers were assigned to image analysis independently. The procedure of analysis was: (1) outlined the area of epidermis or dermis, transformed the selection into the first binary

layer; (2) outlined blood vessels, hair follicles and inflammatory cells semi-automatically, transformed the selected area into the second binary layer; (3) created subtraction of the second layer from the first layer, obtained ROI area; (4) used “subtract white” to effectively discriminate color differences (Prasad & Prabhu, 2012); (5) computed mean staining intensity in ROI area from the pixel intensity histogram in RGB channel, and (4) averaged the mean staining intensity among four images from the same specimen.

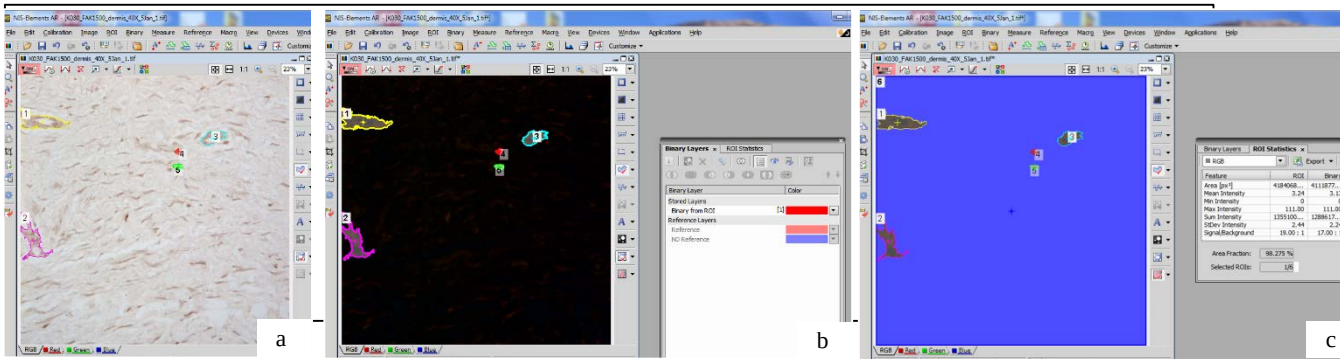


Figure 3-2 Illustration of image processing: a. semi-auto detected blood vessels; b. subtracted white and transform the selection into binary layers; c. created subtraction between two layers, obtain ROI area and intensity of staining per area.

3.3.7 Statistical analysis

Total and sub scores of VSS were computed and listed out. Descriptive analysis was used to generate the mean immunoreactivity of integrin-β1, FAK and ERK in normal skin and HTS. Pearson correlation was used to demonstrate the relationship between post injury days and the immunoreactivity of integrin-β1, FAK and ERK in epidermis and dermis respectively. Pearson correlation coefficient was calculated and P value was set at 0.05 (two-tails). To illustrate the alteration of proteins’ intensity along with the post-injury days,

non-parametric Locally Weighted Scatterplot Smoothing (LOESS) curve was used. LOESS curve was built on linear and nonlinear least squares regression, creating function and regression lines by fitting localized segments of data. There was no specific function or model that required for all data to fit in. It can demonstrate the segmented relationship between two parameters (Cleverland, 1997). IBM SPSS Statistic 23 software was used for statistically analysis.

3.4 Results

3.4.1 Demographic information

Demographic information of subjects was listed in Table 3-3. Thirty-one subjects with forty-three HTS were included in the analysis. In addition, six normal skin specimens were obtained from six out of thirty-one subjects during scar excision, with demographic information listed in Table 3-4.

Table 3-3 Demographic information of enrolled subjects.

Number of patients	31
Number of Scars	43
Age (year, Mean (SD))	40.16 (13.60)
Gender	
Male (No. (%))	22 (71.0%)
Female (No. (%))	9 (29.1%)
PI day (Mean (SD))	183.89 (75.48)
Injury area (% of TBSA, Mean (SD))	18.52 (16.72)
Vancouver Scar Scale (Mean (SD))	
Pigmentation	2.31 (0.74)
Vascularity	2.03 (0.82)
Pliability	1.94 (0.72)
Height	2.31 (0.78)
Total score	8.60 (2.39)

Table 3-4 Demographic information of subjects provided normal skin

Number of patients	6
Number of Scars	6
Age (year, Mean (SD))	39.21 (5.60)
Gender	
Male (No. (%))	6 (100.0%)
Female (No. (%))	0 (0.0%)

3.4.2 IHC presentation of normal skin and HTS

Integrin-β1 staining demonstrated a membranous staining pattern, and strong positive staining was observed predominantly in the basal layer of epidermis. FAK and ERK exhibited mainly cytoplasmic and nuclear immunostaining. For ERK, the positive staining in the basal layer of epidermis was visually weak compared to other parts in epidermis and in dermis. Comparing with our target cell types (keratinocytes, keratinocyte stem cells and fibroblasts), a preferentially stronger expression of mechanosensitive molecules was observed in endothelial cells of capillaries and inflammatory cells in dermis (Figure 3-3).

Since the sample size between HTS group and NS group was small and unbalanced, only descriptive analysis was used to demonstrate the staining intensity of two groups. Comparing to normal skin from the same subject, mean staining intensity of integrin-β1, FAK and ERK was higher both in in the dermis and epidermis of HTS (Table 3-5).

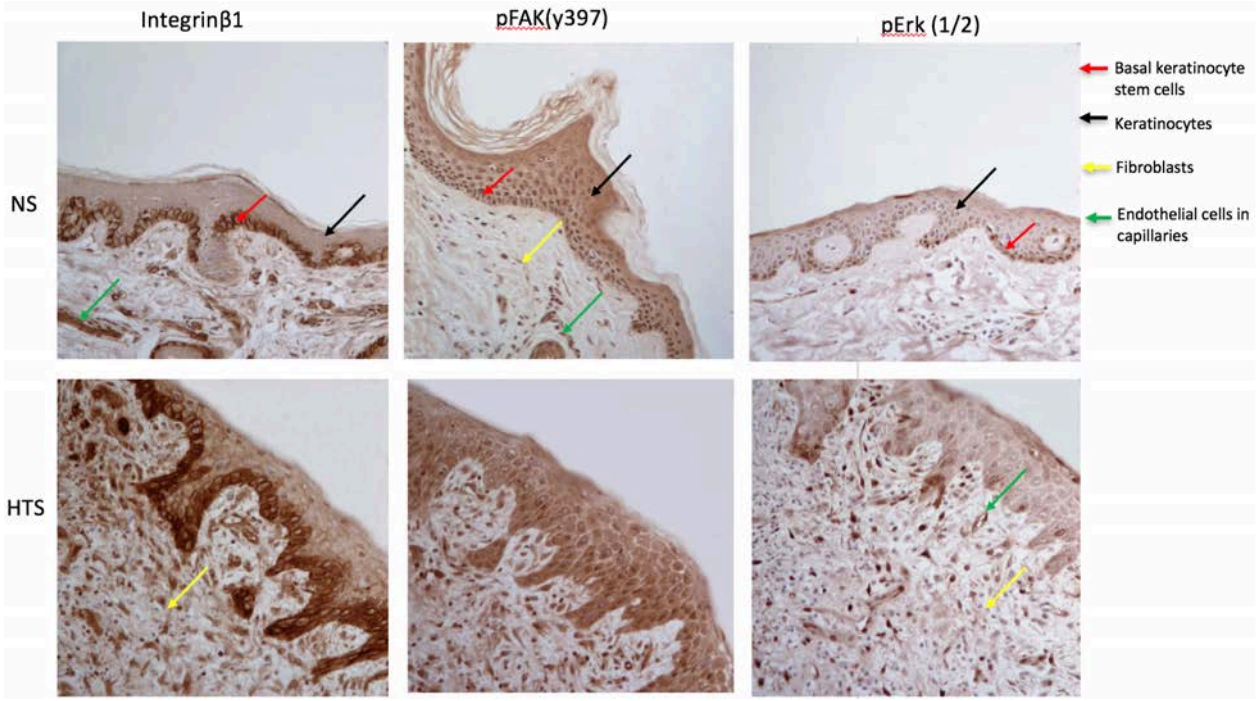


Figure 3-2 Immunohistochemistry photomicrographs (40X) of integrin-β1, FAK(y397) and ERK (1/2) staining in normal skin and HTS from the same subject.

Table 3-5 Mean immunoreactivity of immunoreactivity in HTS and normal skin

	Integrin-β1		Phospho-FAK(Y397)		Phospho-ERK(1/2)	
	HTS (n=43)	NS (n=6)	HTS (n=43)	NS (n=6)	HTS (n=43)	NS (n=6)
Epidermis	19.92(6.00)	5.9(2.0)	18.80(6.73)	6.3(1.8)	17.62(9.87)	6.1(1.1)
Dermis	14.28(5.33)	4.9(1.3)	6.07(2.40)	3.5(0.8)	7.56(4.39)	2.5(0.7)

Note. HTS: hypertrophic scars; NS: normal skin.

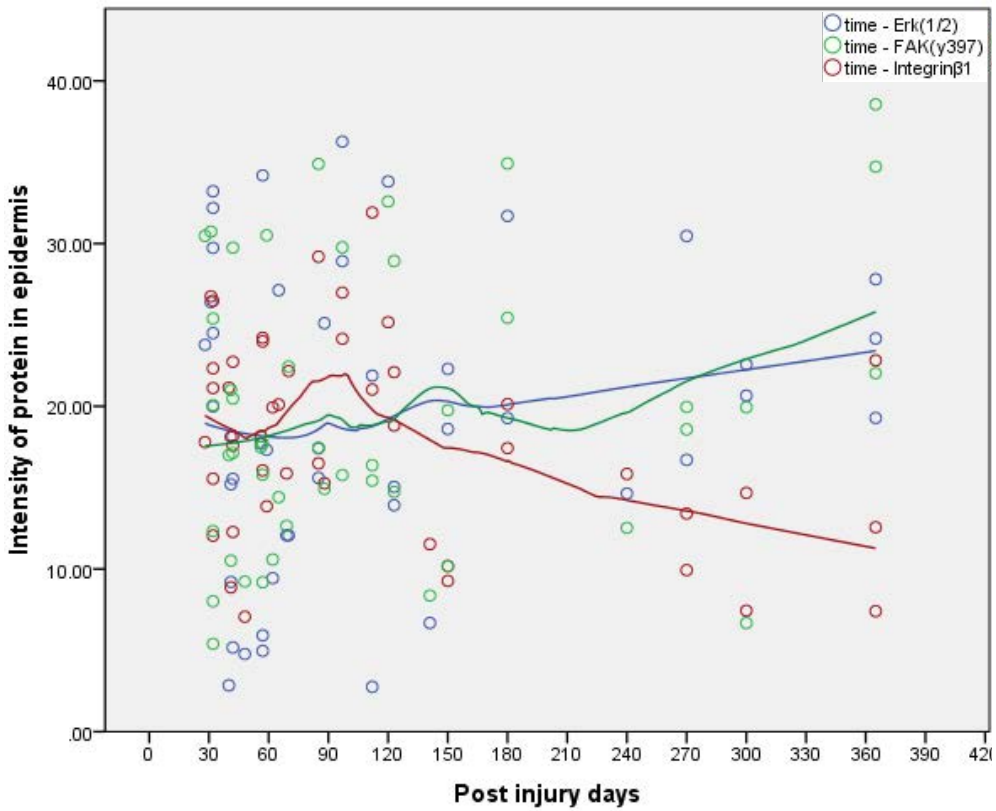
3.4.3 The relationship between immunoreactivity of mechanosensitive molecules and post injury days

Table 3-6 Pearson correlation coefficient between the immunoreactivity of mechanosensitive molecules with post injury days

Epidermal immunoreactivity			
	Integrin β1	Phospho-FAK(Y397)	Phospho-ERK(1/2)
Post injury days	0.33*	-0.03	-0.04
Dermal immunoreactivity			
	Integrin β1	Phospho-FAK(Y397)	Phospho-ERK(1/2)
Post injury days	0.07	-0.34*	-0.23

Note. *: $p<0.05$; **: $p<0.01$

Pearson correlation coefficient between the immunoreactivity of mechanosensitive molecules with post injury days was presented in Table 3-6. The relationship between post-injury days and immunoreactivity of integrin-β1, FAK and ERK was presented through LOESS fit line. The LOESS fit line reveals a declined intensity of integrin-β1, FAK and ERK within 30 to 60 days' (one to two-months), followed by an increase intensity to 90 days (three months) after injury (Figure 3-4). Afterwards, the intensity of integrin-β1 demonstrates a continuous decline along with the increasing post injury days to 360 days (one year) in both epidermis and dermis. Whereas FAK and ERK remain a relatively stable trend.



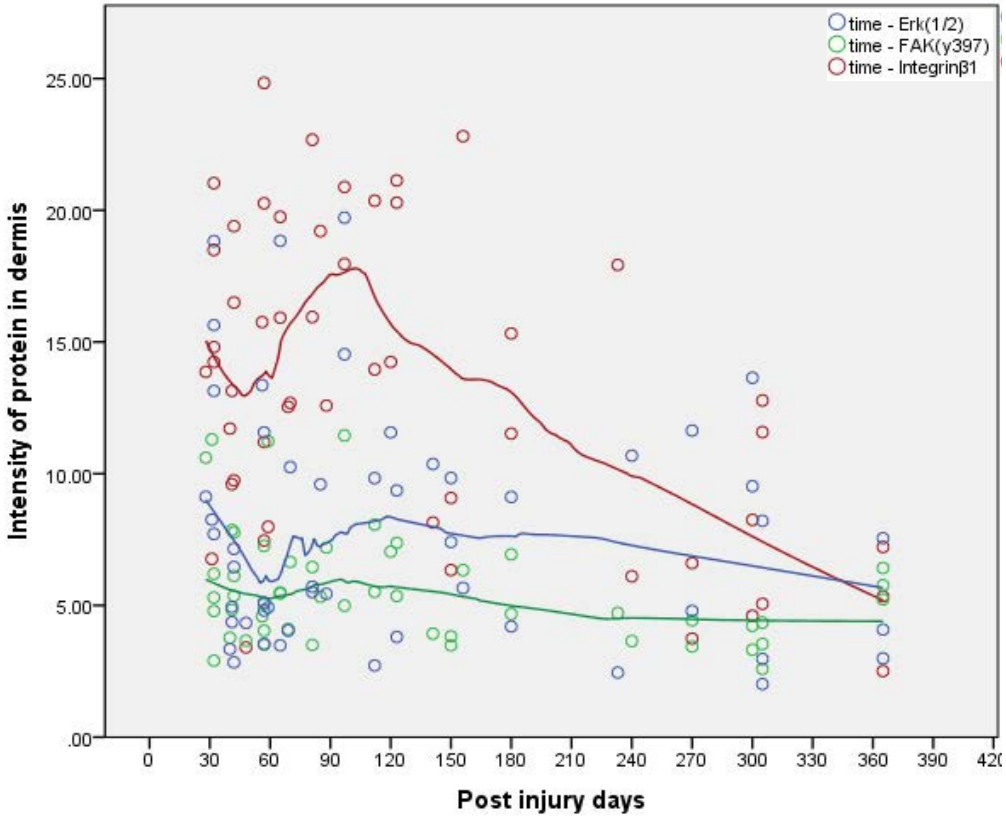


Figure 3-4 Graph of Loess Fit Line (60% of point fit) presented the variation of epidermal and dermal protein intensity along with the post injury days.

