STUDY OF PRESSURE EFFECT ON HYPERTROPHIC SCAR TISSUES

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Study of Pressure Effect on Hypertrophic Scar Tissues

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

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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 which has been accepted for the award of any other degree or diploma, except where due acknowledgement has been made in the text.

Signed: _____

Lai Hoi Yan, Candy

DEDICATION

I dedicate this work to my family members who have been giving me their heartfelt support and understanding throughout my study.

ABSTRACT

Abstract of thesis entitled:

Study of Pressure Effect on Hypertrophic Scar Tissues *submitted by* ***Lai Hoi Yan, Candy*** *for degree of* ***Master of Philosophy*** *at The Hong Kong Polytechnic University in December 2008.*

Hypertrophic scar is a dermal fibroproliferative disorder following dermal skin trauma. This skin abnormality is characterized as raised, rigid and erythematous appearance associated with pain and pruritus. Not only would it cause severe cosmetic disfigurement, but considerable functional impairment often results if scar contracture develops over joints. Pressure therapy has been the widely applied in scar management since the early 1970s because of its non-invasive characteristics and desirable treatment effect. The optimal pressure dose, nevertheless, remains undefined, due to the lack of objective scar assessment tools and precise pressure measurement apparatus.

Pressure therapy has been hypothesized to manage scarring by down-regulating fibroblast activities, however, its mechanisms have not yet been fully understood. To better understand the mechanisms, an *in-vitro* study was conducted to examine the biological activities of hypertrophic scar fibroblasts in response to mechanical pressure. The fibroblasts were cultured on cover-slips with diameter 13mm at a density of 2×10^4 under different loadings of mechanical pressures (0g, 2g, 5g and 10g) for 48 hours. Fibroblasts were harvested on Day 0, Day 2 and two days after unloading (Post-day 2)

for evaluation. Inhibition of fibroblast proliferation was demonstrated upon mechanical pressure loading measured using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay and the inhibition was found to be dose-dependent with higher pressure inducing a more significant effect on cell proliferation as compared to lower pressure. Similar pattern was observed in fibroblast differentiation. Immunocytochemical staining revealed an inverse relationship between pressure loadings and the population of differentiated fibroblasts (myofibroblasts). The external pressure continued to restrain cell differentiation two days after unloading.

Following the *in-vitro* study, a randomized clinical trial with double-blinded design was conducted to explore the relationship between pressure magnitude and scar conditions. To compare the influences of two different levels of pressure on hypertrophic scars, an objective evaluation protocol was adopted to document the pressure dosage and the scar conditions. Fifty-eight post-traumatic scars which were developed for three to nine months (5.23±1.88 months) among 19 subjects were selected according to the inclusion and exclusion criteria. Scar samples were randomly assigned into two groups, namely, the low pressure group (10–15mmHg) and the high pressure group (20–25mmHg). Each patient was prescribed with two sets of tailor-made pressure garment with 5% tensile strength and insertion of pressure padding underneath the garment was used to manipulate the interface pressure generated. Objective quantification of interface pressure generated by pressure garment and padding was obtained using a pressure monitoring system, the Pliance X System, which was validated earlier in the study. For assessment of the scar maturation process, the scar thickness was measured by the

Tissue Ultrasound Palpation System (TUPS), the scar color by the spectrocolorimeter and the pigmentation, vascularity and pliability by the Vancouver Scar Scale (VSS). Pain and pruritus were recorded by means of Visual Analogue Scale (VAS). Monthly assessments were performed throughout the five-month intervention period.

Results demonstrated significant differences in scar conditions between high and low pressure therapy. Static pressure of at least 20mmHg was found to accelerate scar remodeling process with improved clinical presentations. It also demonstrated superior effect on reducing scar thickness. The most apparent improvement was achieved at the first month of intervention. Low pressure group also showed significant decrease in thickness. The decrease was lower and steadily decayed over time as compared to the high pressure group. Significant decrease in scar color, in terms of redness by the spectrocolorimeter, was also recorded. No significant differences were detected in pain and pruritus between the groups. Some subjects, however, reported increased pruritus especially during hot weather probably because of reduced ventilation of pressure padding.

The various scar responses under different pressure magnitudes in both *in-vitro* study and the clinical trial indicated the importance of monitoring the interface pressure onto the scar tissue. These findings suggested that pressure garments with at least 20mmHg static pressure tend to accelerate scar maturation. Careful monitoring of interface pressure should be done to ensure better therapeutic effect in hypertrophic scar management.

PUBLICATIONS ARISING

Paper Publications

Lai, H.Y. C., & Li-Tsang, W.P.C. (2009). *Validation of the Pliance-X system in measuring interface pressure generated by pressure garment. Burns, 35,* 845 - 51 .

Li-Tsang, W.P.C., Lai, H.Y.C., Wang, S.J., Huang, L., Burd, A. & Cheung, K.K.A. (pending submission to *Burns* for review). *Effects of minute mechanical pressure on the fibroblasts-to-myofibroblasts differentiation in cultured human hypertrophic scars.*

Lai, H.Y.C., & Li-Tsang, W.P.C. (pending submission to *Plastic and Reconstructive Surgery* for review). *Different pressure interventions on hypertrophic scar remodeling among the Chinese Population.*

Conference Publications

October 2008 — Li-Tsang, W.P.C., Yu, W.W.M., & Lai H.Y.C. (2008). *Fabric property analysis for pressure garment.* The 6th Pan-Pacific Conference on Rehabilitation, Hong Kong, China. (awarded with merit)

September 2008 — Lai, H.Y.C., & Li-Tsang, W.P.C. (2008). *Interface pressure measurement under pressure garment – a validation study.* The 14th Congress of the International Society for Burn Injuries, Montreal, Canada.

May 2008 — Lai, H.Y.C., Cheung, K.K.A., Burd, A., Huang, L.L., & Li-Tsang, W.P.C. (2008). *Inhibition on the growth of hypertrophic scar fibroblasts upon pressure loading – preliminary report.* The 2nd International Burns and Wound Healing Symposium, Hong Kong, China.

April 2008 — Lai, H.Y.C., & Li-Tsang, W.P.C. (2008). *Evaluation of hypertrophic scars under pressures.* The 1st Workshop on Biomedical Ultrasonic Solutions, Hong Kong, China.

June 2007 — Li-Tsang W.P.C., Guo X., Liu P.Y.K., Lam W. L. T., Choi K. K. P, & Lai H.Y.C. (2007). *Effect of pressure on human burn scar tissue – an in vitro experimental study.* The 4th Asia Pacific Occupational Therapy Congress, Hong Kong, China.

June 2007 — Li-Tsang W.P.C., Guo X., Liu, P.Y.K., Lam, W.L.T., Choi, K.K.P., & Lai, H.Y.C. (2007). *Mechanical pressure on human burn scar tissue – an in-vitro experimental study.* The 6th Asia Pacific Burns Congress, Seoul, Korea.

February 2007 — Li-Tsang, W.P.C., Guo X., Liu, P.Y.K., Lam, W. L.K., Choi, K.K.P., & Lai, H.Y.C. (2007). *Effectiveness of mechanical pressure on human burn scar tissue – an in-vitro experimental study.* The 20th Annual Congress Hong Kong Society for Surgery of the Hand, Hong Kong, China.

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Hypertrophic scar is a dermal fibroproliferative disorder following dermal skin trauma. This skin abnormality is characterized as raised, rigid and erythematous appearance associated with pain and pruritus (Armour, Scott, & Tredget, 2007; Devlin-Rooney, & James, 2005; Van den Kerchove et al, 2005). Hypertrophic scar will also lead to severe cosmetic disfigurement and functional impairment often results if scar contracture develops over joints. Pressure therapy has been the cornerstone of scar management since the early 1970s because of its non-invasive characteristics. Results claimed in clinical settings indicate a superior treatment effect, however, most researches have failed to demonstrate statistically significant improvements in hypertrophic scarring (Stal, Cole, & Hollier, 2008). Empirical observations of prescribed pressures in current practice, due to the absence of a precise pressure measurement apparatus, have been challenged for the therapy. Lacking of objective scar assessment tools is also a defect, though pressure therapy has been widely used for scar control over three decades, the optimal pressure dose is still inconclusive. It is apparent that the relationships of pressure and hypertrophic scar remodeling have to be investigated and the optimal pressure should be well-defined before supporting the use of pressure therapy.

1.1 AIM OF THE STUDY

Considering the pitfalls of current practice, this research study aimed at investigating the relationship between pressure and scar characteristics using in-vitro and clinical trial. This study was divided into three phases: a) validation study of an interface pressure measurement system; b) *in-vitro* model to study biological activities of hypertrophic scar fibroblasts under pressure; and c) clinical trial on pressure therapy

to hypertrophic scar. The results from the study would hopefully shed light to clinicians on the management of scar problems with evidences.

1.2 OBJECTIVES OF THE STUDY

The objectives of this study were:

- to validate the Pliance X System for measurement of interface pressure between skin and pressure garment;
- to reveal the correlation of pressure dosage onto the fibroblast extracted from scar tissue;
- to compare the effect of high pressure versus low pressure on hypertrophic scar; and
- to find out the optimal range of pressure for effective scar control.

1.3 OUTLINE OF THE THESIS

As a preliminary step towards these objectives, a review was conducted in Chapter II on the pathogenesis, histobiological characteristics and impacts of hypertrophic scar. Descriptions of therapeutic effects and mechanisms of pressure therapy and the problems of lacking quantitative measurements in previous studies were highlighted.

Chapter III depicts a pressure monitoring system to measure and record pressures at the scar and dressing interface. In particular, the criteria for an ideal interface pressure sensor were discussed and the unsuitability of existing devices was addressed. The capacitive pressure transducer was testified by a series of laboratory experiments with emphasis on its suitability for use in a clinical environment.

Chapter IV describes the biological activities of hypertrophic scar fibroblasts. An *in-vitro* study was performed to examine the biological reactions of hypertrophic scar fibroblasts towards pressure magnitudes. A clinical trial with double-blinded design was carried out in Chapter V to investigate the pressure effects on hypertrophic scarring. The interface pressure and the scar characteristics were documented in a quantitative approach.

The conclusion of the investigation was presented in Chapter VI. Limitations of the study were also highlighted. Further studies in certain areas for a better understanding of the scar remodeling mechanisms were recommended.

CHAPTER II

LITERATURE REVIEW

2.1 INTRODUCTION

The development of hypertrophic scar is common after dermal skin injury. Pressure therapy has been one of the most widely used modalities to prevent and control hypertrophic scar since the early 1970s. The performance of pressure therapy in the literature, however, has been inconsistent and the optimal dosage of pressure remains controversial. As the background of the study, a literature review on the hypertrophic scarring was conducted. The therapeutic effects and mechanisms of pressure therapy in scar control would also be presented. The pitfalls of pressure therapy would be highlighted in the later part of this chapter.

2.2 ANATOMY OF HUMAN SKIN

Skin is the largest organ in a human being with surface area approximately 1.5 to 1.7m^2 and thickness varying from 2 to 3mm over most areas of body (Falkel, 1994; Johnstone, Farley, & Hendry, 2005). Normal skin, composed of two major layers namely epidermis and dermis, acts as an important protective barrier which is crucial to survival. As shown in Figure 2.1, the outermost epidermis consists of mainly epithelial cells which are continually dividing and migrating outwards to replace the lost surface cells (Devlin-Rooney, & James, 2005). This layer is replaced every three to four weeks through continuous cell division in the basal cell later (Harvey, 2005). The keratin, formed when epithelial cells migrate, is an effective barrier to environmental hazards such as infection and excess water evaporation. The other major layer, the dermis, which is the largest portion of the skin, is responsible for skin durability and flexibility. This layer cushions the body from stress and strain and harbors many nerve endings that provide touch and heat sensation. The dermis

houses the epidermis appendages for instance hair follicles, sweat glands and sebaceous glands and the lymphatic vessels and blood vessels. The dermis takes a major role in reforming the outer epidermis and thus if an injury destroys the dermis, its natural wound healing process can be disturbed. Table 2.1 highlights the skin injury depth and corresponding healing time together with occurrence of abnormal scar.

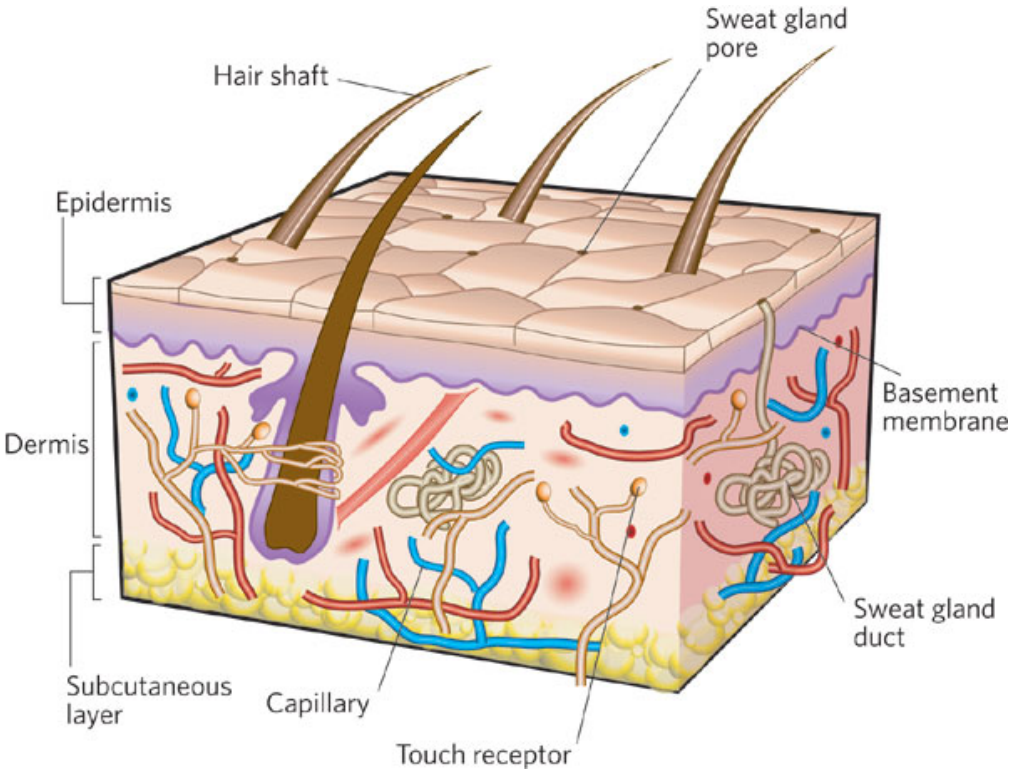


Figure 2.1 Human skin structure (MacNeil, 2007)

Table 2.1 Depth of skin injury

Depth of injury	Portion of skin destroyed	Healing time	Occurrence of abnormal scar
1st degree (e.g. sunburn)	epidermis (outer surface)	less than 7 days	unlikely
Superficial 2nd degree	➢ epidermis ➢ no more than the upper 3rd of dermis	rapid healing occurs from 7 to 14 days	uncommon
Mid 2nd degree	➢ epidermis ➢ about half of dermis	healing is slower (2 to 4 weeks)	➢ minimal to no scarring if healed < 2 weeks ➢ scarring occur if beyond 3 weeks
Deep 2nd degree	➢ most of skin is destroyed except for small amount of remaining dermis	healing requires 4 to 10 weeks or longer	dense scarring is common
3rd degree	➢ completely destroyed leaving no cells to repopulate and heal	usually require excision and skin graft	-

2.3 FORMATION OF HYPERTROPHIC SCAR

2.3.1 Hypertrophic Scar and its Prevalence

Hypertrophic scar formation is a dermal fibro-proliferative disorder that occurs as a prevalent sequel of trauma which involves deep reticular dermis, such as in deep partial thickness burn (Armour, Scott, & Tredget, 2007; Beldon, 2000; Devlin-Rooney, & James, 2005; Van den Kerckhove, et al., 2005). It is characterized as having raised, rigid and erythematous appearance associated with pain and pruritus (Azad, Gerrish, & Dziewulski, 2000; Beldon, 2000; Kawecki, et al., 2008). Its color, rigidity, time of onset, duration and time of involution vary among patients (Shejbal, et al., 2004; Wilhelmi, 2008)

This skin abnormality is unique to humans (Al-Attar, et al., 2006; Sullivan, et al., 2001) subsequent to destructions over large areas of skin to the level of the deep reticular dermis. The reparatory process continues pathologically, resulting in excessive tissue formation. Histologically, an excessive deposition of collagen of an exaggerated wound healing contributes to the growth of atypical scarring; the exact etiology, however, remains elusive. Delayed wound closure and a prolonged inflammatory stage of healing, with either pathological persistence of wound healing signals or a failure of the appropriate down-regulation of wound healing cells, as well as the presence and contents of granulation tissue have been linked to eventual scar formation (Cohen, & McCoy, 1980; Deitch, et al., 1983; Teich-Alasis, & Angela, 1982). The formation of scar not only resulted in severe cosmetic disfigurement, scar contracture developed over joints could reduce patient's functional performance in daily living (Haverstock, 2001; Reid, et al., 1987).

Hypertrophic scar occurs in persons of any age (Wilhelmi, 2008) with equal distribution in both gender (Li-Tsang, Lau, & Chan, 2005). Hypertrophic scar formation has been demonstrated in people of all races, however, a relatively higher incidence rate has been reported among the Africans and Asians (Li-Tsang, Lau, & Chan, 2005; Shaffer, Taylor, & Cook-Bolden, 2002) with the rate being three-fold of that in Caucasians (Alhady, & Sivanantharajah, 1969). It has been suggested that the prevalence rate of hypertrophic scar development in the Caucasian population is 15 to 38% (Deitch, et al., 1983; Elliot, Cory-Pearce, & Rees, 1985) while that in Chinese population can be as high as 75% (Li-Tsang, Lau, & Chan, 2005). Depth of insult, location of injury and time of wound healing are also the determinants of the incidence of hypertrophic scar (Beldon, 1999; 2003; Devlin-Rooney, & James, 2005; Munro, 1995).

2.3.2 Differences between Keloid and Hypertrophic Scar

Tremendous studies have been reported to differentiate hypertrophic scar from keloid as inappropriate management can lead to recurrence and enlargement of the scar. Clinical distinctions have often been applied to discern hypertrophic scar from keloid (Alster & Tanzi, 2003; Urioste, et al., 1999; Wolfram, et al., 2009). Though hypertrophic scar and keloid share the similar characteristics of increased thickness, redness and rigidity the former remains within the confines of the original lesion whereas keloid extends beyond the margins of the skin incision (Atiyeh, Costagliola, & Hayek, 2005; Devlin-Roovey & James, 2005; Robles & Berg, 2007; Shaffer, et al., 2002). Besides, hypertrophic scar is eventually triggered to begin the remodeling phase (Bettinger, et al., 1996; Younai, et al., 1994) and generally regress spontaneously (Wolfram, et al., 2009) whilst keloid continues to grow over time,

sometimes to large pendulous growths without showing tendency towards regression. Figure 2.2 shows the diagram of the development of different scars after wound healing. Hypertrophic scar usually occurs within weeks following the insult while keloid may manifest months to years with an average of 30.4 months (Cosman, et al., 1961), after the initial lesion (Niessen, et al., 1999; Datubo-Brown, 1990). Furthermore, the recurrence rate with surgery for keloid is much higher (Burd & Huang, 2005) than the hypertrophic scar with range from 45 – 100% (Butler, Longaker, & Yang, 2008; Newsome, & Tandon, 2009).

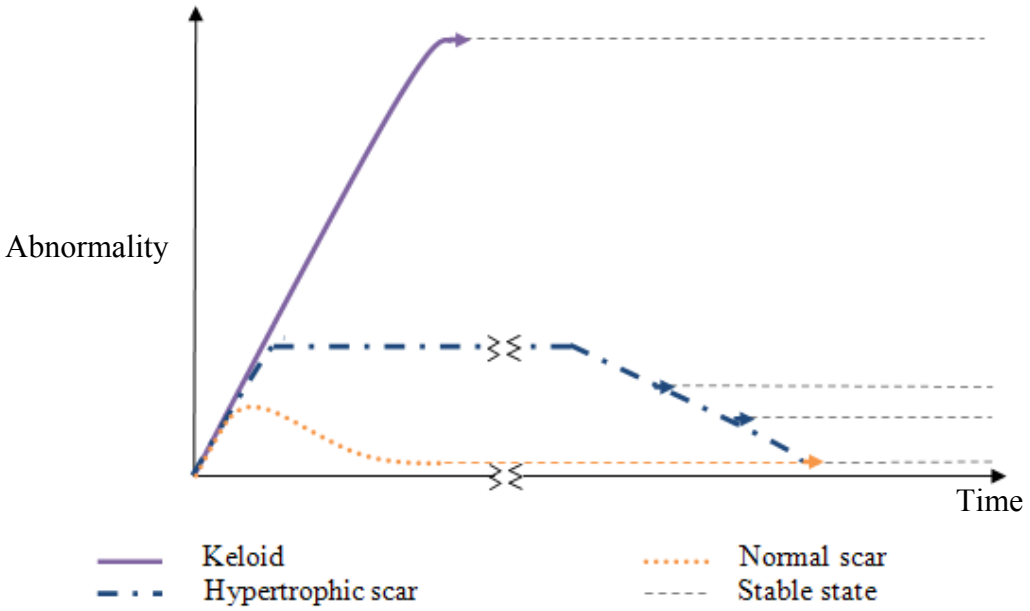


Figure 2.2. The development of two forms of aberrant scars and normal scar after wound closure (simplified figure adapted from Burd & Huang, 2005)

Several studies have examined the nature of hypertrophic scar and keloid at the pathophysiological level. Fibroblast activities have been investigated to delineate the molecular basis of excessive fibrosis resulting in the anomalous scar formations. Both lesions demonstrate overproduction of multiple fibroblast proteins and irregular balance between fibroblast proliferation and cell apoptosis (Akasaka, et al., 2001; Luo, et al., 2001). Hypertrophic scar fibroblasts respond relatively less vigorous to growth factors with a modest increase in collagen production. Collagen synthesis in hypertrophic scar can be four times as much as that in normal skin but varies with age, race, depth and site of the lesion (English & Shenefelt, 1999) whilst collagen synthesisin keloid is about 20 times greater than that in hypertrophic scar (Rockell, et al., 1989). Fibroblasts extracted from keloid, by contrast, show a greater capacity to proliferate and produce high levels of collagen, elastin, fibronectin and proteoglycan (Russell, et al., 1995; Kischer & Hendrix, 1983; Robles & Berg, 2007).