3.5 Discussion

Increased integrins and FAK triggered during early wound healing was found to result in hypertrophic-like scars in animal models, suggesting possible involvements of these mechanosensitive during hypertrophic scarring (Huang, Akaishi, Ogawa, 2012; Wong et al., 2012). Whereas these mechano-regulated proteins may also involve in scar maturation process has not been reported. Our study managed to obtain scar biopsies from burn patients with post injury days ranged from one month (30 days) to one year (360 days), covering the scar maturation period critical for clinical management. Based on this, two research questions were asked: would there be difference in the expression pattern of mechanosensitive proteins between normal and HTS, and what is the relationship between proteins expression and post injury days.

Firstly, to demonstrate the difference between HTS and normal skin regarding the activation of mechanosensitive molecules, a descriptive analysis was used to demonstrate the mean immunoreactivity of target molecules. HTS was distinguished from normal skin for its hyper-proliferative fibroblasts, excessive deposition of extracellular matrix (ECM) substrates, and persistence of myofibroblasts (Ehrlich et al., 1994; Song et al., 2011; Zhu, Ding, Shankowsky, & Tredget, 2013). In the study, we found a higher immunoreactivity of activated dermal and epidermal integrin-β1, FAK and ERK in HTS comparing to normal skin, which was consistent with previous research that the activation of FAK and ERK indicated an increased cell migration and differentiation, as well as increased depositions

in the extracellular matrix (Zebda, Dubrovskyi, & Birukov, 2012; Chiquet et al., 2009; Koivisto et al, 2014).

Secondly, to explore the relationship between immunoreactivity of mechanosensitive molecules and post injury days, LOESS fit line was built based on linear and nonlinear least squares regression of localized subsets of data. It presented a decreasing immunoreactivity of dermal and epidermal integrin-β1, FAK and ERK from one-month to two-month (30 to 60 days') time. The results indicated a decreasing cell migration and differentiation during the process, which was coincident with findings from Koppenol et al (2017) that the density of myofibroblasts (cells/cm^3) and collagen molecules (g/cm^3) was predicted to decrease from one-month to two-month time through a biomechanical mathematical wound healing model (Figure 3-6). In our study, An increasing immunoreactivity of mechanosensitive molecules was observed in HTS from two to three-month time (around 60 to 90 days) and reached peak around three months (90 days) post injury, which was opposite to the trend expected by Koppenol et al in normal wound healing model that a constantly decreased collagen, myofibroblasts was observed after two months post injury (Koppenol et al., 2017) (Figure 3-5). The similarity between LOESS fit line and Koppenol's mathematical prediction indicated that, although the relationship between immunoreactivity of mechanosensitive molecules and post injury days in our study was built on non-consecutive samples from different subjects, the variation observed in LOESS fit line can be used as reference for the further exploration of mechanosensitive molecules' activation in maturation phase.

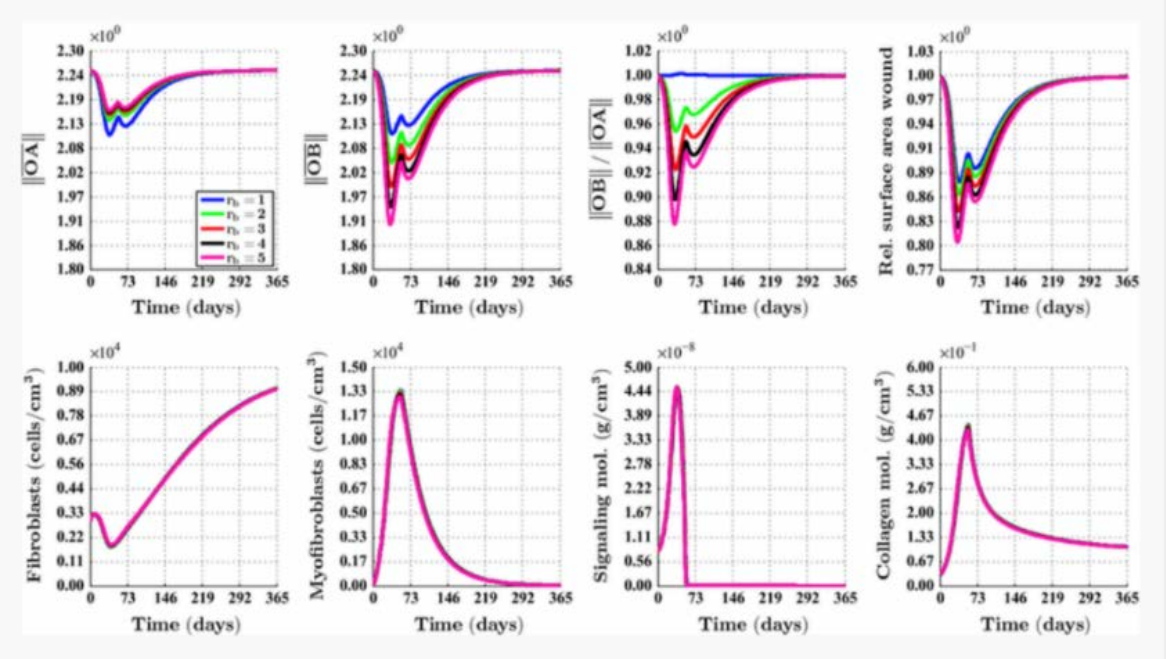


Figure 3-5 Predicted distribution of fibroblasts, myofibroblasts and signalling molecules in wound healing from mathematical modeling. Picture from (Koppenol, Vermolen, Niessen, van Zuijlen, & Vuik, 2017)

Previous research showed that integrin-β1 and FAK are critical in force-modulated scarring process. The elevation of integrin-β1 and FAK under mechanical stretch was reported to result in a higher susceptibility of scars of developing HTS, and the inhibition of integrin β1 and FAKthrough either genetic knockout, pharmacologic blockade or stretch-shielding could suppress mechanotransduction and prevent HTS formation in wound-stretch animal models ((Aarabi et al., 2007; Paterno et al., 2011; Wong et al., 2011; Cheng et al., 2015). Besides, the similar role of integrin-β1 and FAK in controlling fibrosis was also observed in in-vitro cell studies (Zhang et al, 2016; Kuang et al, 2015; Chiquet et al, 2009). These studies indicated that the immunoreactivity of integrin-β1, FAK and ERK may reflect the vulnerability of HTS to mechanical force in aggressive development. From the LOESS fit line in our study, the elevation of dermal integrin-β1 and FAK between two-

month to four-month time may indicate a rapid proliferation of HTS as well as a high sensitivity to mechanical force in this period of time. On one hand, it might be important to control the external skin tension arose from either wound healing or adjacent joints' movement between two-month to four-month time after injury. On the other hand, two-month to four-month time might also be the critical time window for force-based non-invasive scar managements, such as pressure therapy, to obtain its optimal effect in controlling scar development.

The study pioneered to explore the dynamic alteration of mechanosensitive protein expression from growing or matured human HTS along with post injury days. Comparing to scar models constructed by in-vitro cells and animals, results obtained from human HTS samples can better represent the pathophysiology in human beings, and be interpreted from clinical aspects (Yang et al, 2005; Chin et al., 2010; Wong et al., 2011; Germscheid et al., 2012). However, our study had limitations. Since the immunoreactivity of mechanosensitive molecules was generated from non-consecutive samples obtained only once from each HTS, its dynamic alterations along with post injury days should be generalized with caution. A longitudinal observation is needed in the future to monitor the change of mechanotransduction within subjects.

In conclusion, our study unveiled the relationship between immunoreactivity of mechanosensitive molecules and post injury days during scar maturation phase, that two to four month after injury might be critical for application of force-based scar managements. Further work is necessary for a longitudinal observation from consecutive samples. The

study also established a basis for the further research regarding the mechanism of force-based non-invasive scar managements.

Chapter 4 Phase II study: The changes in immunoreactivity of mechanosensitive molecules Integrin-β1 and FAK in human hypertrophic scars upon pressure therapy

4.1 Background of the study

Hypertrophic scar (HTS) is an abnormal yet common consequence of severe burns, arising from excessive growth of dermal fibroblasts, profibrotic cytokines and deposition of substrates in the extracellular matrix (ECM) during wound healing (Zhu, Ding, & Tredget, 2016). It is a leading cause of morbidity for undesirable disfigurement and functional loss (e.g. contracture) (Chapter 2.2**). Non-invasive treatments using external forces are favored in HTS management for their relatively low cost and easy access** (Chapter 2.4). Although more and more non-invasive treatments are blooming, like silicone gel and vacuum massage, **seldom of them is superior to pressure therapy on managing massive scars** (Li-tsang & Zheng, 2010; Saxena et al., 2004; Steinstraesser et al., 2011; Van den Kerckhove et al., 2005).

Pressure therapy has been recommended as the frontline treatment for HTS. Previous clinical trials have demonstrated its effect in improving scar pliability and pigmentation in a magnitude-dependent manner (Anthonissen, Daly, Janssens, & Van den Kerckhove, 2016; Friedstat & Hultman, 2014; Vloemans, Hermans, Van Der Wal, Liebregts, & Middelkoop, 2014). Meanwhile, proliferation of basal keratinocytes and myofibroblasts is

found to be inhibited after consistent pressure, with attenuated TGF-β1 level (Feng, 2012; Kim et al., 2015; Li-Tsang et al., 2015). **However, controversy remains among specialists due to inconsistent results across studies and pressure therapy's unknown mechanism** (Ai et al., 2017; Anzarut, Olson, Singh, Rowe, & Tredget, 2009). Treatment effect quantified through scar thickness, one of the mostly widely assessed objective scar parameters in clinical settings, was found varied across different trials, confusing clinical practitioners and researchers regarding pressure therapy's effect (Ai et al., 2017; Anzarut, Olson, Singh, Rowe, & Tredget, 2009). **Research is needed to further clarify the mechanism of pressure therapy, and bridge the gap between the understanding of molecular modulation and clinical observation.**

Evidence shows that force from either anatomical skin tension or external applications can affect HTS formation (Bouffard et al., 2008; Ogawa et al., 2012; Wong, Levi, Akaishi, Schultz, & Dauskardt, 2012). Mechanotransduction, a process referring to the mechanisms of converting mechanical stimulus into chemical signals is involved. Animal studies have found that, FAK-ERK- monocyte chemoattractant protein-1 (MCP-1) pathway is predominant in force-induced transforming growth factor (TGF-β1) secretion and scar formation (Chiquet et al., 2009; Wong et al., 2012). Fibroblast-specific FAK knockout or pharmacologic FAK or integrin blockade can interfere mechanotransduction and prevent augmentation of inflammation under mechanical stretching, resulting in markedly less scarring (Leask, 2013; Wong et al., 2012; Zhang et al., 2015). **These studies indicate that FAK, integrin-β1 and ERK are candidate proteins to investigate the effect of**

mechanical force in scarring from mechanotransduction perspective (Chapter 2.3). However, the evidence available for the involvement of mechanotransduction in scarring were mostly conducted in cell culture or animal models, which displayed diverse immunological and fibrotic response as human beings, and could hardly be interrelated with clinical application targeting HTS (Wong et al., 2011; Yang, Im, & Wang, 2005)(Chin et al., 2010; Germscheid, Thornton, Hart, & Hildebrand, 2012). **Therefore, a valid model using intact HTS from human subjects is needed for clinical translation when studying the effect of force on HTS from mechanotransduction perspective.**

There are opinions that the effect of pressure therapy on HTS may act through mechanotransduction (Carver & Goldsmith, 2013; Haga, Li, & Chien, 2007). Researchers believe that compression can change the properties of ECM, regulate the activity level of mechanoreceptors, and control scar development (Silver, Siperko, & Seehra, 2003; Yagmur, Akaishi, Ogawa, & Guneren, 2010). Previous research showed that, the inhibition of integrinβ1 and FAK can suppress inflammatory cytokines (TGF-β1, IL-4, and IL-13), activated molecules for protein degradation (MMP-2, 9), and augment apoptosis process, thus to prevent scar formation (Chiquet et al., 2009; Wong et al., 2007). The above-mentioned cytokines and processes activated by mechanical stretch in scarring was found to be controlled after pressure therapy (Feng, 2012; Kim et al., 2015; Li-Tsang et al., 2015). However, whether mechanosensitive molecules (integrin-β1, FAK and ERK) were involved in the upstream modulation remains unknown (Figure 4-1). **To preliminarily explore the effect of pressure therapy on mechanotransduction, an experiment**

investigating the effect of pressure therapy on immunoreactivity of mechanosensitive-molecules is needed.