The differences between the two lesions have further been verified with histological evidence. Both hypertrophic scar and keloid demonstrate increased collagen deposition as compared to normal dermis. Distinct collagen bundles running parallel to the epidermis are found in normal skin, however, in hypertrophic scar, the collagen bundles, primarily type III, are flatter, less demarcated oriented randomly in whorl-like pattern with abundant nodules. In contrast, the keloid is composed of disorganized, pale-staining hypo-cellular collagen bundles in loose, haphazardly oriented sheets with no distinct nodules (Ehrlich, et al., 1994). The contractile characteristic of myofibroblasts plays a crucial role in wound healing, exist only in hypertrophic scar tissue instead of keloid (Su, et al., 1998). Overabundant of myofibroblasts may result in scar contracture which partially explains the scenario

that scar contracture often associates with hypertrophic scar but not keloid (Burd & Huang, 2005). This current study is focused on the responses of hypertrophic scar, which is commonly occurred among the Chinese population.

2.3.3 Pathogenesis of Hypertrophic Scar

Hypertrophic scar is an aberration of the normal healing process (Beldon, 2000). Trauma to any tissue automatically triggers an intricate, yet coordinated, cascade of cellular physiological events which rapidly helps to eradicate the area of damaged cells and other unwanted elements, to protect viable tissue and to reconstitute the damaged area (Fujiwara, Muragaki, & Ooshima, 2005; Ladin, Garner, & Smith, 1995; Niessen, et al., 1999). The complexity of cellular processes, nonetheless, offers much vulnerability that could result in excessive scarring (Scott, et al., 2005; Slemp, & Kirschner, 2006; Tsujita-Kyutoku, et al., 2005).

Wound repair involves several well-orchestrated events (Hess, 2005; Strecker-McGraw, Jones, & Baer, 2007), as shown in Figure 2.3, that could be arbitrarily separated into four overlapping phases: (i) vascular response, (ii) inflammatory response, (iii) proliferation and (iv) wound contraction and remodeling (Alster, & Tanzi, 2003; Armour, Scott, & Tredget, 2007; Devlin-Rooney, & James, 2005; Li, Chen, & Kirsner, 2007; Meenakshi, et al., 2005; Monaco, & Lawrence, 2003; Shejbal, et al., 2004; Singer, & Clark, 1999; Strecker-McGraw, Jones, & Baer, 2007). Any abnormal responses in any of the later three phases could induce the hypertrophic scar formation.

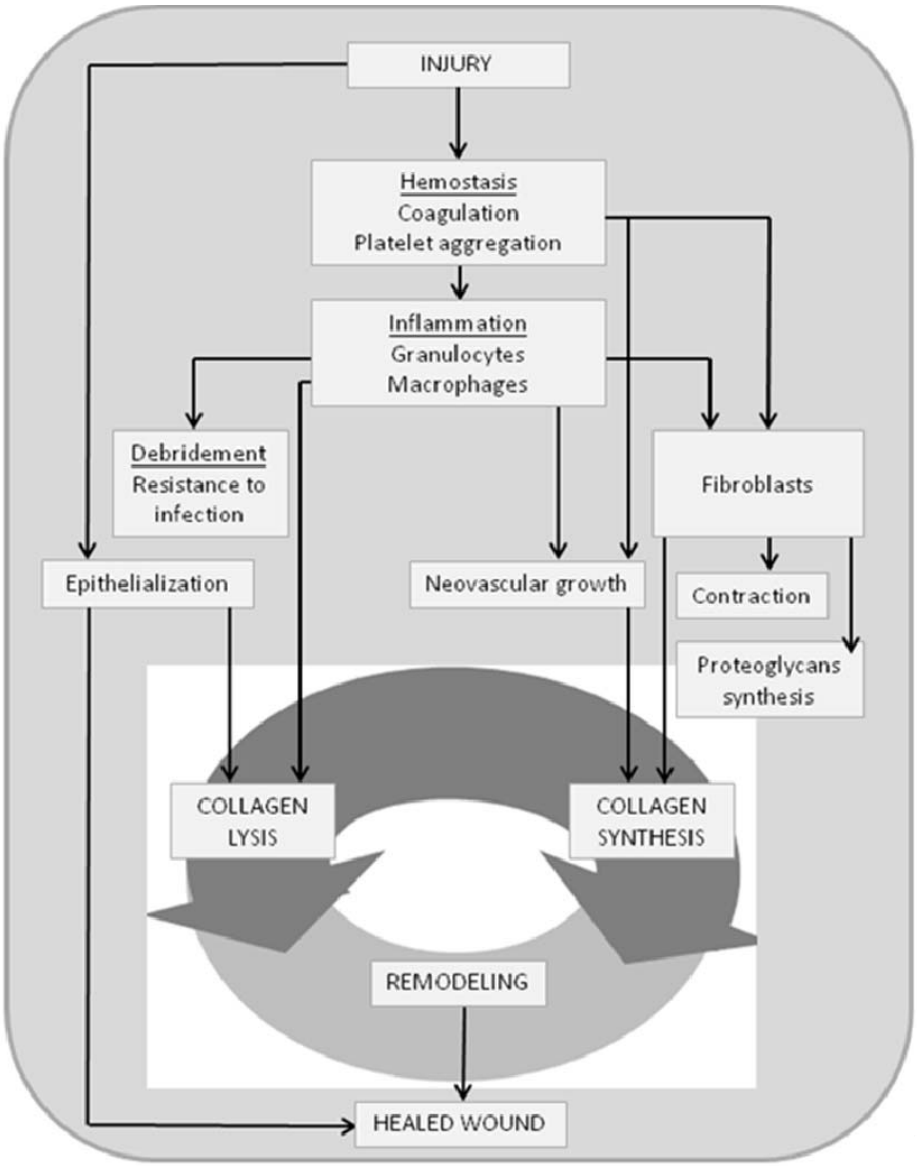


Figure 2.3 Cascade of wound-healing events (Hess, 2005)

(i) Vascular Response

Tissue trauma will cause blood vessel disruption and bleeding. Extra-vasation of blood components stimulates the activation of the endothelial cells with platelet aggregation and clot cascade begins, which spontaneously leads to homeostasis (Kirsner, & Eaglstein, 1993). Within minutes of insult, surrounding blood vessels constrict to reduce the extent of hemorrhage.

(ii) Inflammatory Response

Platelet degranulation is activated in this phase for the release and trigger of an array of potent cytokines, including PDGF (Kohler, & Lipton, 1974; Ross, Glonset, & Kariya, 1974), insulin like growth factor-I (IGF-I) (Karey, & Sirkasku, 1989), epidermal growth factor (EGF) (Oka, & Ort, 1983) and transforming growth factor (TGF-β) (Assoian, Komoriya, & Meyer, 1983), which act as chemotactic agents for the recruitment of inflammatory cells. This phase is marked with higher vascular permeability and migration of inflammatory cells. The skin becomes red, hot and swollen with the production of exudates. Pain may be reported in this phase.

Prolonged inflammatory stage, beyond 21 days (Cubison, Pape, & Parkhouse, 2006), exaggerates the inflammatory responses of healing and increases the activities of fibrogenic cytokines such as TGF-β and IGF-I, thereby increasing the risk of development of pathological scar (Meenakshi, et al., 2005).

(iii) Proliferation

The proliferative phase starts with the deposition of fibrin and fibrinogen matrix together with the activation and turnover of local fibroblasts. It begins approximately from day five post-injury through day 21 in acute wound (Harvey, 2005) depending on the size and depth of wound. After migration of activated fibroblasts over the fibrin matrix, fibroblasts increase collagen synthesis and secretion of extracellular matrix products. Driven by low oxygen and high lactate concentrations (Mulvaney, & Harrington, 1994), the process of angiogenesis occurs concurrently with fibroblast proliferation (Li, Chen, & Kirsner, 2007).

(iv) Wound Contraction and Remodeling

Wound contraction normally occurs soon after wounding and peaks at two weeks and the degree of wound contraction varies with the wound depth. Myofibroblasts, phenotype progressively modulated from fibroblasts during the granulation tissue formation, play a predominant role in this contractile process (Darby, & Hewitson, 2007), which requires cell-cell linkage and cell-matrix communication (Mudera, et al., 2002). Myofibroblast pseudopods extend, together with the binding of cytoplasmic actin to the extracellular fibronectin, retract and attach to collagen fibers and then to the adjacent cells to produce wound contraction. The cells within the wound align along the lines of contraction, and wound contracts in directions of skin tension lines. However, if prolonged wound contraction occurs, disfigurement and loss of function might be resulted (Hinz, 2005).

Once an abundant collagen matrix is fashioned, the fibroblasts stop secreting collagen and the fibroblast-rich granulation tissue, type III collagen predominated, is replaced by a relatively acellular scar with type I collagen (Welch, Odland, & Clark,

1990). Simultaneous apoptosis of cells in the wound, triggered by unidentified signals (Desmouliere, et al., 1995), and the collagen decay, controlled by several proteolytic enzymes termed matrix metalloproteinases from macrophages, epidermal cells, endothelial cells along with fibroblasts (Mignatti, et al., 1996), result in decreasing nodularity and flattening of the scar (Alster, & West, 1997). Since the reduced cellular activity over the wound site, the scar loses its red appearance with the apoptosis of blood vessels (Greenhalgh, 1998). Collagen remodeling, which refers to the deposition of the matrix, commonly starts after two weeks when the process of degradation gradually becomes superior to the process of synthesis. Equilibrium between biosynthesis and matrix degradation is essential for optimal wound healing. Dysregulation of the mentioned processes, either excessive synthesis of collagen or deficient matrix degradation and remodeling might cause fibrotic scarring (Nedelec, Tredget, & Ghahary, 1996; Raghow, 1994).

2.3.4 Etiology of Hypertrophic Scarring

Even if the exact pathophysiologic mechanisms remain epigamic, various factors have been identified for clinicians to predict the risk of hypertrophic scar formation (Alster, & Tanzi, 2003). Certain parts of the body such as pre-sternal area, upper back, anterior chest wall and upper arms, are predisposed to develop peculiar scar (Bayat, McGrouther, & Ferguson, 2003; Devlin-Roovey, & James, 2005). These areas tend to have a relatively high tension, which has been considered as a factor of development of hypertrophic scar (Ketchum, Cohen, & Masters, 1974; Kischer, 1975) since higher tension overstretches the wound area, thus encourages excessive scarring.

The time required for wound closure and wound healing may act as a good indicator for hypertrophic scar formation. Sustained inflammatory or proliferation stages of wound healing have been proven to increase the susceptibility of hypertrophic scar (Singer, & Clark, 2004). The depth of lesion signifies the tendency of hypertrophic scar as it interferes with wound healing process as well (Cohen, & McCoy, 1980). People with darker pigmentation are found to be more prone to develop an anomalous scar (Wolfram et al., 2009). Additional research is warranted to fully elucidate the validity of the possible etiologic factors.

2.3.5 Physical and Histobiological Characteristics of Hypertrophic Scar

Scar hypertrophy differs from normal tissue by its hyper-cellularity and hyper-vascularity (Rayner, 2000; Rockwell, Cohen, & Ehrlich, 1989). Intense erythema is usually the first sign, followed by the raised lesion with pain or pruritus. The scar redness, due to the extensive formation of micro-vasculature with amplified amount of blood flux (Amadeu, et al., 2003; Beldon, 1999, 2000) during the wound healing process, has been reflected by Laser Doppler Flowmetry as double of that normal skin (Leung, et al., 1989). Presence of erythema indicates that active scar turnover is still present. Apart from the markedly increased vasculature, the presence of densely populated fibroblasts and collagen along with the myofibroblasts account for the gross features of a hypertrophic scar. Given ongoing process of excessive scar deposition, the lesion continues to increase in thickness and pliability with its stiffness reported to be eight times higher than that of the normal tissue (Clark, Cheng, & Leung, 1996). The hypertrophic scar begins to decrease with time as collagen lysis begins to exceed the rate of deposition. It decreases as the

inflammatory process diminishes. Ranging from months to years, softening and flattening of the scar could be seen along with loss of scar hyperaemia.

Hyper-cellularity of Hypertrophic Scar

Divergences in histological presentation have been discovered between hypertrophic scar and normal skin. Fibroblasts in hypertrophic scar are found in increased numbers, leading to increased collagen deposition as well as more matrixes (Armour, Scott, & Tredget, 2007). These fibroblasts are more sensitive to growth factors than those in the normal skin. Microscopically, the normal loose tridimensional collagen-fiber arrangement of the dermis is replaced by a disarray of collagen fibrils, which twists and turns to subsequently form the compact whorl-like and nodular arrangement in hypertrophic scar tissue (Bailey, et al., 1975; Baur, et al., 1976; Ehrlich, et al., 1994; Kischer, & Brody, 1981; Linares, et al., 1972). In addition, the diameter of the collagen filaments is about half of the normal skin (Tulley, 1980). Presence of myofibroblasts is unique to hypertrophic scar tissue, and is never found in other scar tissues. The fibroblast content gradually diminishes during scar maturation by apoptosis and the nodules are found absent in an atrophic scar.

Biological analysis also helps differentiate hypertrophy from normal dermis (Thomas, & Critchley, 2006). Significantly different rates of collagen synthesis and degradation have been reported between hypertrophic and normal scars. Considerably higher level of collagen synthesis, with the facilitation of proline hydroxylase, has been detected in hypertrophic scar (Cohen, et al., 1972; Cohen, Keiser, & Sjoerdsma, 1971). It is interesting to note that despite the four-fold increases in collagenase activity for collagen degradation in hypertrophic scar as

compared to adjacent skin (Craig, 1983; McCoy, & Cohen, 1982), it is still insufficient to balance out the disproportionate raise in collagen synthesis (Cohen, et al., 1972; Cohen, Keiser, & Sjoerdsma, 1971). The ultimate disequilibrium between collagen synthesis and catabolism apparently results in the overabundant collagen deposition in hypertrophic scar. A decrease in interferons and cytokines that downregulate collagen and matrix synthesis is also noted (Nedelec, et al., 2001). This abnormality leads to less collagen lysis and matrix degradation with remodeling. Hypertrophic scar, in addition, exhibits a higher level of TGF- β1 and 2 which are believed to promote fibrosis and scar formation (Rumalla, & Borah, 2001). Decorin, interacting with various cytokines such as TGF-β1 and thinning of fibrils via influencing collagen fibillogenesis (Burd, & Huang, 2005), was also demonstrated to have reduced density in active hypertrophic scar fibroblasts (Scott, et al., 1998). The decorin, however, was found increasing along the scar maturation process in another study (Sayani, et al., 2000).

Hyper-vascularity of Hypertrophic Scar

A review of the literature reveals conflicting views of the vascularization pattern in pathological scarring. Rich vascularization (Beldon 1999, 2000) and noticeably increased blood flow (Ehrlich, & Kelley, 1992; Lin, & Li-Tsang, 2008) have been identified in hypertrophic scar. Amadeu and his colleagues (2003) have echoed with these findings by demonstrating a higher amount of vessels in both the papillary and reticular dermis of hypertrophic scar compared with normal skin. The vessels of hypertrophic scar, as well, are more dilated than those of normal skin, increasing oxygen and nutritional supply with rich vasculature that supports the exuberant growth of scar tissue. This is, however, disproved in some studies that reported no

distinct vascular difference between hypertrophic and normal scars. Researchers even postulated the effect of hypoxia, an upshot of significant microvascular occlusion, partially contributes to the deviant scar growth (Hunt, et al., 1978; Kischer, & Shetlar, 1979; Kischer, Shetlar, & Chvapil, 1982; Kischer, Thies, & Chvapil, 1982; Sloan, et al., 1978).

Most of the microvessels discovered in the granulation tissue of the abnormal scar are partially or completely occluded as compared with the normal tissue, which reduces the blood supply to the scar area (Kischer, & Shetlar, 1979). These occluded microvessels are induced by an excess of endothelial cells (Kischer, 1992). The existence of a relatively hypoxic micro-environment has also been documented in various surgical studies in the wound bed (Krighton, & Fiegel, 1991; Steinbrech, et al., 1999). It is speculated that since the fibroblasts are known to be anaerobes and metabolize satisfactorily, or even stimulated, in low oxygen environments, hypoxic environment stimulates their proliferation and collagen secretion and eventually provokes the formation of bulky scar tissue (Detmar, et al., 1997; Kischer, Thies, & Chvapil, 1982). To explain the spontaneous maturation of hypertrophic scar, Kischer (1992) has further suggested that with the continuing loss of microvessels within the nodules, the level of hypoxia gradually increases until the anaerobic fibroblasts could no longer function adequately with the high oxygen tension. This excessive loss of oxygen, nearly anoxic environment, and nutrition triggers the degeneration and apoptosis of the fibroblasts which ultimately sparkle off the scar maturation process. This phenomenon, however, sounds contradictory to the scar clinical outlook of redness and the doubled blood flux reflected by Laser Doppler Flowmetry.

2.3.6 Impact of Hypertrophic Scar

Extensive scarring could have substantial physical and psychological impacts on patients (Kawecki, et al., 2008). Scar hypertrophy interrupts the natural skin functions including serving as a barrier to foreigners, providing sensory information and regulating body temperature and metabolism. As highlighted in Table 2.2, continuous scar deposition results in a raised, hyper-pruritic wound that produces irritations (Thomas, & Critchley, 2006). Persistent and severe itching leads to scratching which would re-traumatize the wound, cause skin breakdown and eventually attract infection. Pain with any scar movement retards activities. Prolonged immobilization, plus the strong contractile feature of scar tissue (Tejero-Trujeque, 2001), might bring the joint surface into stiffness or at worst deformity and contracture (Jensen, & Parshley, 1984). Scar contracture might impede the range of movement if the lesion is located over a joint. With reduced range of motion caused by a stiffened or deformed joint, patient's functional performance will be greatly affected (Haverstock, 2001).

Patients with disfiguring conditions might experience psychosocial difficulties which result in anxiety, sleep disturbance, depression and social avoidance (Leblebici, et al., 2007; Rumsey, & White, 2003; The Scar Information Service, 2007). Other psychosocial sequelae, including development of post-traumatic stress reactions, loss of self esteem, and stigmatization, could lead to diminished quality of life, thus should not be underestimated (Bock, et al., 2006; Carr, Harris, & James, 2000; Douglas, & Way, 2007).

Table 2.2 Physiological characteristics associated with hypertrophic scarring

Presentation	Cause	Impact on patient
Hypo / hyperpigmentation	Unknown	Aesthetically displeasing
Reddening of skin	Changes in vascularity	Aesthetically displeasing The intensity of redness is commonly used as a measure of scar maturity
Thickening of scar and with rough surface	Unnatural deposition of collagen with disorientated bundles	Aesthetically displeasing The reduction of scar thickness is commonly an indicator for scar maturity
Pain	This could be caused by the increased number of C-nerve fibers within a scar	Patient discomfort and stress
Pruritus	Lack of skin lubrication	Patient discomfort and stress Scratching might cause skin breakdown which eventually further stimulates the cell activities
Change in skin elasticity and strength	Scars not containing elastin	Reduced functionality
Contracture	Increasing number of myofibroblasts	Reduced functionality and quality of life

2.4 TREATMENT FOR HYPERTROPHIC SCAR

Excessive fibrosis could be disfiguring and life disturbing. Substantial effort has been devoted to developing rational treatment modalities for hypertrophic scarring. Numerous studies have been conducted to investigate the process of scar formation and wound healing in recent decades. The increasing knowledge has led to the introduction of new treatments as well as to the better understanding of the mechanisms of traditional modalities. All existing treatment approaches of scarring aim to accelerate the process of scar maturity and thereby decreasing pruritus and improving the cosmetic outcome of the scar. Regardless of the current medical progression, there is still no universally accepted treatment modality that results in complete and permanent hypertrophic scar amelioration (Alster, & Tanzi, 2003). Several therapeutic options have been adopted and regarded as satisfactory methods in managing scar hypertrophy, with various degrees of clinical and scientific support. These interventions include surgical excision, radiation, cryotherapy, intralesional injections of corticosteroid, interferon and fluorouracil and pulsed-dye laser treatment (Robles, & Berg, 2007), yet, these techniques are criticized for their invasive nature and considerable side effects, in particular, extreme pain, hyper- or hypo-pigmentation and high recurrence rate. On the other hand, hypertrophic scar has also been proven responsive to a conservative approach named pressure therapy. Given its non-invasive characteristic and desirable treatment effect with fewer complications when compared to the more invasive treatments mentioned above (Rockwell, Cohen, & Ehrlich, 1989), pressure therapy has been widely accepted since 1970s (Staley, & Richard, 1997; Zurada, Kriegel, & Davis, 2006).

2.4.1 Pressure Therapy

Encouraging results of pressure therapy on abnormal scarring were documented by Herman Lawrence of Melbourne as early as 1860 (Ketchum, Cohen, & Masters, 1974; Yan, 2000), and his belief has been endorsed by various scholars in the later years (Cronin, 1961; Fujimori, Hiramoto, & Ofuji, 1968). However, not until late 1960, were more scientific studies conducted to investigate the effects of pressure therapy on scar hypertrophy with mostly positive results (Atiyeh, 2007; Mustoe, et al., 2002; Robertson, et al., 1980; Shih, Waltzman, & Evans, 2007).

Pressure therapy has become a standard protocol for scar management in many centers and is generally accepted by clinical practitioners as the best non-invasive means for preventing and ameliorating hypertrophic scarring (Cheng, et al., 1983; Reno, et al., 2003; Van den Kerckhove, et al., 2005). For instance, a local retrospective survey (Cheng, et al., 1999) shows that over 90% of the patients with hypertrophic scar were referred to the Occupational Therapy Department for pressure therapy. Pressure therapy is currently applied through the elastic garment, which is fabricated in a calculated dimensional tubular form with fabric composites of Lycra and Spandex content (Figure 2.4). Padding materials might be inserted underneath the garment if more localized pressure is needed.

Figure 2.4 Samples of pressure garment (sock and gloves)

Early intervention, preferably within two weeks after the burn wound or the skin-grafted area has healed, is essential for optimal results (Cheng, et al., 1983). To achieve the maximum benefit, compression should be applied continuously for 18 to 24 hours per day with short rest periods for meals and hygienic measures (Puzey, 2002; Staley, & Richard, 1997; Su, et al., 1998). Pressure might be discontinued only after the scar becomes atrophic (Baur, et al., 1976), but normally requires at least six months from wound closure (Leung, et al., 1984; Linares, Larson, & Willis-Galstaun, 1993).

Even though pressure therapy has been advocated as a non-invasive but effective means with few complications (Li-Tsang, et al., 2004), it has several drawbacks (Zurada, Kriegel, & Davis, 2006). Compression is effective only while the scar is active (Burke, et al., 1996), and has been reported to be less effective after six months of treatment (Staley, & Richard, 1997). Discomfort from heat and perspiration, particularly in warm weather, affects the compliance of wear among patients (Brown, 2001; Macintyre, & Baird, 2006; Stewart, et al., 2000). Not only the pressure dressing reportedly being uncomfortable and cumbersome as complained by patients, but the easy breakdown of skin due to friction or undue dosage of pressure (Carr-Collins, 1992), limit their compliance to the lengthy wear regime (Gallagher, et al., 1992; Johnson, et al., 1994).