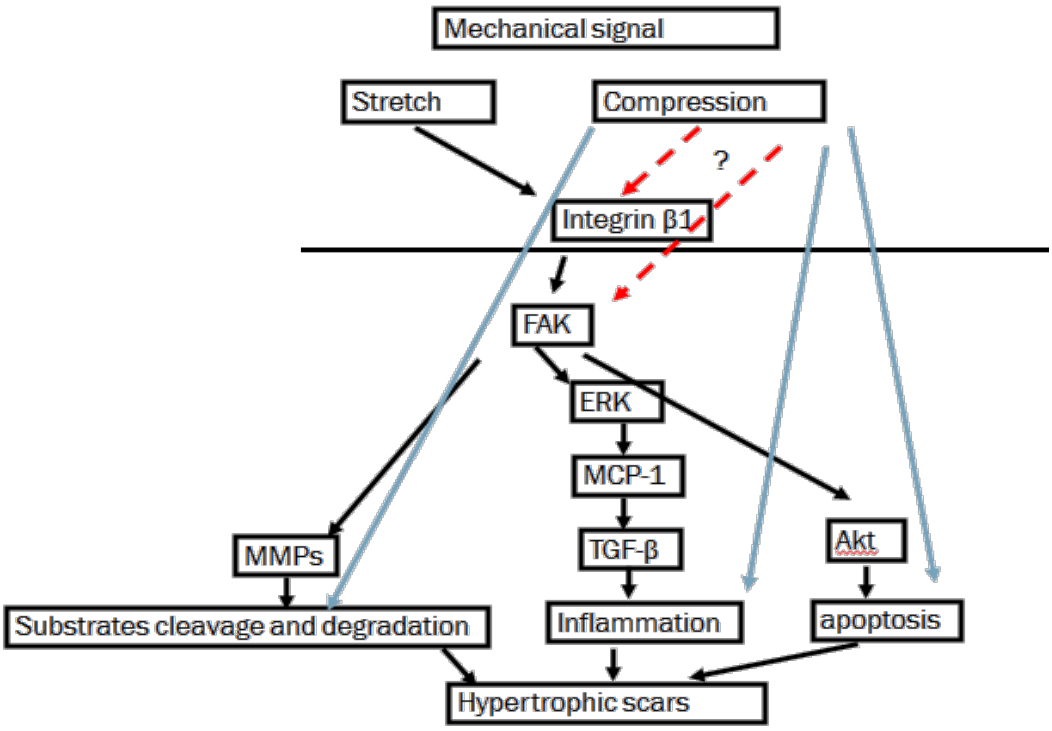


Figure 4-1 The logistic of studying the effect of pressure therapy on mechanosensitive molecules and related pathways in mechanotransduction and scarring

As for confounding factors that may influence the effect of pressure therapy, magnitude and time of force application play an important role in force-regulated scarring process (Chapter 2.5). In-vitro studies demonstrated that, magnitude and duration of cyclic stretch can affect behavior of dermal fibroblasts such as proliferation, differentiation, apoptosis and secretion of inflammatory cytokines and ECM substrates (Tokuyama et al., 2015; Kuang, et al., 2015; H.Ishise et al., 2015; Peters et al., 2015; Rolin et al., 2014; Miyazaki, et al., 2013; Paterno et al., 2011; Kanazawa et al., 2009). The force application time after wound healed, and the duration of force applied on scar were also found to alter the

outcomes of scarring in animal studies (Cheng et al., 2015; Paterno et al., 2011; Wong et al., 2011; Gurtner et al., 2011; Aarabi et al., 2007). Meanwhile, in Chapter 3 (Phase I study), we found that the immunoreactivity of mechanosensitive molecules varied along with post injury days during scar maturation process. **These studies indicated that, the time of treatment implementation may influence the effectiveness of pressure therapy, and there is a need to control the pressure dosage and treatment duration in the study design**.

Therefore, for a preliminary understanding of the mechanism of pressure therapy from mechanotransduction perspective, a study was conducted to explore the change of immunoreactivity of mechanosensitive molecules (FAK, integrin-β1 and ERK) on HTS before and after pressure therapy, as well as the change of clinical manifestation of HTS. Intact human HTS specimens (epidermis and dermis) were obtained for a valid clinical interpretation. The influence of pressure application time (post injury days) on the effect of pressure therapy was explored, providing support for the optimal timing in clinical practice. At the same time, the relationship between objective HTS assessment (scar thickness) and the immunoreactivity of mechanosensitive molecules was examined, aiming to predict the activity level of mechanotransduction through non-invasive clinical assessments. To minimize the confounding factors that may affect the interpretation, dosage and duration of pressure application were standardized in the study.

4.2 Aims of the study

The study aims (1) to examine the clinical manifestation of post-burn HTS before and after pressure therapy; (2) to examine the effect of pressure therapy through comparing activated FAK, integrin-β1 and ERK in HTS before and after standardized pressure therapy; (3) to explore the influence of post-injury days on the changes of FAK, integrin-β1 and ERK; (4) to explore the relationship between the change of clinical scar thickness and the change of immunoreactivity of mechanosensitive molecules.

4.3 Methodology

4.3.1 Study design

A pretest-posttest study was conducted to examine the clinical and histological manifestation of HTS before and after 3-month pressure garment therapy. Asian subjects aged between 18 to 60 years old were recruited from Chengdu Second People's Hospital (Sichuan, China) and Southwest Hospital of Third Military Medical University (Chongqing, China) between May 2013 and April 2016 by convenience sampling (Figure 4-2). Written consent was obtained from subjects, and ethical approval was obtained from hospitals and Hong Kong Polytechnic University. Demographic information, such as age,

gender, post-injury days of subjects was described. For burn severity, type of injuries, total burn surface area (TBSA) and scar sites were obtained.

4.3.2 Inclusion and exclusion criteria

Female and male subjects were recruited according to the following criteria: (1) had second or third degree burns, (2) wounds healed up no less than 21 days after injury or split-thickness skin grafting, (3) scars were scored ≥ 4 in Vancouver Scar Scale, with each item scored ≥ 1, (4) scars were over four extremities, (5) scars were of 4×4 cm2 or above. Subjects were excluded if: (1) the scars were over head, hands, trunk, or face, (2) the scars were having open wounds or infection, (3) the depth of burn was not confined due to the type of injury, such as chemical burns, (4) the patients received other scar treatments before our study, such as corticosteroid injection and laser therapy, (5) the patients were accompanied with medical conditions that affect wound healing like diabetes mellitus, (6) the patients were accompanied with other severe complications such as multiple organ dysfunction syndrome. The flow chart of inclusion and treatment process were presented in Figure 4-2

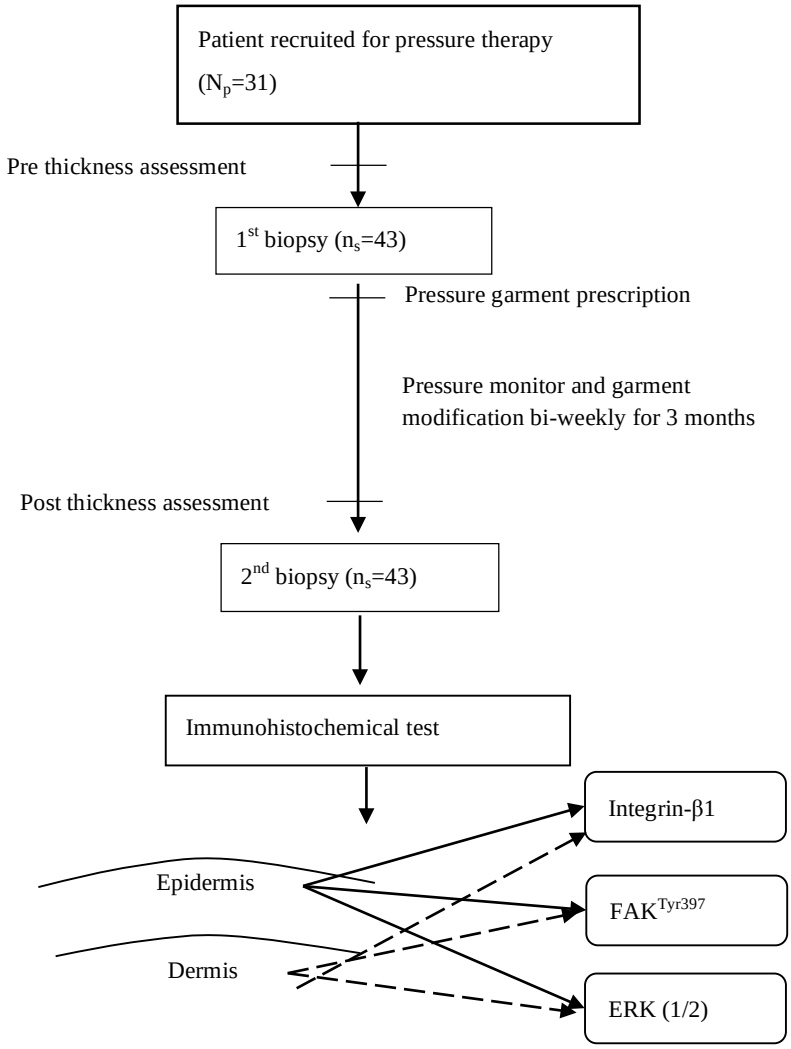


Figure 4-2 Flow chart of intervention and assessment procedure.

4.3.3 Intervention

A research assistant (WXY) was employed and trained for the pressure garment (PG) measurement, prescription, and pressure dosage monitor for a standardized treatment and good compliance from the subjects. Necessary modification was made during 3-month follow-up to ensure a consistent interface pressure generated by PG. Regular clinical assessments were conducted by an experienced therapist (LP) under standardized protocol during whole process to ensure reliable assessment results.

4.3.3.1 Standardized pressure garment production

Experienced occupational therapists conducted PG measurement, pressure dosage monitor and education on PG wearing regime under standardized protocol. The tailor-made PG pattern was generated by YUKA system (Figure 4-3), a computerized software with standardized measuring and data-input protocol (Feng, Pao, Wu, Li, & Li-Tsang, 2013). PG then fabricated by a skilled tailor, and prescribed to subjects after the initial assessment. Therapists instructed subjects and their carers on the 23-hour daily wearing regime, PG caring methods, and precautions during activities.

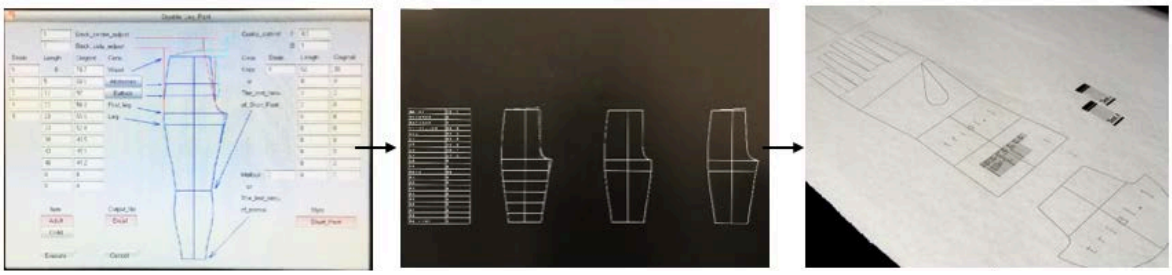


Figure 4-3 Data input and pattern generating in YUKA system

4.3.3.2 Pressure dosage monitor

A pressure dosage of 15mmHg is widely accepted as a minimal ‘‘dose’’ necessary for an effect, and an interface pressures over than 40 mmHg could lead to discomfort and complications (Engrav et al., 2010; Van den Kerckhov et al., 2005; Giele et al., 1998; Naismith et al., 1980). Our team found a statistically difference between the improvement in high pressure group (20– 25 mmHg) and low pressure group (10–15 mmHg) (Lai et al., 2010). Therefore, an optimal interface pressure of 15-25 mm Hg was defined in the study. Pressure dosage generated by PG was monitored by a validated pressure sensing tool, Pliance X System (Germany-Novel Electronics, Munich, Germany) (Figure 4-4) (Lai &Li-Tsang, 2009). Consistent pressure was ensured through bi-weekly follow-ups and corresponding garment modifications if necessary.

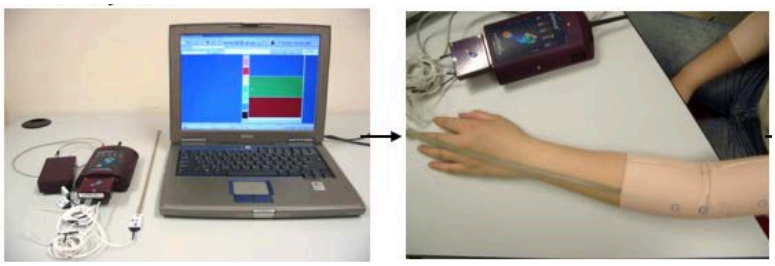


Figure 4-4 Interface pressure dosage monitor using Pliance X System

4.3.4 Clinical assessment

4.3.4.1 Vancouver Scar Scale

Clinical scar manifestation such as height, vascularity, pigmentation and pliability was measured by Vancouver Scar Scale (Baryza & Baryza, 1995), a validated scar assessment

tool widely used to assess scar appearance. A higher score indicates a more progressive scar.

Table 4-1 Scoring of Vancouver Scar Scale

Pigmentation (M)	Pliability (P)	Height (H)	Vascularity (V)
0: Normal	0: Normal	0: Flat	0: Normal
1: Hypopigmented	1: Supple (flexible with minimal resistance)	1: <2mm	1: Pink
2: Mixed	2: Yielding (giving way to pressure)	2: 2-5mm	2: Red
3: Hyperpigmented	3: Firm (inflexible; resistant to manual pressure) 4: Banding (rope like tissue that blanches) 5: Contracture (permanent shortening of scar producing deformity or distortion)	3: >5mm	3: Purple

4.3.4.2 Scar thickness

A portable ultrasound scanner (Mindray M5, Mindray Medical International Limited, Shenzhen, China) was used to measure the scar thickness monthly. Each measurement was conducted 10 minutes after removing the PG. The convex transducer was placed perpendicularly to the target HTS point with an appropriate amount of ultrasonic gel to separate the face of the probe and the epidermis. Scar thickness was measured by built-in electronic caliper in the two-dimensional B-mode image, defining as the mean length (in mm) between surface–epidermis and dermis-subcutaneous tissue interfaces.

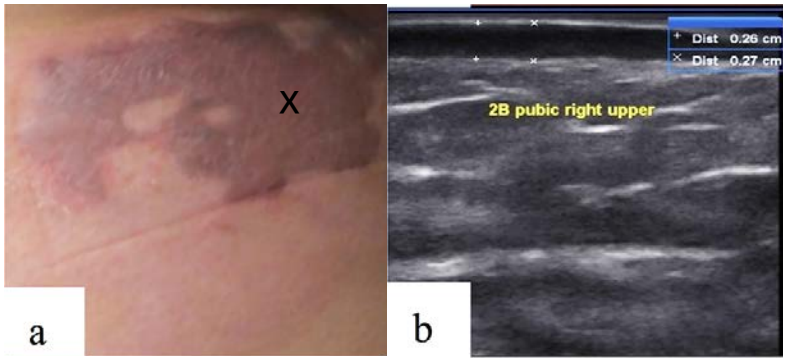


Figure 4-5 Scar thickness measurement using built-in caliper in ultrasound scanning system. a: HTS with target measuring point (marked with a cross); b: length between surface–epidermis and dermis-subcutaneous tissue interfaces was measured and averaged.

4.3.5 Tissue preparation, immunohistochemical staining, image processing

From each subject, either one or two scars met the predefined criteria were selected. In case for two scar biopsies taken from the same subject, they should be obtained from different body parts. Location of scars and the selected points for biopsy were recorded via photography. 3 mm HTS biopsy was used to collect scar tissue (comprising both the epidermis and dermis) before and after 3-month pressure therapy. Considering the new wound caused by pre-biopsy-collecting procedure may affect the immunohistochemical outcome of HTS tissue on the same point, HTS specimen adjacent to the pre-collected points from the same scar was obtained after pressure therapy. Afterwards, specimens were preserved with 4% paraformaldehyde before paraffin embedding procedures as previously mentioned (Li-Tsang et al., 2015).