2.4.2 Therapeutic Effects of Pressure Therapy on Hypertrophic Scar

As mentioned above, pressure therapy has been a common modality for preventing and treating hypertrophic scar. Many clinical observations have shown that persistent

and adequate pressure could produce favorable results in both the physical and histological features of the scar (Cheng, et al., 2001; Deitch, et al., 1983; Giele, et al., 1995; Haq, & Haq, 1990). Pressure therapy, as a first-line treatment, is recommended for patients with wound healed after 14 to 21 days for scar prevention (Deitch, et al., 1983). A success rate of 60 to 85% has been reported (Deitch, et al., 1983; Haq, & Haq, 1990; Reno, et al., 2003) with no evidence of recurrence in patients with good compliance. It is assumed that compression could hasten scar maturation and reduce the incidence of contractures and thereby the need for surgical interventions (Atiyeh, 2007; Larson, et al., 1974; Macintyre, 2007; Williams, Knapp, & Wallen, 1998). Application of pressure also alleviates the itchiness and pain associated with active hypertrophic scar (Kloti, & Pochon, 1982; Leung, & Ng, 1980; Pratt, & West, 1995; Ward, 1991), together with the scar thickness (Groce, et al., 2000; Groce, et al., 2000; Li-Tsang, et al., 2004; Moore, et al., 2000; Tredget, et al., 1997; Van den Kerckhove, et al., 2005) and redness (Van den Kerckhove, et al., 2005).

Despite these positive results, several researchers claimed that their studies were not able to harvest the supporting evidence of pressure therapy. By comparing pressure and non-pressure treated groups through a prospective randomized study, Chang and co-workers (1995) have concluded that pressure therapy does not play a role in the rate of scar maturation of closed burn wounds and the length of hospital stay. Their disproving findings, yet, are based on a relatively subjective measurement named Vancouver Scar Scale and the length of hospital stay is an unusual variable of pressure therapy. Moreover, the pressure dosages in their study were not reported therefore it is difficult to comment on the results. Moore and his research team (2000)

have also challenged the therapeutic effect of pressure. Their research also showed no significant differences in scar pliability, vascularity, thickness and pigmentation between treatment and control groups. Moreover, excessive pressure has been condemned of retarding the dental and skeletal growth of children (Fricke, et al., 1999; Leung, et al., 1984), though researchers have suggested the recovery of growth could pace up after the treatment session (Rappoport, Muller, & Flore-Mir, 2008). The effectiveness of pressure therapy has also been questioned because of its undefined optimal pressure, which is further convoluted by anatomical variations (Giele, et al., 1998; Leung, & Ng, 1980). Furthermore, some recent experimental studies have indicated that uncontrolled pressure would adversely affect certain aspects of the normal physiologic balance (Bourget, et al., 2007; Harumi, et al., 2001). Problems inherent to the fabric materials (Boone, 1995; Macintyre, & Baird, 2006), pressure loss over time (Cheng, et al., 1983; Ng, 1993, 1994) and poor compliance of patients involved with garment usage (Johnson, et al., 1994) are reportedly other factors affecting its treatment efficacy. Addressing the above problems is hence urgently needed in order to advance the therapeutic effects of pressure therapy.

2.4.3 Mechanisms of Pressure Therapy

Although pressure therapy has been so widely used, its underlying mechanisms of action remain hypothetical. Over the years, extensive use of in vitro systems has aided the identification and characterization of many of the components that interact with one another during wound healing process and the understanding of histological changes under pressure therapy. Most studies have investigated the topological and

cytological features of pressure-treated scars by comparing with non-pressure-treated scars, non-hypertrophic scars or normal skin. Up to now, two predominant hypotheses have been introduced to explain the treatment mechanisms: the reduction of blood perfusion and pressure-induced alterations of histobiological structure.

The application of pressure on scar has been assumed to accelerate the wound healing process by compressing patent microvessels (Lin, & Li-Tsang, 2008). It has been first suggested by Kischer and his co-workers (1975) that continuous loss of blood supplies by sustained pressure results in a hypoxic environment, where to such an anoxic level fibroblasts could no longer function well. This undue loss of oxygen and nutrition accelerates the apoptotic process of fibroblasts and the subsequent scar assembly (Hunt, et al., 1978; Jensen, & Parshley, 1984; Kischer, 1992; Kischer, & Shetlar, 1979; Kischer, Shetlar, & Chvapil, 1982; Kischer, Thies, & Chvapil, 1982; Reid, et al., 1987; Sloan, et al., 1978). Another proposed interpretation (Baur, et al., 1976; Cohen, Diegelmann, & Bryant, 1974) is that the decrease in capillary blood flow secondary to the pressure allows diminished delivery of α_2 macroglobulin levels. Since α_2 macroglobulin is known to have inhibitory effect on collagenases for collagen degradation, collagen lysis in the pressure-treated areas is increased with lower level of α_2 macroglobulins.

Compression, on the other hand, has also been postulated to exert its effect by changing scar histological and biochemical features (Jensen, & Parshley, 1984). Distinct morphological differences with realignment of the contorted collagen fibers and reduction of collagen nodules have been discovered in pressure-treated areas (Costa, et al., 1999; Kischer, Shetlar, & Shetlar, 1975; Larson, et al., 1971; Longacre,

1976; Reid, et al., 1987; Shetlar, et al., 1972). Researchers also showed significant pressure-induced apoptotic cell percentage under continuous pressure (Costa, et al., 1999; Reno, et al., 2003). Pressure has been found enhancing scar maturation through intracellular communication and fibroblast matrix remodeling (Swartz, et al., 2001). Apart from the histological reorganization, modulation in scar biochemistry occurs with adequate pressure. A significant decrease in the relative amount of chondroitin sulfate A (Kischer, Shetlar, & Shetlar, 1975; Longacre, 1976; Roques, 2002) and TNF-α secretion (Reno, et al., 2003) was reported to play a key role in hypertrophy regression induced by pressure therapy. Other findings include pressure-induced release of metalloproteinase-9 (Reno, et al., 2002), lL-1β (Reno, et al., 2003), prostaglandin E2 (Ajubi, et al., 1999; Reno, et al., 2001; Saito, et al., 1990; Saito, et al., 1991) and Epilsin (Reno, et al., 2005) which are involved in ECM remodeling in hypertrophic scar. Pressure, furthermore, controls the availability of water in the tissue (Kischer, & Shelter, 1974) and in turn reduces the amount of ground substances being released or produced by mast cells, favoring collagen formation (Baur, et al., 1976). They hence put forward the hypothesis that pressure therapy accelerates the remission phase of the post-injury-repair process.

To date, despite the fact that enormous attention has been paid to explain the mechanisms by which continuous pressure appears prominent in hypertrophic scar management, its exact mechanisms have not been completely understood yet. Research in a retrospective manner possibly limits our extent of knowledge with pressure therapy. Previous studies also fail to document the minimal pressure value required in order to generate the mentioned positive effects. Further studies are warranted to achieve comprehensive understanding of the pressure mechanism.

2.4.4 Optimal Dosage for Pressure Therapy

Though pressure has been utilized for almost four decades, its optimal magnitude remains controversial. There is a general lack of documentation of pressure dose in numerous studies which is likely to hinder the research fundamentally (Atiyeh, 2007; Chang, et al., 1995; Robertson, et al., 1980; Wertheim, et al., 1999; Zurada, Kriegel, & Davis, 2006). Pressure values stated in most studies are merely based on the formula provided by the garment suppliers without considering the influences of tissue variability among human subjects or body areas and/or the properties of the fabric materials. Even though attempts have been made to determine the therapeutic pressure range, it remains augmentative probably due to the unavailability of an accurate and reliable pressure measurement instrument (Ferguson-Pell, Hagisawa, & Bain, 2000; Maklewska, et al., 2007; Mann, et al., 1997; Norman, 2004; Van den Kerckhove, et al., 2007).

2.4.5 Fabric Properties Affecting Interface Pressure

Pressure garment is a major element in pressure therapy; its fabrication is therefore important to successful treatment. The importance of fabric properties has been stressed on clinical effectiveness of pressure garments (Cheng, et al., 1983; Macintyre, Baird, & Weedall, 2004; Macintyre, & Baird, 2006; Maklewska, et al., 2007; Naismith, 1980; Ng, 1990, 1993, 1994; Ng, & Hui, 2001), yet, it is often underestimated in various clinical studies. A wide range of pressure from 10 to 55mmHg among fabrics is obtained despite following a standard construction technique (Macintyre, & Baird, 2005). Immense elastic deterioration varying from 15% to 40% has been detected in some fabric materials in 30 minutes (Ng, 1994) or a few hours (Boone, 1995; Naismith, 1980) after stress test. Pressure loss over time

is an additional pitfall to current practice (Cheng, et al., 1983; Ng, 1990, 1993, 1994). A significant reduction of 50% elasticity after four weeks impels frequent garment renewal and therefore is not cost-effective (Cheng, et al., 1983; Giele, et al., 1995). Discomfort brought about by poor perspiration of some fabric materials also limits patient's compliance (Brown, 2001; Macintyre, & Baird, 2006; Stewart, et al., 2000) and inadequate knowledge of fabric properties further brings complexity in definitive scientific studies of pressure therapy.

CHAPTER III

PHASE I OF THE STUDY – MEASUREMENT OF INTERFACE PRESSURE

3.1 INTRODUCTION

Persistent and adequate pressure application to the skin surface has been established for decades as one form of conservative treatments for hypertrophic scars (Linares, Larson, & Willis-Galstaun, 1993; Puzey, 2002; Staley, & Richard, 1997; Zurada, Kriegel, & Davis, 2006). Precise and reliable interface pressure measurement system, however, remains unavailable in current practice. In this phase of study, a validation study was conducted on the application of a pressure measuring device for static interface pressure measurement. Sensor properties would also be evaluated through a series of laboratory tests in the first part of the study. Part II of the study would be a clinical study using the apparatus to differentiate the loading generated on the patients' scars through additional inserts and pressure garment. By performing a systematic experiment on the properties of the sensor, its feasibility in clinical use could be evaluated.

3.2 REVIEW ON PRESSURE SENSING SYSTEMS

Pressure monitoring is crucial for effective pressure therapy. Nevertheless, recommendations for the therapeutic pressure range are based merely on empirical observations in most clinical settings. This extrapolated advice may be attributed to the paucity of easy-to-use, accurate and reliable measuring equipment (Rayner, 2000). To investigate the relationship between pressure and hypertrophic scar remodeling, it is essential to quantify the magnitudes of the applied pressure generated by pressure garment. Over the years, the importance of maintaining desired magnitude of pressure has led to the development of various devices to measure the therapeutic pressure range.

Nonetheless, it remains augmentative probably due to the unavailability of a precise and reliable instrument (Ferguson-Pell, Hagisawa, & Bain, 2000; Maklewska, Nawrocki, Kowalski, Andrzejewska, & Kuzanski, 2007; Mann, Yeong, Moore, & Engrav, 1997; Norman, 2004; Yan, 2000).

3.2.1 Definition of an Ideal Pressure Monitoring Device

An ideal interface pressure device, usually in the form of a force sensor, should be small, thin and highly flexible and be sensitive in detecting the range of pressure as low as 0 to 50mmHg (Ashruf, 2002; Cheng, Evans, Leung, Clark, Choy, & Leung, 1983; Ferguson-Pell, 1980; Grant, 1985; Ian, 2005; Naismith, 1980; Partsch, et al. 2006; Wolsley, & Hill, 2000). It should be capable of displaying a continuous output and free from error of measurement on curved surfaces and from the effects of temperature and moisture. The sensor should not distort the actual geometry and highly conformed to the body contour. Sensors developed over the years were, nonetheless, unable to fulfill this ascertained definition.

3.2.2 Criticisms on Pressure Sensors in Current Use

Since the 1980s, sensors with diverse technologies have been developed for pressure garments. The earlier techniques include electro-pneumatic (Cheng, Evans, Leung, Clark, Choy, & Leung, 1983; Harries, & Pegg, 1989; Partsch, 2005; Robertson, Druett, Hodgson, & Druett, 1980; Sawada, 1993; Steinberg, & Cooke, 1993) and fluid-filled pressure transducers (Barbenel, & Sockalingham, 1990; Horner, Lowth, & Nicolaides, 1980) which have been refuted with low sensor accuracy and hysteresis (Ferguson-Pell, & Cardi, 1993; Norman, 2004; Steinberg, & Cooke, 1993; Wolshey, & Hill, 2000) and

poor conformity to body curvature (Rayner, 2000). Many systems employing the above techniques are also found incapable to measure pressures below 20mmHg (Clark, 1994) and with differing time (Hafner, Luthi, Hanssle, Kammerlander, & Burd, 2000). Measuring pressures sub-dermally by a 19-gauge needle has been suggested to monitor the actual value transmitted to skin tissue (Giele, Liddiard, Booth, & Wood, 1998; Giele, Liddiard, Currie, & Wood, 1997), yet, stemming from its invasive nature, the system is not popularized in both clinical and research industry.

With regards to the unresolved dilemma outlined above, pressure systems have been thereafter revolutionized. By means of piezoresistive elements such as strain gauges (Figure 3.1) and force-sensing resistors, resistive pressure systems, for instance, the Iscan (Mann, Yeong, Moore, & Engrav, 1997) and the FlexiForce Sensor (Ferguson-Pell, Hagisawa, & Bain, 2000; Yan, 2000), can map the force and translate it into pressure readings. Once the sensor is under-pressure, the internal resistance of the strained or deflected element changes until this element's original position is restored. Although better accuracy has been noted, the piezoresistive elements have been reported sensitive to temperature (Bethaves, 2002). The sensor durability has also been challenged with its dropping resiliency to return to original position after several measurements. In addition, the sensor sensitivity is only limited at forces less than 10mmHg (Ferguson-Pell, Hagisawa, & Bain, 2000). This indicates that resistive pressure systems have reduced accuracy of readings at low forces and with temperature changes.

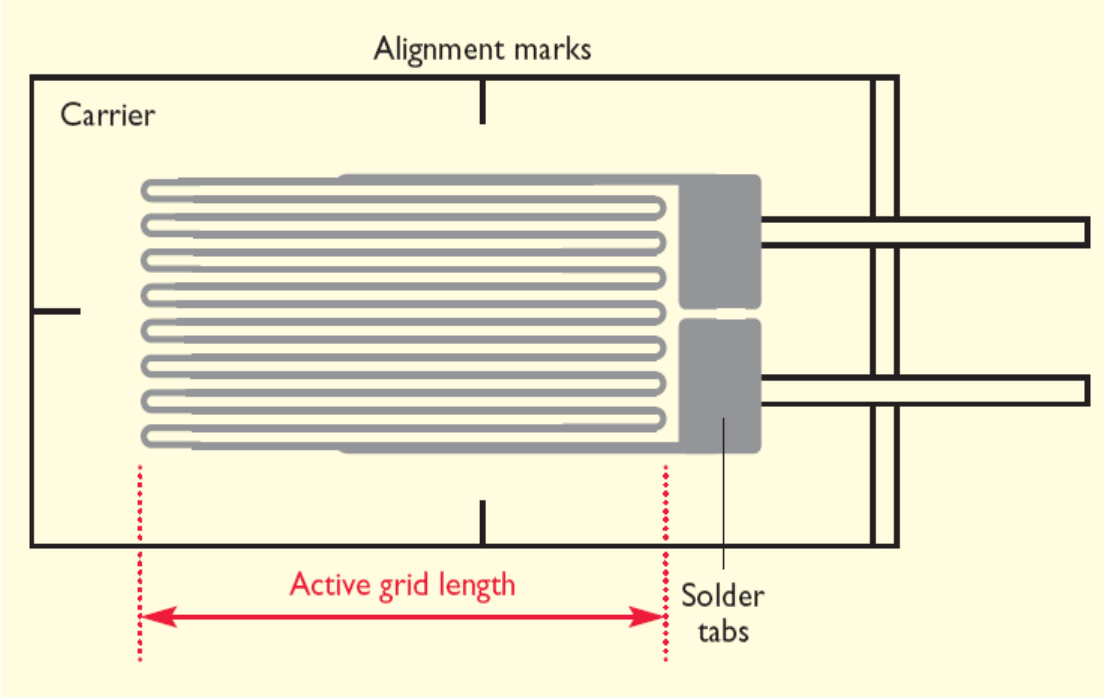


Figure 3.1 Metal wire bonded strain gage (Bethaves, 2002)

Capacitive pressure sensor, an alternative technique used in pressure measurement, is an electrical element that stores energy in the form of an electrical field, in which their electrical characteristics change in response to external pressure. By translating signals of pressure changes into electrical capacitance variation, capacitive transducer is found able to provide not only higher sensitivity and flexibility, lower temperature dependency, but also more robust structure and lower power consumption than piezoresistive devices (Bethaves, 2002; Chavan, & Wise, 2001; Lee, & Wise, 1982; Naismith, 1980; Zhou, Huang, Qin, & Zhou, 2005). It is commonly performed on support systems such as seating for paraplegics and geriatrics (Hsi, Kang, & Lee, 2005; Kernozek, Wilder, Amundson, & Hummer, 2002) and also on rehabilitation engineering prosthesis and orthosis (Jia, Suo, & Wang, 2007; Meyring, Diehl, Milani, Hennigm, & Berlit, 1997) with high interface pressure. A recently developed commercial capacitive sensor, the Pliance X System (Germany-Novel Electronics, Munich, Germany), has claimed its effectiveness in low interface pressure application. Besides the advantages of general capacitor transducers, this device is also designed with a small (10mm in diameter) and ultra-thin (less than 1mm) sensor which appears to be a good device for measurement of interface pressure generated by pressure garment to the skin tissue. The apparatus has been employed in a previous study (Hu, Zhu, Lu, Yeung, & Yeung, 2007) for the measurement of the pressure between skin and pressure garment or bandages and demonstrated to be feasible. This study therefore aims to evaluate the performance of this potential apparatus for measurement of interface pressure through series of laboratory tests and clinical trial.

3.3 OBJECTIVES OF THE PHASE I OF THE STUDY

In order to define the optimal dosage of pressure for effective scar control, a quantitative measurement technique on the interface pressure should be identified prior to the clinical trial. As mentioned above, a newly available apparatus, the Pliance X System, could be a potential quantitative assessment tool for measuring interface pressure. This phase of the study was therefore set to examine its performance in terms of its mechanical properties and its applicability in clinical practice.

Laboratory investigation is a common practice to testify the mechanical properties of a pressure device, the sensors are often assessed on a rigid surface, yet, this has been challenged for its clinical applicability (Ashruf, 2002; Clark, 1994; Norman, 2004) in particular, the effect of human tissue elasticity and inter-subject variability. Human skins with and without scars were thus included in the second part of the investigation. Little research on the reliability of the measurement is another disparagement (Clark, 1994). Taking into account of these criticisms, this study intended to investigate the mechanical properties of the novel device, to test its inter-rater, test-retest reliability and repeatability and to examine its generalization onto human body.

Therefore, the objectives of this study were:

- to examine the sensor properties of the Pliance X System;
- to find out the differences of interface pressure between normal skin and hypertrophic scar;
- to identify the interface pressure under different layers of padding inserts; and
- to testify if scar maturation and location affect the interface pressure.

3.4 EQUIPMENT

With the technological advancement, the Pliance X System (Germany-Novel Electronics, Munich, Germany) was developed initially for measuring pressure generated in shoe-wear and sitting. The sensors were recently modified to suit the low pressure in pressure garment. The system consists of data acquisition hardware, analysis software with a data acquisition display and capacitive sensor. The local force generated by the pressure garment and padding transmits through the sensor and is converted into a pressure profile for data acquisition and analysis. Data acquired by the Pliance X System (Figure 3.2a) is in the unit of millimeter mercury (mmHg). The sensor photo captured in Figure 3.2b is designed with a small (10mm in diameter) and ultra-thin (less than 1mm thick) sensing area connected to the system via an extended conductive strip which favors insertion to clothing with long sleeves. Due to its advantages of capacitor transducers of high sensitivity, durability (Bethaves, 2002), ability to produce continuous data and low temperature-dependency natures, the Pliance X System encompasses the potential for pressure therapy application. The system also allows flexibility in sensor configuration as a single sensor or in a matrix (with multiple sensors) for multiple measurements simultaneously. It appears to be a good device for measurement of interface pressure generated by pressure garment, yet there is a lack of validation studies to testify its suitability. This study therefore aimed to evaluate the performance of the Pliance X System for measurement of interface pressure through series of laboratory tests and clinical trial.