The procedures of immunohistochemical staining and image processing were described previously (Chapter 3.3.5, 3.3.6). In general, immunohistochemical staining for Phosphorylated FAK^{Tyr397}, integrin-β1, and phosphorylated Erk (1/2)$^{Thr202/Tyr204}$ were

performed using ImmPRESS™ Excel Amplified HRP Polymer Staining Kit (Anti-Rabbit IgG, MP-7601, Vector Laboratories, USA). Detailed solution and duration of antigen retrieval, and working dilutions of primary body (incubated at 4℃ overnight) were listed in Table 4-1. Color development was conducted by staining the sections with ImmPACT™ DAB EqV working solution. Counter-stained with hematoxylin for nuclear visualization and mounted with DPX. The air-dried sections were ready for visualized under light microscopy for subsequent image capturing and analysis. The brown color product after DAB staining represented cells or tissues immunopositive for the respective protein molecules.

Table 4-2 Summary of working dilution of primary antibodies, solution and duration of antigen retrieval.

Antibody	Cat no.	Vendor	Host	Dilution	Antigen retrieval	Antigen retrieval time
Anti-FAK (phospho Y397)	ab39967	Abcam	Rabbit polyclonal	1:1500	-	-
Anti-Integrin beta 1	ab179471	Abcam	Rabbit monoclonal	1:5000	Tris/EDTA, pH 9.0	5 min
Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204)	4370s	Cell Signaling Technology	Rabbit monoclonal	1:800	Citrate acid, pH 6.0	3 min

For image analysis and quantification, counterstained and positive-stained-only images were captured by microscope (40X magnification, Eclipse 80i, Nikon) using Spot Advance Software (Diagnostic Instruments, Inc. USA) with consistent settings. NIS-Element-Advanced Research program (Version 4.10, Nikon, Japan) was used to analyze images

(Fedchenko et al., 2014; Kraan et al., 2000). Mean staining intensity in the outcome was defined as the mean intensity values of pixels in the stained area (NIS-Element AR manual). Epidermis and dermis were analyzed separately. Two analyzers were assigned to image analysis independently. Blood vessels, hair follicles and inflammatory cells were outlined semi-automatically and subtracted from the ROI area. Mean staining intensity in ROI area was obtained from the pixel intensity histogram in RGB channel, and averaged among four images from the same specimen.

4.3.6 Statistical analysis

Wilcoxon signed rank test was used to compare categorical scoring data from VSS before and after pressure therapy. For continuous data of scar thickness, paired sample t test was used examine the difference before and after pressure therapy.

Pearson correlation was used to examine if there was potential correlation between the expression pattern of integrin-β1, FAK and ERK. A moderate correlation would suggest the use of multivariate test ($0.3 < r < 0.7$) (Maxwell, 2001). Afterwards, One-Way Repeated-Measures Multivariate Analysis of Variance (MANOVA) was used to compare the intensity of integrin-β1, FAK and ERK immunoreactivities before and after pressure therapy.

Multiple Regression Analysis was used to examine the influence of post injury days and scar thickness on the change of immunoreactivity of mechanosensitive molecules. The

contribution of post-injury days and scar thickness to the change of intensity in integrin-β1, FAK and ERK was examined.

Prior to conducting the analysis, assumptions of normal distribution of intensity differences was examined. The assumption was considered satisfied, as if (1) the null hypothesis was accepted in Shapiro-Wilk test and Kolmogorov-Smirnov test ($p > 0.05$); (2) the skewness and kurtosis levels were less than the allowable values for a paired t-test (skewness<|2.0| and kurtosis <|9.0|; Posten, 1984). Besides, assumptions for One-way Repeated-Measures MANOVA was examined as well, such as detection of outliers using boxplots, checking normality through Shapiro-Wilk test of normality, testing homogeneity of variance using Levene's test. Significance level was set at 0.05 (two-tailed). IBM SPSS Statistic 23 software was used for statistically analysis.

4.4 Results

4.4.1 Demographic information

Thirty-one subjects were enrolled in pressure therapy group, with forty-three HTS specimens obtained before and after treatment. The post-injury (PI) days of subjects ranged from 28 to 277 days, with Total Burn Surface Area (TBSA) from 1% to 65% (Table 4-3).

Table 4-3 Demographic information of recruited subjects

	Number
Number of patients	31
Number of Scars	43
Age (year, Mean (SD))	40.16 (13.60)
Gender	

Male (No. (%))	22 (71.0%)
Female (No. (%))	9 (29.1%)
Post injury days (PI day) (Mean (SD))	91.88 (75.48)
Injury area (% of TBSA, Mean (SD))	18.52 (16.72)
Type of injury	
Fire burn	9 (29.0%)
Scalded	17 (54.8%)
Electrical	5 (16.1%)
Scar site	
Hand	6 (13.6%)
Forearm	8 (18.2%)
Upper arm	6 (13.6%)
Thigh	9 (20.5%)
Calf	8 (18.2%)
Foot	7 (15.9%)

4.4.2 Histological manifestation

- Downregulation of integrin-β1, FAK and ERK in dermis of HTS after pressure therapy.

For the general staining pattern, integrin-β1 was found expressing in the cell membrane, while expression of FAK and ERK was predominantly cytoplasmic. Strong immunopositivity of integrin-β1 was detected predominantly in the basal layer of epidermis. After pressure therapy, intensity of integrin-β1, FAK and ERK staining was reduced in the dermis of HTS. In epidermis, intensity of integrin-β1 in the basal layer was lower in HTS after pressure therapy, whereas the difference of FAK and ERK before and after pressure therapy was not obvious (Figure 4-6).

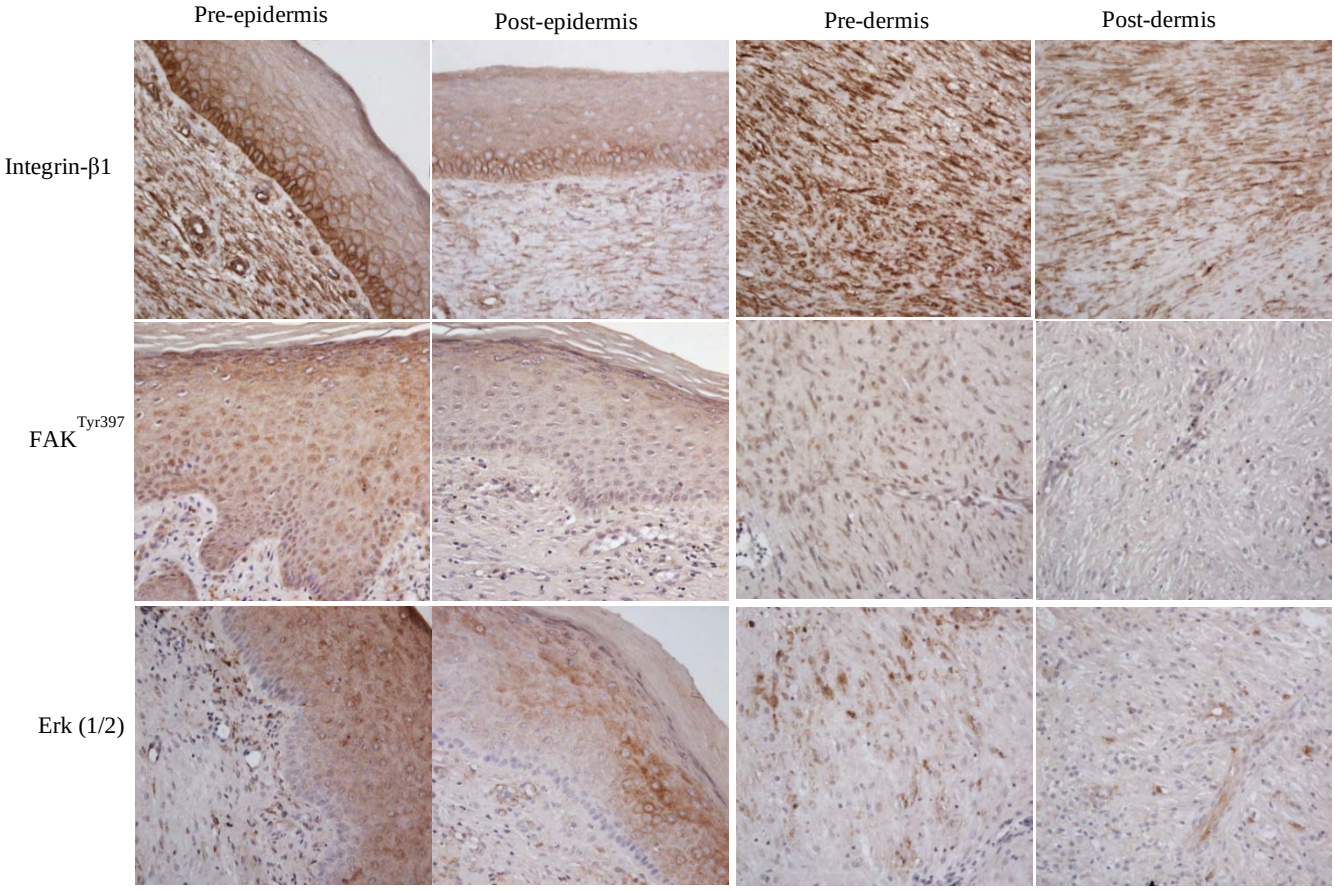


Figure 4-6 Immunohistochemistry photomicrographs (40X) of Integrin-β1, FAK^{Tyr397} and Erk (1/2) staining in HTS before and after pressure therapy. Integrin-β1 was observed predominantly in the cell membrane. FAK^{Tyr397} and Erk(1/2) exhibited mainly cytoplasmic and nuclear immune-staining, especially in epidermis. After pressure therapy, decreased positive staining density was observed in dermal integrin-β1, FAK^{Tyr397} and Erk (1/2).

4.4.3 Pre-post comparison

- Clinical scar manifestation

Comparing to HTS before 3-month, improved clinical characteristics (color, height and pliability) were observed in most of the HTS (Figure 4-7). For scoring from VSS, significant difference before and after pressure therapy was found in vascularity and height (Table 4-4). However, objective scar thickness before (Mean=2.90, SD=1.28) and after (Mean=2.89, SD=1.36) pressure therapy did not show significance

difference (t=0.05, p=0.96), and the decrease of scar thickness was observed in 47.10% of patients.

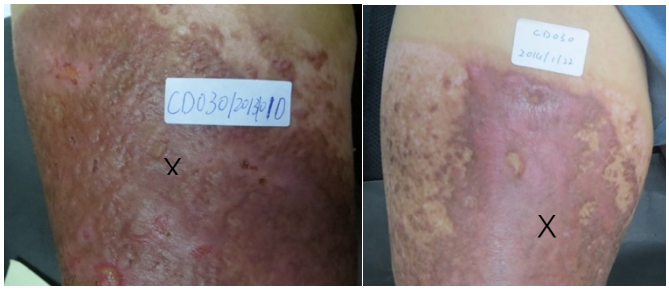


Figure 4-7 Clinical manifestation of HTS before and after 3-month standardized pressure therapy. Improved scar color and height were observed.

Table 4-4 Wilcoxon signed rank test and paired sample t test was used to compare VSS scores and scar thickness before and after pressure therapy.

Vancouver Scar Scale (Mean (SD))	Pre	Post
Pigmentation	2.31 (0.74)	2.30 (0.65)
Vascularity	2.03 (0.82)	1.77 (0.73)*
Pliability	1.94 (0.72)	1.87 (0.90)
Height	2.31 (0.78)	2.67 (0.96)*
Total score	8.60 (2.39)	8.60 (2.49)
Scar Thickness	2.90 (1.28)	2.89 (1.36)

- The immunoreactivity of integrin-β1, FAK and ERK was downregulated in dermis after pressure therapy.

  Table 4-5 presents the means and standard deviations of immunoreactivity of three mechanosensitive molecules. The mean immunoreactivity of three mechanosensitive molecules was generally downregulated after 3-month pressure therapy except for epidermal FAK. To further examine if the difference was statistically significant, a within subject comparison was conducted.

Table 4-5 Means and Standard Deviations for Three Protein Variables at Pretest and Posttest.

Measure	**Mean (SD)**	
	Pretest	Posttest
Epidermis		
Integrin-β1	19.92(6.00)	19.83(7.78)
FAK	18.80(6.73)	20.10(8.61)
ERK	17.62(9.87)	18.87(9.53)
Dermis		
Integrin-β1	14.28(5.33)	11.54(4.38)
FAK	6.07(2.40)	5.17(1.71)
ERK	7.56(4.39)	5.98(3.44)

Prior to the within subject comparison, Pearson Correlation Coefficient was computed among the changes of proteins' intensity (Table 4-6). The increase of immunoreactivity in integrin-β1 was consistently associated with positive change of FAK and ERK in both skin layers. Therefore, MANOVA was used for overall comparison of the immunoreactivity of three molecules before and after pressure therapy after assumptions examined and fulfilled.

Table 4-6 Pearson correlation between the differences of mean intensity among integrin-β1, FAK, ERK

Epidermis	Δ Integrin-β1	Δ FAK	Δ ERK
Δ Integrin-β1	1	.431**	.379*
Δ FAK	.431**	1	.489**
Δ ERK	.379*	.489**	1
Dermis	Δ Integrin-β1	Δ FAK	Δ ERK
Δ Integrin-β1	1	.318*	.329*

Δ FAK	.318*	1	.205
Δ ERK	.329*	.205	1

As Table 4-7 shows, time had significant effect on the mean immunoreactivity of dermal integrin-β1 ($F(1,39)=15.54$, $p<0.001$), FAK ($F(1,39)=7.51$, $p= .009$), and ERK ($F(1,39)= .636$, $p= .016$). There was no significant effect of time on the immunoreactivity of proteins in epidermis.

Table 4-7 One-way Repeated Measures MANOVA examined the effect of time on three proteins

Measure	**Mean (SD)**		**Effect of time**			
	Pretest	Posttest	F	p	Partial	Observed
Epidermis						
Integrin-β1	19.92(6.00)	19.83(7.78)	0.15	0.705	0.004	0.066
FAK	18.80(6.73)	20.10(8.61)	0.91	0.348	0.026	0.152
ERK	17.62(9.87)	18.87(9.53)	0.20	0.656	0.006	0.072
Dermis						
Integrin-β1	14.28(5.33)	11.54(4.38)	15.54**	0.000	0.285	0.97
FAK	6.07(2.40)	5.17(1.71)	7.51**	0.009	0.161	0.762
ERK	7.56(4.39)	5.98(3.44)	6.36*	0.016	0.140	0.691

4.4.4 Exploration of influence of confounding factors on the change of immunoreactivity of mechanosensitive molecules

Post injury days and the change of thickness could predict the change of immunoreactivity in dermal integrin-β1 and FAK.

A Multiple Regression Analysis was conducted to determine the best linear combination of post-injury (PI) days and scar thickness for predicting the change of mean immunoreactivity. Table 4-8 shows that, the prediction of models involved PI days and thickness-related variables was statistically significant in integrin-β1 ($F(3, 40)=5.17$, $p= .005$) and FAK ($F(3, 40)=3.16$, $p= .014$). PI days negatively contributes to the variation in the change of immunoreactivity in dermal integrin-β1 and FAK. The change in scar thickness is positively associated with the change of immunoreactivity in dermal integrinβ1 and FAK. As for the staining intensity in epidermis, expect for FAK, the contribution of PI days and thickness was not statistically significant in any of the other proteins.