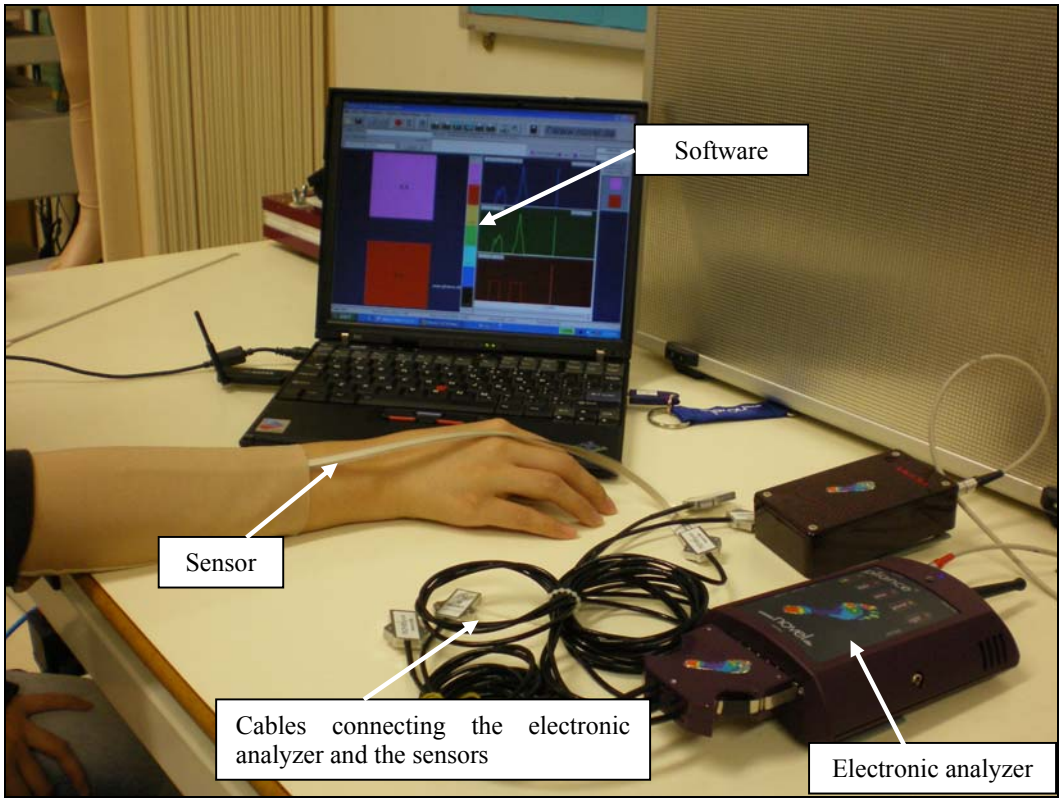


Figure 3.2a The Pliance X System

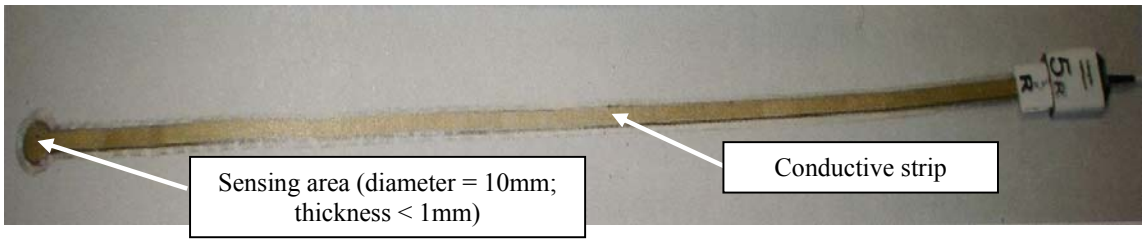


Figure 3.2b The sensor of the system

3.5 PART I – MECHANICAL PROPERTIES OF THE SENSOR

In order to assess the mechanical properties of the system, a series of laboratory tests were exercised with reference to previous studies (Ferguson-Pell, Hagisawa, & Bain, 2000; Lee, Goonetilleke, Cheung, & So, 2001; Parsons, 2003; Wolsley, & Hill, 2000; Yan, 2000). The device was calibrated before the experiment implemented. The sensor properties were tested with pressure limited to 50mmHg since adverse effects, namely maceration and paresthesia, were reported with higher pressures (Naismith, 1980). A series of mechanical performance tests, including linearity, repeatability and drift tests, were testified with the experimental setup sketched in Figure 3.3. A 10 x 10 x 4mm plastic disc was placed under the pressure sensor such that all forces of the weight were transferred only to the sensor. The data of weight recorded was converted to force in millimeter mercury (mmHg) by the equation:

$$\text{Applied Pressure (mmHg)} = \frac{\text{Force}}{\text{Area}} = \frac{\text{Weight (kg) X } 9.81\text{m/s}^2}{\text{(Contact surface area) mm}^2} \text{ x } 7500.637554$$

(Equation 3.1)

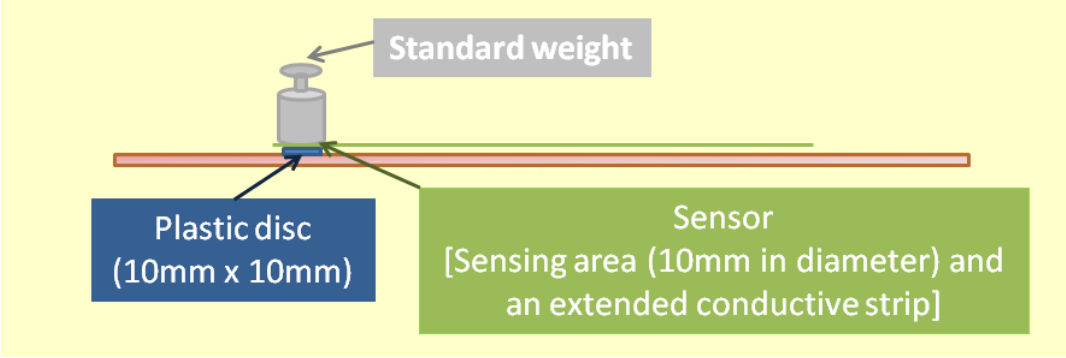


Figure 3.3 Sketched experimental setup for linearity, repeatability and drift tests

3.5.1 Procedures

(I) Linearity and Repeatability Test

The test was to examine the accuracy of the pressure device by measuring the proportionality of the sensor response to a range of standardized loads. Standard weights were applied to the sensor and 1,000 readings per weight were recorded one minute after loading with 10 repetitions. Pearson's Moment Correlation was used to measure the linear relation between the applied forces and sensor values. Evaluation of the test-retest reliability of the Pliance X System was also represented by the analysis of intra-class correlation coefficient, ICC (3, 10). The same procedure but with five trials was repeated by three independent assessors for its inter-rater reliability which was analyzed by ICC (2, 5).

(II) Drift Test

This test was to verify the time-dependency factor of which changes of output values subjected to a constant force over an extended period of time. The sensor stability was tested by placing a weight (20g) with fixed area on the sensor for 30 minutes for 10 trials and readings were recorded every five minutes. The same procedure was repeated with a 50g weight and the data generated by the system was recorded.

(III) Hysteresis Test

Hysteresis influences minimal to static pressure measurement, however, in applications that involve loading increasing and decreasing, that is dynamic measurement, significant

errors may be introduced to sensors with poor hysteretic property which cannot be improved by calibration.

Error of hysteresis was studied by observing the differences in sensor response during loading and unloading with the same force. The hysteretic property of the sensor was assessed by pneumatic calibrator where pressure was monitored through a digital manometer. The sensor was tested with consecutive increasing and decreasing forces over a range of 0 to 50mmHg with 10mmHg increments. The process was replicated ten times and the descriptive statistics were depicted.

(IV) In-vivo Pressure Measurement

Adopting previous protocols (Barbenel, & Sockalingham, 1990; Hafner, Luthi, Hanssle, Kammerlander, & Burd, 2000) to testify the effect of human skin elasticity on the sensor accuracy, the Pliance X System was assessed with the measurement of the interface pressures between skin surface and a sphygmomanometer cuff. Five healthy volunteers (three males and two females; age ranged from 23 to 50) were recruited with a total of four assessment locations for each subject, namely lateral aspect of upper arm (10cm above elbow), forearm (10cm below elbow), thigh (10cm above knee) and calf (10cm below knee) to evaluate the effect of human skin elasticity (Harries, & Pegg, 1989; Sawada, 1993). All measurements on the upper limbs were taken in sitting position with the arm in natural resting position while those on the lower limbs were taken in standing

position (Teng, & Chou, 2006). Measurements were taken starting from 10 to 50mmHg with increments of 10mmHg and the procedure was repeated five times.

3.5.2 Results

(I) Linearity and Repeatability Test

The results of linearity and repeatability on rigid surface are shown in Table 3.1 and the correlation between the applied pressure and the sensor output is illustrated in Figure 3.4. Reasonable coefficient of variation, ranging from 1.097 to 8.450%, was obtained in which lower forces resulted in higher relative variations. Minor differences between the applied pressure and sensor output were found in the test with less than 1mmHg (0.175 ± 0.264mmHg). Correlation between the applied pressure and sensor output was revealed with Pearson's Moment Correlation r = .998 and adjusted r^2 = .997 and test-retest reliability was revealed with an ICC (3, 10) of .998. The inter-rater reliability ICC (2, 5) among the three independent assessors was 95% with CI from .995 to 1.00. The results demonstrated high correlation on linearity and repeatability of the system.

Table 3.1 Results of linearity and repeatability test

Standard Weight	*Applied Pressure (mmHg)	Sensor Value Mean (mmHg)	S.D.	Coefficient of Variation (%)
5 g	4.215	4.059	0.343	8.450
10 g	9.393	9.717	0.648	6.669
20 g	18.276	18.512	0.641	3.463
30 g	28.139	28.646	0.643	2.245
40 g	37.513	38.114	0.418	1.097
50 g	46.396	46.662	0.748	1.603

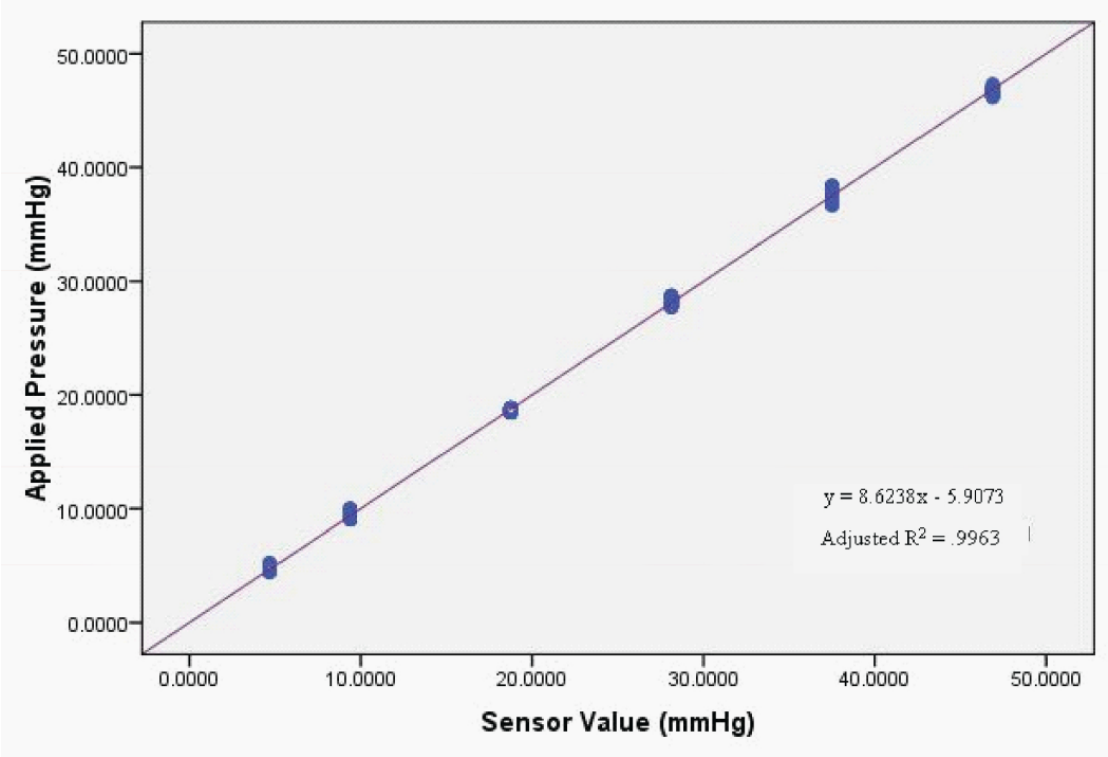


Figure 3.4 Association between sensor output and applied pressure

(II) Drift Test

Drift is the percentage change increase in output over a period of time when subjected to a constant force. The device showed minor degradation in performance in terms of stability with only a slight increase of 1.509mmHg for both 20g (7.99%) and 50g (3.33%) weights after 15-minute loading.