Table 4-8 Multiple Regression Analysis Summary Predicting Positive Staining Intensity of Proteins from Post Injury Days, Initial Thickness and Change of Thickness after Pressure Therapy. **<0.05 **<0.01**

Dermis	**β**	**R^2**	**Adjusted R^2**	**F**
Δ Integrin-β1				
(Model)		0.47	0.42	8.65**
PI day	-0.51**			
1st thickness	0.12			
Δ thickness	0.47**			
Δ FAK				
(Model)		0.34	0.266	4.86**
PI day	-0.47**			
1st thickness	0.30			
Δ thickness	0.41*			
Δ ERK				
(Model)		0.11	0.016	1.18
PI day	-0.07			

1st thickness	-0.16			
Δ thickness	0.21			
Epidermis	**β**	**R2**	**Adjusted R2**	**F**
Δ Integrin-β1				
(Model)		0.10	0.004	1.05
PI day	-0.20			
1st thickness	0.18			
Δ thickness	0.26			
Δ FAK				
(Model)		0.26	0.190	3.58*
PI day	-0.35			
1st thickness	0.47*			
Δ thickness	0.36*			
Δ ERK				
(Model)		0.16	0.080	1.95
PI day	-0.11			
1st thickness	-0.09			
Δ thickness	0.33			

Note. * Significance level $p<0.05$; ** Significance level $p<0.01$

4.5 Discussion

The study investigated the difference of immunoreactivity of mechanosensitive molecules (FAK, integrin-β1 and ERK) before and after 3-month standardized pressure therapy to preliminarily explore the mechanism of pressure therapy from a mechanotransduction perspective. To facilitate the interpretation and translation of outcomes from molecular to clinical aspects, intact human HTS specimens were selected in the study. Based on confounding factors found from previous literature review (Chapter 2.5.2), the influence of post injury days on the effect of pressure therapy was examined, providing evidence for the timing of pressure therapy intervention. Besides, the influence of scar thickness, one of the commonly assessed objective scar parameters, on the effect of pressure therapy was also explored, aiming to find the potential explanations for inconsistent results observed in clinical practice.

In the study, the difference of scar height measure by VSS before and after 3-month pressure therapy was found to be statistically significant, which is consistent with findings from meta-analysis conducted by Anzarut's group (Anzarut, et al., 2009). However, for the objective scar thickness obtained by ultrasound, the difference was not statistically significant. The population of subjects and the duration of follow up may be the important factors that contribute to the different outcomes. In previous clinical trials that objectively assessed scar thickness, the follow-up time of pressure therapy ranged from 3 to 12 months. Bernadette et al

found a significantly decrease in scar thickness in the time spans of 3-6 months, 6-12 months and 3-12 months after burns (Bernadette et al, 2014). In a 3-month pressure therapy program with an average pressure dosage of 20mmHg, Kerckhove et al found a 52.9% decrease in scar thickness comparing to the first measurement (Kerckhove et al., 2005). It should be noticed that the included subjects in these two studies were all or primarily Caucasian, which were different from our subjects (Asian). Comparing to Caucasian, Asian population was reported to have a higher chance of developing severe HTS (Li-Tsang et al., 2005). A more progressive HTS might be one of the reasons that the non-significant results observed in our study. Meanwhile, a 5-month pressure therapy study conducted in Chinese population found a significant reduction of thickness after pressure therapy (20-25mmHg) (Lai et al., 2010), indicating that the inadequate follow-up time may be the other reason that lead to non-significant effect.

However, we did find a significant effect of the 3-month pressure therapy on downregulation of immunoreactivity of dermal integrin-β1, FAK and ERK. Previous research found that mechanosensitive molecules such as integrins and their downstream FAK are critical in mediating response of cells to the mechanical signal from ECM or cytoskeleton, thus to regulate cell adhesion, proliferation, migration and apoptosis (Zebda, Dubrovskyi, & Birukov, 2012; Chiquet et al., 2009; Koivisto et al, 2014 Golubovskaya, 2010). FAK was found significantly elevated in invasive and metastatic tumors comparing to normal tissues, and the inhibition of FAK has been applied into cancer treatment (Tai et al., 2015; Golubovskaya, 2010; Golubovskaya, 2014; Sulzmaier et al., 2014). For research focusing on skin fibrosis, the

inhibition of FAK through either pharmalogical blockade or genetic knockout was found to result in scarless wound healing in animal studies (Leask, 2013; Wong, et al., 2012; Zhang et al., 2015). Besides, in Phase I study (Chapter 3.4), we found a relatively increased immunoreactivity of mechanosensitive molecules in HTS comparing to normal skin, suggesting that a stronger immunopositivity of mechanosensitive molecules might indicate a more active HTS. In the study, although objective scar thickness was not found to statistically decrease, the downregulated immunoreactivity of mechanosensitive molecules may suggest a controlled progression of HTS after 3-month treatment, and the thickness measured by ultrasound may not be sensitive enough to detect the change in the 3-month time in our study. Afterwards, the potential relationship between immunoreactivity of mechanosensitive molecules and scar thickness was explored. It was found that the change in scar thickness was positively associated with the change of immunoreactivity in dermal integrin-β1 and FAK. The results could support the statement that there was a downregulated immunoreactivity of mechanosensitive molecules, which could reflect the activity level of HTS progression, may not be able to reflect in terms of scar thickness within 3-month treatment time.

With previous understanding of importance of treatment implementation time on the scar progression (Chapter 2.5.2), the relationship between PI days and the change of immunoreactivity of mechanosensitive molecules was further explored. PI days was found to negatively contributes to the variation in the change of immunoreactivity in dermal integrin-β1 and FAK. In phase I study, we discussed the evidence that the immunoreactivity of integrin-β1 and FAK may reflect the vulnerability of HTS to mechanical force in aggressive

development (Chapter 3.5). And in LOESS fit line constructed in phase I study (Chapter 3.4.3), an increasing immunoreactivity of mechanosensitive molecules was observed in HTS obtained two to three-month post injury, followed by a constantly decrease in the immunopositivity, which may indicate a decreasing ability of HTS response to mechanical force along with post injury days. The negative relationship between the increase in the PI days and the decrease in the immunoreactivity of dermal integrinβ1 and FAK could be explained by decreasing sensitivity of HTS to compression force, which was consistent with statements that the earlier the implementation of pressure therapy, the better outcome can be obtained in scar managements.

Chapter 5 Phase III Clinical application of pressure therapy on aggressive hypertrophic scars concerning the concept of mechanotransduction: a case report

5.1 Introduction

Hypertrophic scars (HTS) are aggressive cutaneous proliferative condition characterized by raised, rigid, hypervascular scarring within the boundary of wounds (Jumper, Paus, & Bayat, 2015). Genetic factors and severity of injury are reported to affect development of HTS. Prolonged inflammation is the predominant mechanism, which involves persistent imbalance between excessive deposition of extracellular matrix and insufficient substrate degradation (Zhu, Ding & Tredget, 2015). In recent years, researchers find that skin equivalent tension is associated with shape and incidence of HTS. More and more evidence support the involvement of mechanotransduction in HTS formation (Akaishi et al., 2008).

Complex pathogenesis increase difficulty of effective management on HTS. Compared with invasive treatments, non-invasive treatments are welcomed for their skin-protecting properties (Gauglitz & Korting, 2011; Monstrey et al., 2014). As the first-line conservative treatment, pressure therapy and silicone gel are reported to be effective in managing hypertrophic scars (HTS) (Li-Tsang & Zheng, 2010; Li-Tsang et al., 2015; Anthonissen et al., 2016). As the morphology of HTS is associate with topographical skin tension, pressure application over different body parts should be site-specific (Akaishi et al., 2008; Akaishi et al., 2010). HTS management over pubic area is far more challenging because of its concave contour and

limited area of supporting surface. Strategies for pressure therapy on HTS over pubic area is yet to be established (Sand et al., 2007).

Notwithstanding the inadequate elucidation of the mechanism and effective treatment of aggressive HTS over pubic area, few reports have illustrated the longitudinal characteristics and management of HTS during their development across time. In this report, the authors present an adolescent case with aggressive HTS developing over the pubic area. A novel 3-dimensional (3D) pressure therapy intervention regime incorporating the application of pressure as well as silicone gel lined padding was introduced.

5.2 Case presentation

A 12-year-old girl got hot water scald injury during dinner on 13th August, 2012, and was diagnosed with second degree burn of 9.5% total body surface area (TBSA). Infection of Staphylococcus aureus was reported in the third week. Daily dressing was implemented and the wound was healed within five weeks. No surgery was performed. One month after the injury, the scar appeared red and rose within the border over the groin area, pubic area and the medial side of the thigh region, which altogether covered about 3% TBSA.

The girl was then referred to our clinic for scar management. Eight sites were chosen for the measurements: two on the right thigh (scar 1a, 1b), four in the groin and pubic areas (scar 2a, 2b, 2c, 2d), and two on the left thigh (scar 3a, 3b) (Figure 5-1).

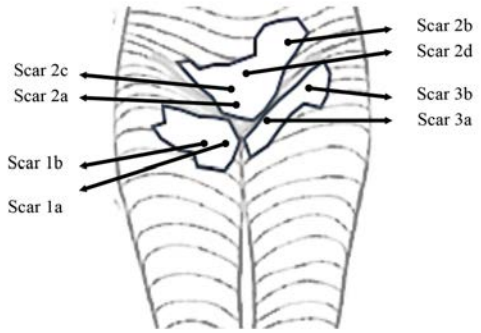


Figure 5-1 Selected scar sites for clinical measurements.

Objective non-invasive scar measurement was conducted to monitor the scar progress. Thickness of the scar was measured by the ultrasound system (Mindray, M5) in musculoskeletal (MSK) mode. Photographs were taken to record scar appearance. Based on the measured scar thickness (around 3mm) and appearance, conventional pressure therapy using pressure pants, plastazote padding was prescribed. The pressure applied was about 20 mmHg based on the measurement of the Pliance-X system (with a sensitive pressure measurement device ranging from 5 mmHg to 60 mmHg). Improved redness and pliability over the scar was observed one week later. The girl had no complaint of discomfort.

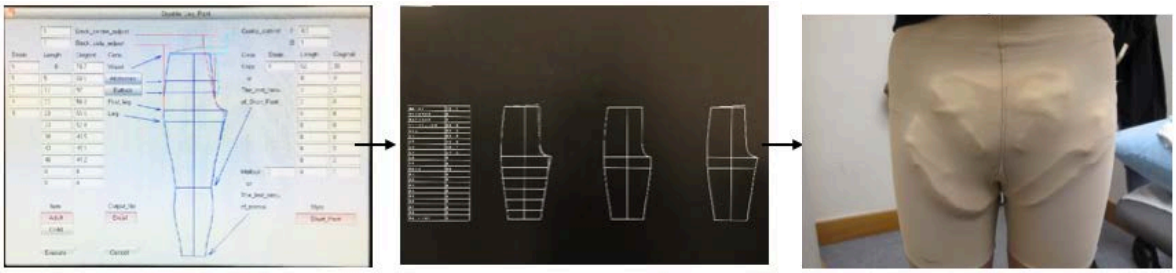


Figure 5-2 Illustration of conventional pressure therapy program: (a, b) YUKA system; (c) conventional pressure pants with plastazote inserts.

However, in the subsequent monthly follow-ups, despite the girl's fair compliance with the intervention protocol, the scars showed an increase in thickness by an average of 5.5mm within 6 months, along with along with the increased Vancouver Scar Scale (VSS) score for pigmentation and pliability. The parents reported no family history of hypertrophic scar or keloid and there was no history of drug intake or any other trauma recorded.

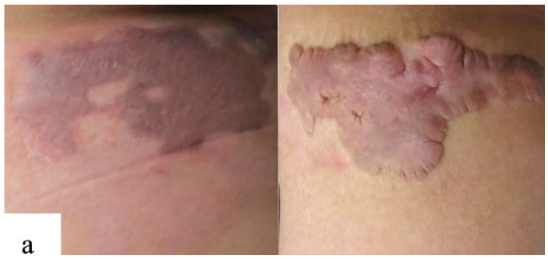


Figure 5-3 Scar appearance at 2 (left), 11 (right) months after conventional pressure therapy.

To control the aggressive scar, a newly developed scar-care gel padding and a 3D conforming splint were introduced to the girl 11 months after the injury (Figure 5-4). The scar-care gel padding had a layer of studs covering the silicone gel sheets. The flexibility of the gel padding enabled optimal fitting and uniform application of pressure to the whole scar tissue without restricting movements. Its silicone gel lining helped preserving the moisture of the scar. In addition to traditional pressure management, in which pressure is applied vertically over the scar surface, supplementary compression was applied at the lateral side through modifying the height of the studs (Figure 5-5). A 3D conforming splint was made by thermoplastic material, molding to surround the whole HTS. Concave area was also shaped thus to restrained excessive growth of HTS (Figure 5-6). The programme was planned such that the scar-care gel padding plus pressure pants would be worn during the day, while the 3D conforming splint was worn at night time. Emu oil was prescribed with education on scar massage. In the

subsequent monthly follow up sessions, renewal of the scar-care gel paddings and pressure pants was arranged at 3-month intervals. Optimal pressure application was monitored by the Pliance-X pressure system. The 3D conforming splint was replaced and remolded every two months to control the lateral growth of the aggressive HTS. A longitudinal follow up was conducted until 42 months since the injury when the HTS appeared mature and became stabilized. The girl and her parents were reminded to comply diligently with the new pressure intervention protocol. Close liaison with the case therapist in any event of discomfort or complication was required. Both the girl and her parents rejected surgical procedure.

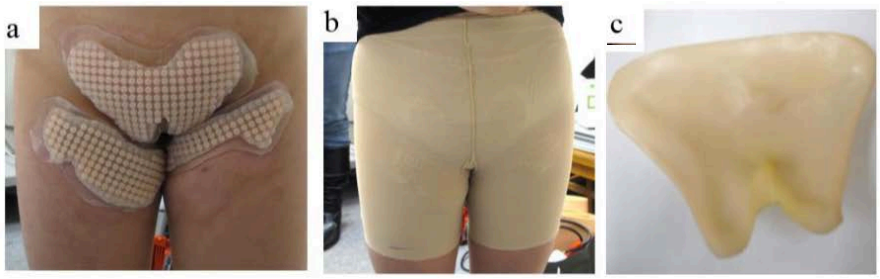


Figure 5-4 The Scar-care padding (a) and SPMS (b) were dressed in the day-time; Splint conformer (c) and SPMS were dressed at night for scar management.

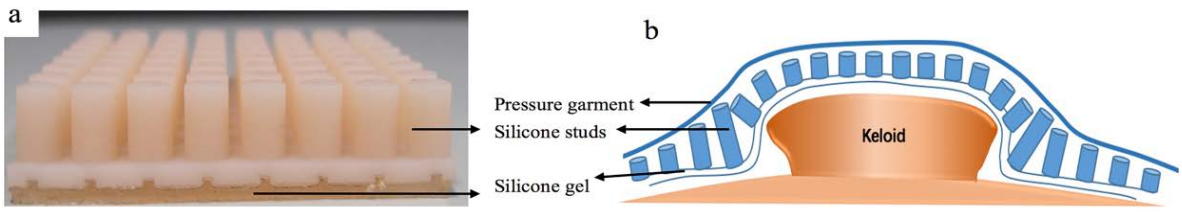


Figure 5-3 Schematic of Scar-care padding: supplementary compression was applied at the lateral side through modifying the height of the studs

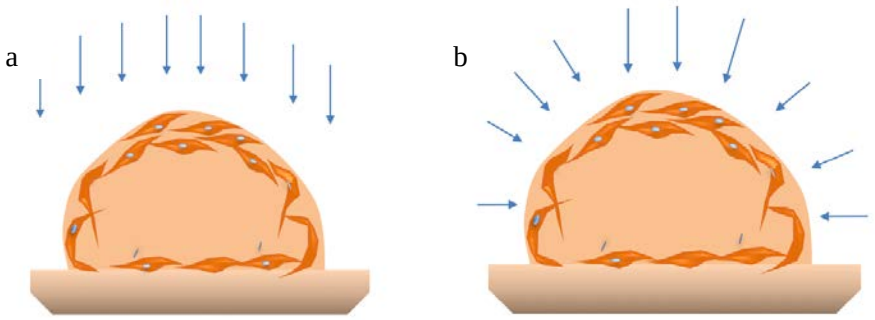


Figure 5-6 Force displayed upon 3-dimentional compression (b) compared to conventional pressure therapy (a)

5.3 Results

The thickness of the hypertrophic scars ranged from 2.2 mm (at the left groin area) to 3.6 mm (at the lower right pubic area) at the initial assessment, compared with < 1 mm in normal skin. The thickness underwent a rapid increase of 0.64 mm monthly in the initial stage when only the conventional pressure garment was implemented. The upsurge of thickness was suspended since the 11th month when the new 3D pressure intervention regime was introduced, and transited to a 3-month modest increase followed by a 30-month downward trend of 0.81 mm monthly. In terms of appearance of the scar, reduction of thickness and shrinkage over the border was observed (Figure 5-7). Regarding the difference among anatomical regions, the thickness of the scars over the groin and the pubic area (Scar 2) was higher than that of the scars on either the left or the right thigh roots (Scar 1 and 3) (Figure 5-8).

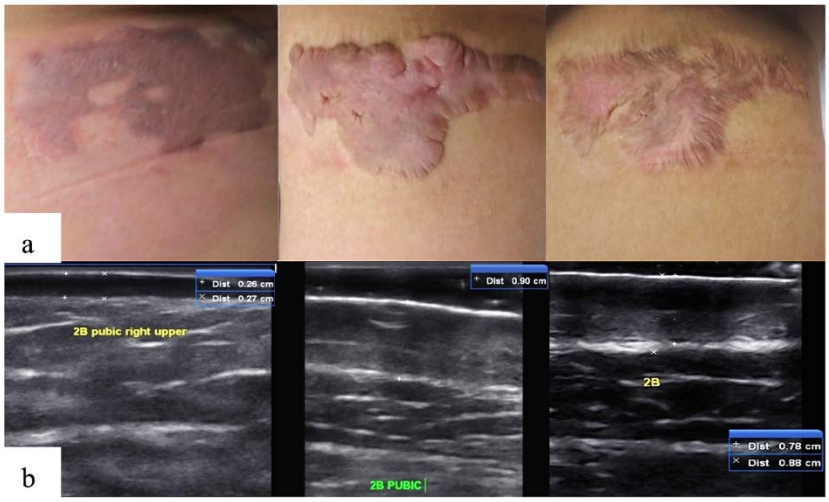


Figure 5-7 The appearance (a) and thickness (b) of scars over the right thigh at 2 months (left), 11 months (middle), and 31 months (right) after the injury.

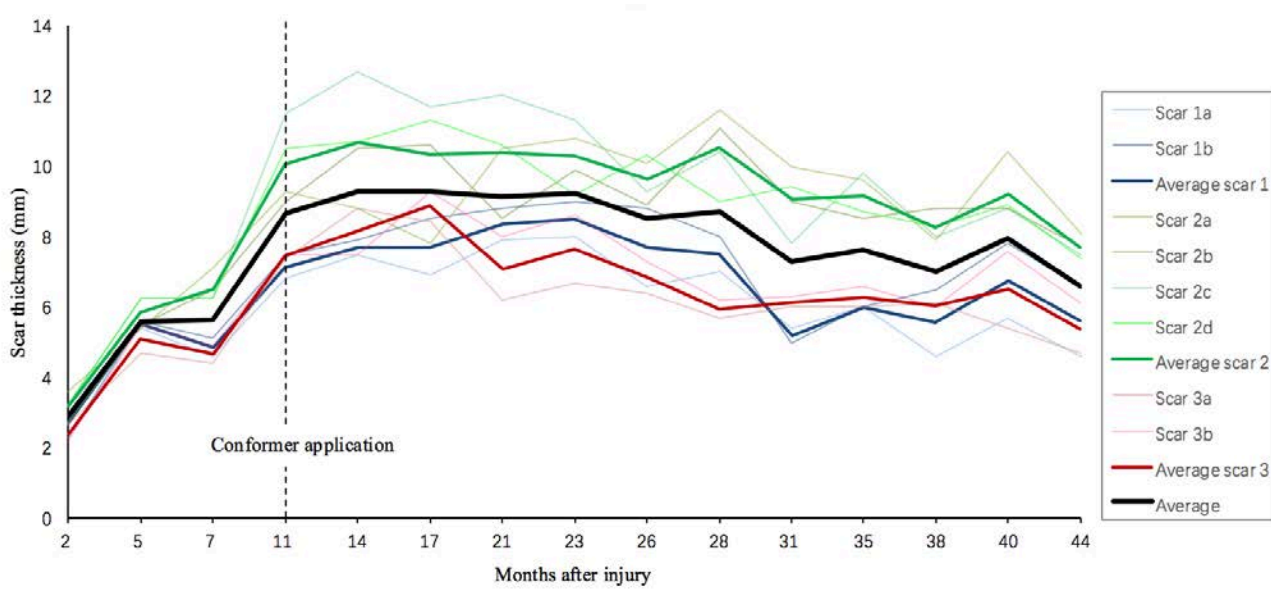


Figure 5-8 Dynamic variation of scar thickness over pubic area (Scar 2), right (Scar 1) and left (Scar 3) thigh root.

5.4 Discussion

This is the first report on a longitudinal review of how aggressive hypertrophic scars responded to a novel 3D pressure therapy programme, on body regions with flat and soft skin surface that are difficult to apply sufficient pressure to control the scars. Regulation of pressure using standardized pressure measurement system, good compliance of the case and support from the family were ensured, and contributed to the effectiveness of this comprehensive programme.

The pathogenesis of hypertrophic scars is complex. In addition to genetic predisposition, multiple factors that are involved in the wound healing process could precipitate aberrant scars formation. In this case, the infection which exposed the wound to prolonged inflammation may serve as a key factor for scar formation. Modulation of androgen and estrogen in puberty is also considered to contribute to the keratinocyte migration, fibroblasts proliferation and accumulation of proteinase in scars, especially in the injured skin of the pubic area with androgen receptors and the upper legs with estrogen receptors (Akaishi et al., 2008; Ogawa et al., 2012; Shin et al., 2015). In addition, mechanical stretch is suggested to alter the behavior and phenotype of fibroblasts. Tension generated from physical activities after discharge might also be a risk factor for generating aggressive scars. Hypertrophic scars over the groin and pubic areas showed constantly higher thickness. It is likely that the groin and pubic areas are subject to more traction force during hip movements compared to the proximal thigh. Prior to providing advices on

modality and intensity of activities after burn injury, further research is necessary to discover the timeframe in which scars are more vulnerable to force-induced proliferation.

Pressure therapy is one of the widely accepted treatments for excessive scars. The regularly applied compression alters the production of inflammatory cytokines and fibroblasts in the scars through generating hypoxia environment, converting mechanical signals, and reducing scar tension (Suarez, et al., 2014). Regardless of distinguished manifestations and features, numerous studies conflated the pressure therapy strategies of hypertrophic scars in different regions (Tredget, Levi & Donelan, 2014; Ogawa, 2010). Inspired by the above-mentioned research, we firstly implemented the concept of “height” in pressure management of hypertrophic scars. A newly developed scar-care gel padding with silicone lining and a conforming splint was designed and administered on this case as a pilot trial. The aim of applying the scar care gel padding and the conforming splint was to restrict the proliferation of the hypertrophic scars through reducing the radius of curvature and to apply additional pressure at the peripheral regions. The foundation of this design lies on the higher proliferation speed of collagen over the perilesional sites and the effect of compression force on fibroblast (Huang, et al., 2014). Regression in scar thickness was observed after renewing the treatment plan, suggesting the 3D pressure intervention programme was promising in managing aggressive hypertrophic scars, with reduced risk of scar recurrence and deterioration. Nevertheless, due to the lack of a negative control, the effect of this pressure therapy programme requires further investigation by systematic clinical trials.

Chapter 6 Conclusion

6.1 Restatement of the purpose of study

- Phase I of the study: An observational study was conducted to explore the relationship between post injury days and immunoreactivity of integrin-β1, FAK and ERK on human HTS, establishing a reference of mechanosensitive molecules on growing or matured HTS.

- Phase II of the study: A pretest-posttest study was designed in phase II to explore the effect of pressure therapy on mechanotransduction. The influence of post injury days and scar thickness on the change of immunoreactivity was also explored.

- Phase III of the study: A case report of modified pressure therapy generating compression force on aggressive hypertrophic scars from three dimensions (3D) was described, aiming to explore the effect of modified 3D pressure therapy on scar thickness.

6.2 Summary of major findings

In Phase I study, a cross-sectional study was conducted to observe the variation in immunoreactivity of mechanosensitive molecules along with post injury days. Higher immunoreactivity of integrin-β1, FAK and ERK was found in dermal hypertrophic scar (HTS) comparing to normal skin. The LOESS fit line reveals a declined intensity of integrin-β1, FAK and ERK within one to two months' time, followed by an increased

intensity to three months after injury. Afterwards, the intensity of integrin-β1 demonstrates a continuous decline throughout a year in both epidermis and dermis. Whereas FAK and ERK remain a relatively stable trend.

In Phase II study, a pretest-posttest comparison was conducted between immunoreactivity of mechanosensitive molecules before and after pressure garment therapy. The difference of scar thickness before and after pressure therapy was not statistically significant, while the difference in immunoreactivity of integrin-β1, FAK and ERK in dermis was significant. Increased post injury days and scar thickness both negatively contributed to the decrease in immunoreactivity in dermal integrin-β1 and FAK.

In Phase III study, a case with aggressive hypertrophic scars over pubic area was reported. Scar thickness underwent a rapid monthly increase when conventional pressure therapy was implemented. The upsurge of thickness was suspended since the 11th month when the new 3D pressure intervention regime was introduced. For scar appearance, reduction of thickness and shrinkage over the border was observed. Regarding difference between anatomical regions, scar thickness over groin and pubic area was higher than thickness over thigh root.

6.3 Significance of the study

Increased integrins and FAK triggered during early wound healing was found to result in hypertrophic-like scars in animal models. Whereas the change of these mechano-regulated proteins over scar maturation process has not been reported. Comparing to scar models constructed by in-vitro cells and animals, results obtained from human HTS samples can better represent the biophysiological process in human beings, and be interpreted from clinical aspects (Wong et al., 2011; Yang, Im, & Wang, 2005).

Our study managed to obtain scar biopsy from burn patients with PI days ranged from one month to one year, covering the scar maturation period critical for clinical management. And we pioneered to explore the dynamic variation of mechanosensitive molecules from formed human HTS along with post injury days. Concerning skin tension and its relation to scar progression, we firstly implemented a 3-dimensional pressure therapy program on aggressive hypertrophic scars over contoured pubic area. And from longitudinal observation, we found scars being controlled after applying compression from lateral side.

To explore the relationship between mechanotransduction and scar maturation could provide a better understanding of scar treatments in the form of force, such as pressure therapy, massage, taping etc.

6.4 Limitations of the study and prospects for future study

There are several limitations for the study. Firstly, an ideal design for investigating dynamic variation of mechanosensitive molecules in formed HTS should measure the immunoreactivity of molecules consecutively after constant time intervals. While it would be unethical to obtain HTS biopsy from same subject for several times, and follow-up rate was expected to be low. Secondly, for pre-post comparison in the effect of pressure therapy, a controlled group without any treatment is needed to form a control group. However, under the circumstances, it would be challenge to ensure a high compliance in the follow-up. In addition, subject recruitment with delayed or without treatments would violate ethical issue. Therefore, the results from dynamic variation of mechanosensitive molecules, as well as the effect of pressure therapy from mechanotransduction perspective, should be generalized with caution.

In the exploration of mechanotransduction, only three molecules in mechanotrasduction were selected in the study. To further understand the mechanism of pressure therapy, the relationship between mechanotransduction and related signaling pathway (such as TGF-β signalling) need to be explored. Meanwhile, compare to immunohistochemical tests, western blot or total protein quantification would be better to quantify protein amount. In addition, to study the mechanism of compression on matured scar, appropriate animal models should be established. Further study could examine the effect of compression on mechanotransduction and HTS, and the effect of compression on progression of HTS when mechanotransduction is inhibited.

In the phase III study, a case report was used to elucidate the clinical application of mechanotransduction in controlling aggressive scars. To demonstrate the clinical effect of a modified treatment, systematically designed clinical trials with adequate sample size and standardized treatment protocol are needed.

6.5 Concluding remarks

To sum up, the study reports the dynamic variation of activated integrin-β1, FAK and ERK was found to vary during scar maturation phase. A rapid proliferation of HTS and a potential “window time” appeared around two months to four months after injury, which indicate a controlled skin tension (range of motion) before four-month time to prevent HTS progression.

After standardized 3-month pressure therapy, the immunoreactivity of integrin-β1, FAK and ERK was found to significantly decreased in dermis. And the earlier the treatment, the more decreased in mechanosensitive molecules. Meanwhile, for clinically measured scar thickness, increased trend was found after pressure therapy, and the extend of decrease in mechanosensitive molecules was negatively associated with extend of increase in thickness.

Furthermore, in a case study which implemented compression over lateral side of hypertrophic scars, the progression of hypertrophic scars was controlled comparing to the conventional treatments. According to the findings, mechanotransduction may be involved

in the mechanism of pressure therapy. The clinical measured scar thickness on limited time points (eg. pre and post) may not reveal the true effect of scar management.

Meanwhile, outcomes and conclusion generated from our study need to be implement with cautions in clinical practice. The focus of our study is to examine the management and mechanism of hypertrophic scars from mechanotransduction perspective, while in clinical settings, we also need to consider the physical function of the client. We recommend the early implementation of scar management to prevent aggressive scar formation, as well as the early splint positioning with gentle and constant force to prevent soft tissue contracture and scar progression at the same time.

Chapter 7 References:

Aarabi, S., Bhatt, K. a, Shi, Y., Paterno, J., Chang, E. I., Loh, S. a, …Gurtner, G. C. (2007). Mechanical load initiates hypertrophic scar formation through decreased cellular apoptosis. *The FASEB Journal*, *21*(12), 3250–3261.

Ai, J.-W., Liu, J.-T., Pei, S.-D., Liu, Y., Li, D.-S., Lin, H.-M., &Pei, B. (2017). The effectiveness of pressure therapy (15-25 mmHg) for hypertrophic burn scars: A systematic review and meta-analysis. *Scientific Reports*, *7*, 40185.

Alrobaiea, S. M., Ding, J., Ma, Z., &Tredget, E. E. (2016). A Novel Nude Mouse Model of Hypertrophic Scarring Using Scratched Full Thickness Human Skin Grafts. *Advances in Wound Care*, *5*(7), 299–313.

Anthonissen, M., Daly, D., Janssens, T., &Van denKerckhove, E. (2016). The effects of conservative treatments on burn scars: A systematic review. *Burns*, *42*(3), 508–518.

Anzarut, A., Olson, J., Singh, P., Rowe, B. H., &Tredget, E. E. (2009). The effectiveness of pressure garment therapy for the prevention of abnormal scarring after burn injury: a meta-analysis. *Journal of Plastic, Reconstructive & Aesthetic Surgery : JPRAS*, *62*(1), 77–84.

Atiyeh, B. S., El Khatib, A. M., & Dibo, S. A. (2013). Pressure garment therapy (PGT) of burn scars: evidence-based efficacy. Annals of Burns and Fire Disasters, 26(4), 205–12.

Atkinson, J.-A. M., McKenna, K. T., Barnett, A. G., McGrath, D. J., &Rudd, M. (2005). A randomized, controlled trial to determine the efficacy of paper tape in preventing hypertrophic scar formation in surgical incisions that traverse Langer's skin tension lines. *Plastic and Reconstructive Surgery*, *116*(6), 1648-1656-1658.

Baker, M. (2011). Tissue models: A living system on a chip. *Nature*, *471*(7340), 661–665.

Baryza, M. J., &Baryza, G. A. (1995). The Vancouver Scar Scale: an administration tool and its interrater reliability. *The Journal of Burn Care & Rehabilitation*, *16*(5), 535–8.

Bethke, K., 2005. The second skin approach: skin strain field analysis and me- chanical counter pressure prototyping for advanced spacesuit design. Mass. Inst. Technol. M.S. Thesis, 1-148.

Bergers, L. I. J. C., Reijnders, C. M. A., van denBroek, L. J., Spiekstra, S. W., deGruijl, T. D., Weijers, E. M., &Gibbs, S. (2016). Immune-competent human skin disease models. *Drug Discovery Today*, *21*(9), 1479–1488.

Bombaro, K. M., Engrav, L. H., Carrougher, G. J., Wiechman, S. A., Faucher, L., Costa, B. A.,Honari, S. (2003). What is the prevalence of hypertrophic scarring following burns? *Burns*, *29*(4), 299–302.

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

Chang, L., Deng, W., &Yeong, E. (2008). Pressure Effects on the Growth of Human Scar Fibroblasts. *J Burn Care Res*, *29*, 835–841.

Chen, X., Nam, J. O., Jean, C., Lawson, C., Walsh, C. T., Goka, E., Schlaepfer, D. D. (2012). VEGF-Induced Vascular Permeability Is Mediated by FAK. *Developmental Cell*, *22*(1), 146–157.

Cheng, L., Sun, X., Yu, J., Guo, Q., Jin, R., Sun, B., Zhang, Y. (2015). A facilely fabricated in vivo hypertrophic scar model through continuous gradient elastic tension. *RSC Adv.*, *5*(130), 107430–107444.

Cheung, K.-K., Mok, S. C., Rezaie, P., &Chan, W. (2008). Dynamic expression of Dab2 in the mouse embryonic central nervous system. *BMC Developmental Biology*, *8*(1), 76.

Cheung, K. K., Chan, W. Y., &Burnstock, G. (2005). Expression of P2X purinoceptors during rat brain development and their inhibitory role on motor axon outgrowth in neural tube explant cultures. *Neuroscience*, *133*(4), 937–945.

Chin M.S., Lancerotto L, Helm DL, et al. Analysis of neuropep- tides in stretched skin. Plast Reconstr Surg 2009;124:102e13.

Chiquet, M., Renedo, A. S., Huber, F., &Flück, M. (2003). How do fibroblasts translate mechanical signals into changes in extracellular matrix production? *Matrix Biology*, *22*(1), 73–80.

Chiquet M, Gelman L, Lutz R, et al. From mechanotransduction to extracellular matrix gene expression in fibroblasts [J]. Biochimica et Biophysica Acta. 2009, 1793 (5): 911–20.

Cho, Y. S., Jeon, J. H., Hong, A., Yang, H. T., Yim, H., Cho, Y. S., Kim, D.-H., Hur, J., Kim, J. H., Chun, W., Lee, B. C., & Seo, C. H. (2014). The effect of burn rehabilitation massage therapy on hypertrophic scar after burn: A randomized controlled trial. *Burns, 40*(8), 1513-1520.

Czubryt, M. P. (2012). Common threads in cardiac fibrosis, infarct scar formation, and wound healing. *Fibrogenesis Tissue Repair*, *5*(1), 19.

Dewey, W. S., Richard, R. L., & Parry, I. S. (2011). Positioning, splinting, and contracture management. *Physical Medicine and Rehabilitation Clinics of North America*, *22*(2), 229-247.

Du, Q. C., Zhang, D. Z., Chen, X. J., Lan-Sun, G., Wu, M., & Xiao, W. L. (2013). The effect of p38MAPK on cyclic stretch in human facial hypertrophic scar fibroblast differentiation. *PLoS ONE 8*(10), e75635.

Ehrlich, H. P., Desmoulière, A., Diegelmann, R. F., Cohen, I. K., Compton, C. C., Garner, W. L., Gabbiani, G. (1994). Morphological and immunochemical differences between keloid and hypertrophic scar. *The American Journal of Pathology*, *145*(1), 105–13.

Elsner, P., Berardesca, E., Wilhelm, K.P., Maibach, H.I., 2002. Bioengineering of the Skin: Skin Biomechanics. Dermatology: Clinical & Basic Science Series. CRC Press LLC.

Eto, H., Suga, H., Aoi, N., Kato, H., Doi, K., Kuno, S., …Yoshimura, K. (2012). Therapeutic potential of fibroblast growth factor-2 for hypertrophic scars: upregulation of MMP-1 and HGF expression. *Laboratory Investigation*, *92*(2), 214–223.

Feng, B., Ye, W.-L., Ma, L., Fang, Y., Mei, Y.-A., &Wei, S.-M. (2012). Hydrogen peroxide enhanced Ca2+-activated BK currents and promoted cell injury in human dermal fibroblasts. *Life Sciences*, *90*(11–12), 424–431.

Gabriel, V. (2011). Hypertrophic Scar. *Physical Medicine and Rehabilitation Clinics of North America*, *22*(2), 301–310.

Godleski, M., Oeffling, A., Bruflat, A. K., Craig, E., Weitzenkamp, D., & Lindberg, G. (2013). Treating burn-associated joint contracture: Results of an inpatient rehabilitation stretching protocol. *Journal of Burn Care and Research, 34*(4), 420-426.

Gurtner, G. C., Dauskardt, R. H., Wong, V. W., Bhatt, K. A., Wu, K., Vial, I. N., …Longaker, M. T. (2011). Improving Cutaneous Scar Formation by Controlling the Mechanical Enviroment. *Annals of Surgery*, *254*(2), 217–25.

Halka, A. T., Turner, N. J., Carter, A., Ghosh, J., Murphy, M. O., Kirton, J. P., …Walker, M. G. (2008). The effects of stretch on vascular smooth muscle cell phenotype in vitro. *Cardiovascular Pathology*, *17*(2), 98–102.

Ham, S. A., Kim, H. J., Kim, H. J., Kang, E. S., Eun, S. Y., Kim, G. H., …Seo, H. G. (2010). PPARdelta promotes wound healing by up-regulating TGF-beta1-dependent or -independent expression of extracellular matrix proteins. *Journal of Cellular and Molecular Medicine*, *14*(6B), 1747–59.

Hart, D. W., Wolf, S. E., Mlcak, R., Chinkes, D. L., Ramzy, P. I., Obeng, M. K., …Herndon, D. N. (2000). Persistence of muscle catabolism after severe burn. *Surgery*, *128*(2), 312–319.

Hong, S.-Y., Jeon, Y.-M., Lee, H.-J., Kim, J.-G., Baek, J.-A., &Lee, J.-C. (2010). Activation of RhoA and FAK induces ERK-mediated osteopontin expression in mechanical force-subjected periodontal ligament fibroblasts. *Molecular and Cellular Biochemistry*, *335*(1–2), 263–72.

Huang, C., Holfeld, J., Schaden, W., Orgill, D., &Ogawa, R. (2013). Mechanotherapy: revisiting physical therapy and recruiting mechanobiology for a new era in medicine. *Trends in Molecular Medicine*, *19*(9), 555–64.

Huang, C., Miyazaki, K., Akaishi, S., Watanabe, A., Hyakusoku, H., &Ogawa, R. (2013). Biological effects of cellular stretch on human dermal fibroblasts. *Journal of Plastic, Reconstructive and Aesthetic Surgery*, *66*(12), e351–e361.

Huang, D., Liu, Y., Huang, Y., Xie, Y., Shen, K., Zhang, D., &Mou, Y. (2014). Mechanical compression upregulates MMP9 through SMAD3 but not SMAD2 modulation in hypertrophic scar fibroblasts. *Connective Tissue Research, 55*(5–6), 391–6.

Huang C., Akaishi S., Hyakusoku H., Ogawa R.. Are keloid and hypertrophic scar different forms of the same disorder? A fibroproliferative skin disorder hypothesis based on keloid findings. *International Wound Journal* 2014; 11: 517-522.

Ishise, H., Larson, B., Hirata, Y., Fujiwara, T., Nishimoto, S., Kubo, T., …Kawai, K. (2015). Hypertrophic scar contracture is mediated by the TRPC3 mechanical force transducer via NFkB activation. *Scientific Reports*, *5*.

Jean, C., Gravelle, P., Fournie, J.-J., &Laurent, G. (2011). Influence of stress on extracellular matrix and integrin biology. *Oncogene*, *30*(24), 2697–706.

Jumper, N., Paus, R., &Bayat, A. (2015). Functional histopathology of keloid disease. *Histology and Histopathology, 30*(9), 11624.

Junker, J. P. E., Kratz, C., Tollbäck, A., & Kratz, G. (2008). Mechanical tension stimulates the transdifferentiation of fibroblasts into myofibroblasts in human burn scars. *Burns, 34*(7), 942-946.

Kanazawa, Y., Nomura, J., Yoshimoto, S., Suzuki, T., Kita, K., Suzuki, N., &Ichinose, M. (2009a). Cyclical cell stretching of skin-derived fibroblasts downregulates connective tissue growth factor (CTGF) production. *Connective Tissue Research, 50*(5), 323–9.

Kim, J. Y., Willard, J. J., Supp, D. M., Roy, S., Gordillo, G. M., Sen, C. K., &Powell, H. M. (2015). Burn Scar Biomechanics after Pressure Garment Therapy.

Kishi, K., Imanishi, N., Kobayashi, N., Nakajima, H., &Nakajima, T. (2012). New technique for releasing tension to prevent widening of an abdominal scar. *Journal Of Plastic Surgery And Hand Surgery*, *46*(5), 364–366.

Kloeters, O., Tandara, A., &Mustoe, T. A. (2007). Hypertrophic scar model in the rabbit ear: A reproducible model for studying scar tissue behavior with new observations on silicone gel sheeting for scar reduction. *Wound Repair and Regeneration*, *15*(SUPPL. 1), S40–S45.

Koivisto L, Heino J, Häkkinen L, et al. Integrins in Wound Healing [J]. Advances in Wound Care. 2014, 3 (12): 762–783.

Kolmus, A. M., Holland, A. E., Byrne, M. J., & Cleland, H. J. (2012). The effects of splinting on shoulder function in adult burns. *Burns*, *38*(5), 638-644.

Kratz, Anna Tollbäck, Gunnar Kratz, C. (2001). Effects of Continuous Stretching on Cell Proliferation and Collagen Synthesis in Human Burn Scars. *Scandinavian Journal of Plastic and Reconstructive Surgery and Hand Surgery*, *35*(1), 57–63.

Kuang R, Wang Z, Xu Q, et al. Influence of mechanical stimulation on human dermal fibroblasts derived from different body sites [J]. International Journal of Clinical and Experimental Medicine. 2015, 8 (5): 7641–7647.

Kuang, R., Wang, Z., Xu, Q., Cai, X., &Liu, T. (2015). Exposure to Varying Strain Magnitudes Influence the Conversion of Normal Skin Fibroblasts Into Hypertrophic Scar Cells. *Annals of Plastic Surgery*, *76*(4), 388–393.

Lai, Hoi Yan, C., Li-Tsang, Wai Ping, C., &Zheng, Y. (2010). Effect of different pressure magnitudes on hypertrophic scar in a Chinese population. *Burns : Journal of the International Society for Burn Injuries*, *36*(8), 1234–41.

Lai, C. H. Y., &Li-Tsang, C. W. P. (2009). Validation of the Pliance X System in measuring interface pressure generated by pressure garment. *Burns : Journal of the International Society for Burn Injuries*, *35*(6), 845–51.

Langer, Karl. (1861), ‘On the anatomy and physiology of the skin’, The Imperial Academy of Science, Vienna. Reprinted in (1978): British Journal of Plastic Surgery 17(31), 93–106.

Larson, D.L., Abston, S., Willis, B., Linares, H., Dobrkovsky, M., Evans, E.B, & Lewis, S.R. (1974). Contracture and scar formation in the burn patient. Clinics in Plastic Surgery, 1(4), 653-656.

Lau, J. C. M., Li-Tsang, C. W. P., &Zheng, Y. P. (2005). Application of tissue ultrasound palpation system (TUPS) in objective scar evaluation. *Burns : Journal of the International Society for Burn Injuries*, *31*(4), 445–52.

Lawrence, J. W., Mason, S. T., Schomer, K., &Klein, M. B. (2012). Epidemiology and Impact of Scarring After Burn Injury.

Leask, A. (2013). Focal Adhesion Kinase: A Key Mediator of Transforming Growth Factor Beta Signaling in Fibroblasts. *Advances in Wound Care*, *2*(5), 247–249.

Li-Tsang, C. W. ., Lau, J. C. ., &Liu, S. K. . (2003). Validation of an objective scar pigmentation measurement by using a spectrocolorimeter. *Burns*, *29*(8), 779–784.

Li-Tsang, C. W. P., Lau, J. C. M., &Chan, C. C. H. (2005). Prevalence of hypertrophic scar formation and its characteristics among the Chinese population. *Burns : Journal of the International Society for Burn Injuries*, *31*(5), 610–6.

Li-Tsang, C.W. (2009). “Method for manufacturing Smart Pressure Monitored Suits”. (Patent Publication No. US-2009-0165688-A1).

Li-tsang, C. W. P., &Zheng, Y. P. (2010). A Randomized Clinical Trial to Study the Effect of Silicone Gel Dressing and Pressure Therapy on Posttraumatic Hypertrophic Scars.

Li-Tsang, C. W. P., Feng, B., Huang, L., Liu, X., Shu, B., Chan, Y. T. Y., & Cheung, K.-K. (2015). A histological study on the effect of pressure therapy on the activities of myofibroblasts and keratinocytes in hypertrophic scar tissues after burn. *Burns : Journal of the International Society for Burn Injuries*, *41*(5), 1008–16.

Lynam, E. C., Xie, Y., Dawson, R., McGovern, J., Upton, Z., &Wang, X. (2015). Severe hypoxia and malnutrition collectively contribute to scar fibroblast inhibition and cell apoptosis. *Wound Repair and Regeneration*, *23*(5), 664–671.

Macarak, E. J., Korostoff, J. M., Howard, P. S., &He, Y. (2004). Compression and tension: differential effects on matrix accumulation by periodontal ligament fibroblasts in vitro. *Connective Tissue Research*, *45*(1), 28–39.

Martins, V. L., Caley, M., &O’Toole, E. a. (2013). Matrix metalloproteinases and epidermal wound repair. *Cell and Tissue Research*, *351*(2), 255–268.

Monstrey, S., Middelkoop, E., Vranckx, J. J., Bassetto, F., Ziegler, U. E., Meaume, S., & Téot, L. (2014). Updated scar management practical guidelines: Non-invasive and invasive measures. *Journal of Plastic, Reconstructive and Aesthetic Surgery, 67*(8), 1017-1025.

Momtazi, M., Kwan, P., Ding, J., Anderson, C. C., Honardoust, D., Goekjian, S., &Tredget, E. E. (2013). A nude mouse model of hypertrophic scar shows morphologic and histologic characteristics of human hypertrophic scar. *Wound Repair and Regeneration, 21*(1), 77–87.

Monsuur, H. N., Boink, M. A., Weijers, E. M., Roffel, S., Breetveld, M., van denBroek, L. J., &Gibbs, S. (2016). Methods to study differences in cell mobility during skin wound healing in vitro. *Journal of Biomechanics, 49*(8), 1381–1387.

Morien, A., Garrison, D., & Smith, N. K. (2008). Range of motion improves after massage in children with burns: A pilot study. *Journal of Bodywork and Movement Therapies, 12*(1), 67-71.

Ogawa, R. (2010) The most current algorithms for the treatment and prevention of hypertrophic scars and keloids. *Plast Reconstr Surg* 2010; 125: 557-568.

Ogawa, R. (2011). Mechanobiology of scarring. *Wound Repair And Regeneration: Official Publication Of The Wound Healing Society [And] The European Tissue Repair Society, 19 Suppl 1*, s2–s9.

Ogawa, R., Okai, K., Tokumura, F., Mori, K., Ohmori, Y., Huang, C., …Akaishi, S. (2012). The relationship between skin stretching/contraction and pathologic scarring: the important role of mechanical forces in keloid generation. *Wound Repair And Regeneration: Official Publication Of The Wound Healing Society [And] The European Tissue Repair Society*, *20*(2), 149–157.

Okhovatian, F., & Zoubine, N. (2007). A comparison between two burn rehabilitation protocols. *Burns, 33*(4), 429-434.

Paterno, J., Vial, I. N., Wong, V. W., Rustad, K. C., Sorkin, M., Shi, Y., …Gurtner, G. C. (2011). Akt-mediated mechanotransduction in murine fibroblasts during hypertrophic scar formation. *Wound Repair and Regeneration, 19*(1), 49–58.

Patiño, O., Novick, C., Merlo, A., & Benaim, F. (1999). Massage in hypertrophic scars. *Journal of Burn Care and Rehabilitation, 20*(3), 268-271.

Penn, J. W., Grobbelaar, A. O., &Rolfe, K. J. (2012). The role of the TGF-β family in wound healing, burns and scarring: a review. *International Journal of Burns and Trauma, 2*(1), 18–28.

Perera, A., Perera, C., & Karunanayake, A. (2015). Effectiveness of early stretching exercises for the quality of recovery of the upper limb in burnt patients. *Physiotherapy, 101*(Suppl 1), e1194.

Peters, A. S., Brunner, G., Krieg, T., &Eckes, B. (2015). Cyclic mechanical strain induces TGFβ1-signalling in dermal fibroblasts embedded in a 3D collagen lattice. *Archives of Dermatological Research*, *307*(2), 191–197.

Reiffel, R. S. (1995). Prevention of hypertrophic scars by long-term paper tape application. *Plastic and Reconstructive Surgery*.

Reinke, J. M., &Sorg, H. (2012). Wound Repair and Regeneration. *European Surgical Research*, *49*(1), 35–43.

Renò, F., Sabbatini, M., Lombardi, F., Stella, M., Pezzuto, C., Magliacani, G., &Cannas, M. (2003). In vitro mechanical compression induces apoptosis and regulates cytokines release in hypertrophic scars. *Wound Repair and Regeneration : Official Publication of the Wound Healing Society [and] the European Tissue Repair Society*, *11*(5), 331–6.

Roh, Y. S., Cho, H., Oh, J. O., & Yoon, C. J. (2007). Effects of skin rehabilitation massage therapy on pruritus, skin status, and depression in burn survivors. *Journal of Korean Academy of Nursing*, *37*(2), 221-226.

Roh, Y. S., Seo, C. H., & Jang, K. U. (2010). Effects of a skin rehabilitation nursing program on skin status, depression, and burn-specific health in burn survivors. *Rehabilitation Nursing*, *35*(2), 65-69.

Rolin, G., Binda, D., Tissot, M., Viennet, C., Saas, P., Muret, P., &Humbert, P. (2014b). In vitro study of the impact of mechanical tension on the dermal fibroblast phenotype in the context of skin wound healing. *Journal of Biomechanics*, *47*(14), 3555–3561.

Roques, C. (2002). Massage applied to scars. *Wound Repair and Regeneration*, *10*(2), 126-128. doi: 10.1046/j.1524-475X.2002.02107.x

Sand M., Sand D., Boorboor P. *et al.* Combination of surgical excision and custom designed silicon pressure splint therapy for keloids on the helical rim. *Head Face Med* 2007; 3: 14.

Sarifakioglu, E., &Sarifakioglu, N. (2006). Dressing spray enhances the adhesive strength of surgical dressing tapes. *Indian Journal of Dermatology, Venereology and Leprology*. https://doi.org/10.4103/0378-6323.27751

Saxena, V., Hwang, C.-W., Huang, S., Eichbaum, Q., Ingber, D., &Orgill, D. P. (2004). Vacuum-assisted closure: microdeformations of wounds and cell proliferation. *Plastic and Reconstructive Surgery*, *114*(5), 1086-1096-1098.

Shin, T. M., & Bordeaux, J. S. (2012). The role of massage in scar management: A literature review. *Dermatologic Surgery*, *38*(3), 414-423. doi:10.1111/j.1524-4725.2011.02201.x

Shin, J. U. *et al.* Estrogen Upregulates Slug to Enhance the Migration of Keratinocytes. *J. Invest. Dermatol.* **135,** 3200–3 (2015).

Silver, F. H., Siperko, L. M., &Seehra, G. P. (2003). Mechanobiology of force transduction in dermal tissue. *Skin Research and Technology : Official Journal of International Society for Bioengineering and the Skin (ISBS) [and] International Society for Digital Imaging of Skin (ISDIS) [and] International Society for Skin Imaging (ISSI)*, *9*(1), 3–23.

Song, R., Bian, H.-N., Lai, W., Chen, H.-D., &Zhao, K.-S. (2011). Normal skin and hypertrophic scar fibroblasts differentially regulate collagen and fibronectin expression as well as mitochondrial membrane potential in response to basic fibroblast growth factor. *Brazilian Journal of Medical and Biological Research*, *44*(5), 402–410.

Steinstraesser, L., Flak, E., Witte, B., Ring, A., Tilkorn, D., Hauser, J., …Al-Benna, S. (2011). Pressure garment therapy alone and in combination with silicone for the prevention of hypertrophic scarring: randomized controlled trial with intraindividual comparison. *Plastic and Reconstructive Surgery*, *128*(4), 306e–13e.

Suarez, E., Syed, F., Rasgado, T. a, Walmsley, A., Mandal, P., &Bayat, A. (2014). Skin equivalent tensional force alters keloid fibroblast behaviour and phenotype. *Wound Repair and Regeneration : Official Publication of the Wound Healing Society [and] the European Tissue Repair Society*, *22*(5), 1–38.

Tejiram, S., Zhang, J., Travis, T. E., Carney, B. C., Alkhalil, A., Moffatt, L. T., …Shupp, J. W. (2015). Compression therapy affects collagen type balance in hypertrophic scar. *Journal of Surgical Research*, (November), 1–7.

Todd, M. (2011).Compression bandaging: types and skills used in practical application. British Journal of Nursing, 20(11), 681-2, 684, 686-687.

Tokuyama, E., Nagai, Y., Takahashi, K., Kimata, Y., & Naruse, K. (2015). Mechanical stretch on human skin equivalents increases the epidermal thickness and develops the basement membrane. *PLoS ONE 10*(11), e0141989.

Van denKerckhove, E., Stappaerts, K., Fieuws, S., Laperre, J., Massage, P., Flour, M., &Boeckx, W. (2005). The assessment of erythema and thickness on burn related scars during pressure garment therapy as a preventive measure for hypertrophic scarring. *Burns*, *31*(6), 696–702.

Verhaegen, P. D., Schouten, H. J., Tigchelaar-Gutter, W., VanMarle, J., VanNoorden, C. J., Middelkoop, E., &VanZuijlen, P. P. (2012). Adaptation of the dermal collagen structure of human skin and scar tissue in response to stretch: An experimental study. *Wound Repair and Regeneration*, *20*(5), 658–666.

Wang, J., Hori, K., Ding, J., Huang, Y., Kwan, P., Ladak, A., &Tredget, E. E. (2011). Toll-like receptors expressed by dermal fibroblasts contribute to hypertrophic scarring. *Journal of Cellular Physiology*, *226*(5), 1265–1273.

Wang, Z., Fong, K. D., Phan, T.-T., Lim, I. J., Longaker, M. T., &Yang, G. P. (2006). Increased transcriptional response to mechanical strain in keloid fibroblasts due to increased focal adhesion complex formation. *Journal of Cellular Physiology*, *206*(2), 510–7.

Ward, R. S. (1991). Pressure therapy for the control of hypertrophic scar formation after burn injury. A history and review. *The Journal of Burn Care & Rehabilitation*, *12*(3), 257–62.

Wille, J. J., Ambrosi, C. M., &Yin, F. C.-P. (2004). Comparison of the Effects of Cyclic Stretching and Compression on Endothelial Cell Morphological Responses. *Journal of Biomechanical Engineering*, *126*(5), 545.

Wong, V.W., Kirit, A.B., Ivan, N.V., Kenneth, W., Karine, P., Reinhold, H.D., Michael, T.L., Geoffrey, C.G., 2010. Beyond Langer's lines: manipulating wound mechanical forces to control hypertrophic scar formation in the red Duroc pig. J. Am. Coll. Surg. 211 (3), S77. Supplement.

Wong, V. W., Levi, K., Akaishi, S., Schultz, G., &Dauskardt, R. H. (2012). Scar Zones: Region-Specific Differences in Skin Tension May Determine Incisional Scar Formation.

Wong, V. W., Longaker, M. T., &Gurtner, G. C. (2012). Soft tissue mechanotransduction in wound healing and fibrosis. *Seminars in Cell & Developmental Biology*, *23*(9), 981–6.

WHO methods and data sources for global burden of disease estimates 2000-2015 [Internet]. WHO Geneva: Department of information, evidence and research; January 2017 [cited April 2018]. Available from: http://www.who.int/healthinfo/global_burden_disease/GlobalDALYmethods_2000_2015.pdf?ua=1

Yagmur, C., Akaishi, S., Ogawa, R., &Guneren, E. (2010). Mechanical receptor-related mechanisms in scar management: a review and hypothesis. *Plast. Reconstr. Surg.*, *126*(2), 426–34.

Yano, S., Komine, M., Fujimoto, M., Okochi, H., &Tamaki, K. (2004). Mechanical stretching in vitro regulates signal transduction pathways and cellular proliferation in human epidermal keratinocytes. *The Journal of Investigative Dermatology*, *122*(3), 783–90.

Yelvington M, Brown S, Castro MM, et al. The use of neoprene as a scar management modality. Burns 2013; 39: 866–875.

Zachary, I. (2003). VEGF signalling: integration and multi-tasking in endothelial cell biology. *Biochemical Society Transactions*, *31*(Pt 6), 1171–7.

Zebda, N., Dubrovskyi, O., &Birukov, K. G. (2012). Focal adhesion kinase regulation of mechanotransduction and its impact on endothelial cell functions. *Microvascular Research*, *83*(1), 71–81.

Zhang, K., Ding, W., Sun, W., Sun, X.-J., Xie, Y.-Z., Zhao, C.-Q., &Zhao, J. (2015). Beta1 integrin inhibits apoptosis induced by cyclic stretch in annulus fibrosus cells via ERK1/2 MAPK pathway. *Apoptosis : An International Journal on Programmed Cell Death*.

Zhang, S. J., Truskey, G. A., &Kraus, W. E. (2007). Effect of cyclic stretch on beta1D-integrin expression and activation of FAK and RhoA. *American Journal of Physiology. Cell Physiology*, *292*(6), C2057-69.

Zhang, Y. T., Li-Tsang, C. W., & Au, R. K. A Systematic Review on the Effect of Mechanical Stretch on Hypertrophic Scars after Burn Injuries [J]. Hong *Kong Journal of Occupational Therapy*, 2017, *29*: 1-9.

Zhu, Z., Ding, J., Shankowsky, H. A., &Tredget, E. E. (2013). The molecular mechanism of hypertrophic scar. *Journal of Cell Communication and Signaling*, *7*(4), 239–52.