(III) Hysteresis Test

The hysteretic performance of the device is illustrated in Figure 3.5. As shown in Equation 3.2 listed below, by dividing the maximal difference in the readings between loading and unloading output values at the same applied force by the maximum output reading in the range of loading, the mean ± SD of the maximum difference in output for increasing and decreasing loads could be determined (4.58 ± 0.88%).

$$\frac{\text{maximal} \left| \text{loading} - \text{unloading} \right|}{\text{maximum output}} \times 100\%$$

(Equation 3.2)

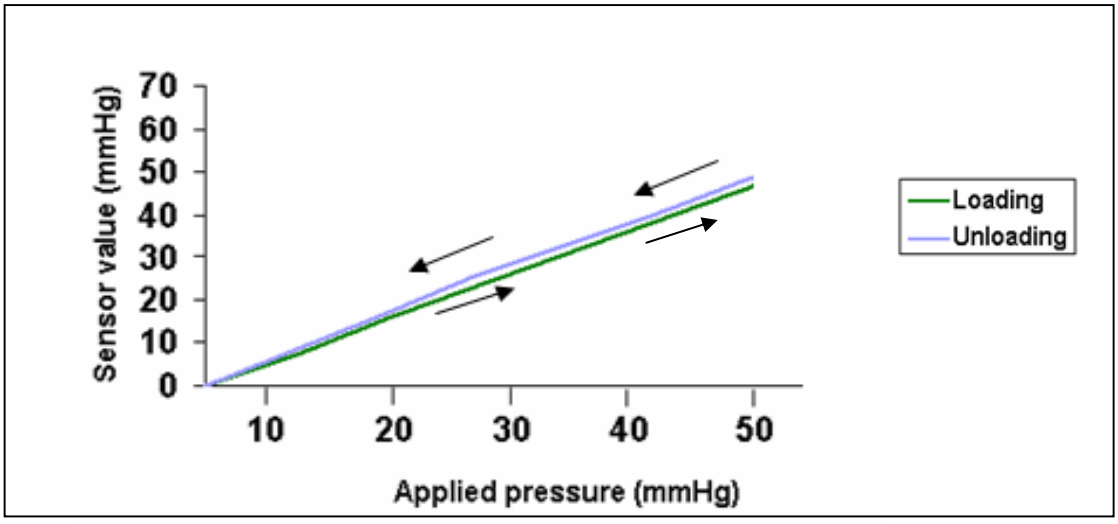


Figure 3.5 Results of Hysteresis Test

(IV) In-vivo Pressure Measurement

The sensor accuracy was evaluated by placing the sensor between the human limbs and a sphygmomanometer cuff for five healthy volunteers. Table 3.2 summarizes the average pressures in each test site. No significant discrepancies between the readings of sphygmomanometer and the device were obtained in the test with a maximum deviation of -1.451mmHg.

Table 3.2 Sensor accuracy measured on human limbs

Measurement Site	Applied pressure (mmHg)	Output Value Range (mmHg)	Output Value Mean (mmHg)	SD	Mean Percentage Difference
Upper arm	10	9.81 – 11.22	10.179	0.609	1.79%
	20	21.12 – 21.58	21.285	0.206	6.42%
	30	31.02 – 31.70	31.451	0.279	4.84%
	40	39.68 – 41.43	40.511	0.644	1.28%
	50	48.92 – 51.53	49.815	1.011	0.37%
Forearm	10	9.93 – 11.34	10.492	0.642	4.92%
	20	19.72 – 21.13	20.757	0.590	3.78%
	30	28.90 – 30.19	29.752	0.511	0.83%
	40	39.77 – 40.76	40.416	0.463	1.04%
	50	49.37 – 49.96	49.692	0.213	0.62%
Thigh	10	9.81 – 11.32	10.314	0.696	3.14%
	20	19.62 – 20.69	19.840	0.476	0.80%
	30	30.17 – 30.25	30.212	0.031	0.71%
	40	39.99 – 40.79	40.486	0.398	1.22%
	50	49.62 – 52.58	50.734	1.138	1.47%
Calf	10	9.80 – 9.94	9.835	0.059	1.65%
	20	19.64 – 21.05	20.487	0.726	2.43%
	30	29.38 – 30.31	29.865	0.337	0.45%
	40	39.24 – 40.71	39.978	0.705	0.05%
	50	49.83 – 51.21	50.230	0.570	0.46%

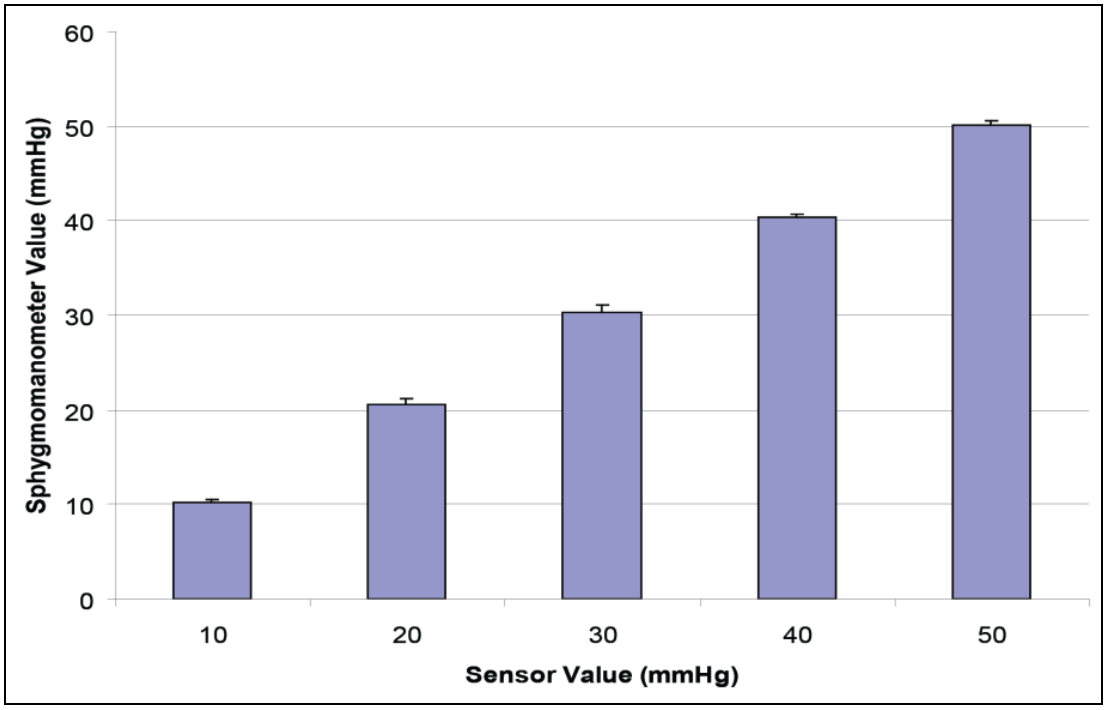


Figure 3.6 Histogram of sensor values under sphygmomanometer cuff

3.6 PART II – CLINICAL PRESSURE MEASUREMENT

3.6.1 Sampling Method

To test the discriminant validity of the instrument subjected to three levels of pressure loading, with $\alpha = 0.05$, power = 0.8, and f-value = 0.6, the sample size was estimated as a total of 24. To reduce the heterogeneity of scar due to the scar location with different body contours, only scars developed over upper or lower extremities were included in this investigation. Subjects, fulfilling the following criteria, were recruited from the Rehabilitation Department of Longgang Central Hospital in Shenzhen:

Inclusion Criteria

- co-operative and comply well with medical intervention
- size of hypertrophic scar with at least 4 x 4 cm^2 over upper or lower extremities

Exclusion Criteria

- hypertrophic scar with open wound
- scar developed over small joints (such as fingers and toes)

3.6.2 Procedures

Each patient was prescribed with a tailor-made pressure garment (PG) with 5% tensile strength by a registered occupational therapist. To evaluate the discriminant validity of the Pliance X system by using the known group method, as localized pressure is believed to increase with additional inserts, the same measuring sites were subjected to three levels of pressure loading for five trials: PG; PG with a layer of padding (PG + 3mm); and PG with two layers of padding (PG + 6mm). PG alone was expected to

generate the minimal force to skin tissue while the highest force was assumed for PG with two-layered padding. Pressure paddings were mounded according to the body curvature and inserted underneath the PG to vary the pressure exerted onto the measuring sites.

The most severe and prominent site on each scar was chosen for interface pressure measurement. The scar was outlined on a translucent sheet with the assessment site spotted. The exact location on the opposite limb with normal skin was measured for comparison. Before putting on pressure garment and inserts, the sensor was stabilized with tape onto the skin surface. Measurements over upper limbs were taken in sitting position with arm in natural position while those over lower limbs were taken in standing position (Teng, & Chou, 2006). This was to evaluate the discriminant validity of the Pliance X system by using the known group method.

3.6.3 Results

(I) Demographic data

Eight patients, five males and three females, aged from 18 to 40 (27.88 ± 8.31 years of age), with multiple hypertrophic scars were recruited for investigation. In total, 24 hypertrophic scars over limbs with onset ranging from three to eight months (5.48 ± 1.58 months of scar age) were selected and divided into $Scar_a$ (≤ 6 months, n = 16) and $Scar_b$ (> 6 months, n = 8) scar groups for analysis. Eleven hypertrophic scars over upper limbs and 13 over lower limbs were recorded. The same location on the opposite limb of

each scar was also measured to observe the differences in the interface pressure measured on the scar tissue and normal skin.

Table 3.3 Distribution of subjects for the cause, location and maturity of scars

		Frequency	Percentage (%)
Cause			
Burn		18	75
Scald		4	16.7
Trauma		2	8.3
	Total	**24**	**100**
Location			
Upper Limb		11	45.8
Lower Limb		13	54.2
	Total	**24**	**100**
Scar Maturity			
$Scar_a$ (≤ 6 months)		16	66.7
$Scar_b$ (> 6 months)		8	33.3
	Total	**24**	**100**

(II) Discriminant Validity

The average pressures under various conditions are presented in Table 3.4. Based on the assumption, greater interface pressure would be exerted by increasing the number of padding inserts. The discriminant ability of the device was proven with its positive findings in differentiating pressure with various layers of paddings as illustrated in Table 3.5 (H-value = 82.545, $p < 0.01$, Kruskal Wallis ANOVA). A multiple post-hoc analysis Dunnett T3 test demonstrated a significant increase in pressure with an additional layer of pressure padding ($p < 0.01$). An extra layer of padding induced approximately two times of interface pressure to underlying skin.

(III) Additional Factors affecting Interface Pressure

Other variables affecting interface pressure under pressure garment were also identified in the trial. The results revealed that under the same levels of pressure loading, hypertrophic scar was subjected to higher interface pressure than normal skin with *t*-value = 4.563 at $p < 0.01$. Pressure was shown approximately two times greater in hypertrophic scar than normal skin for both upper and lower limbs.

Table 3.4 Interface pressure measured under different conditions

Location	Type of Skin	Level of Pressure Loading	Interface Pressure (mmHg) Mean ± SD
Upper Limb	Hypertrophic scar (n = 11)	PG	8.00 ± 0.92
		PG with a layer of padding (3mm)	12.64 ± 1.94
		PG with two layers of padding (6mm)	23.32 ± 0.78
	Normal skin (n = 11)	PG	3.28 ± 0.92
		PG with a layer of padding (3mm)	8.33 ± 0.56
		PG with two layers of padding (6mm)	17.68 ± 0.74
Lower Limb	Hypertrophic scar (n = 13)	PG	5.41 ± 1.23
		PG with a layer of padding (3mm)	8.85 ± 0.77
		PG with two layers of padding (6mm)	14.07 ± 0.94
	Normal skin (n = 13)	PG	1.77 ± 0.58
		PG with a layer of padding (3mm)	4.83 ± 0.87
		PG with two layers of padding (6mm)	10.88 ± 1.37

Table 3.5 Test statistics under different conditions

	Test value		*p*-value
Level of Pressure Loading	Kruskal Wallis ANOVA	82.545[a]	< 0.01**
Type of Skin (N vs HS)	Independent t-test	4.563[b]	< 0.01**
Location (UL vs LL)	Kolmogorov-Smirnov Z	2.205[a]	< 0.01**

Dependent variable: Interface Pressure (mmHg)
[a] *Unequal variance formula*
[b] *Equal variance formula*
***The mean difference is significant at the .01 level*

Significant variations were also observed between the upper (13.66 ± 5.91mmHg) and lower limbs (9.44 ± 3.74mmHg) with Kolmogorov-Smirnov *Z*-value = 2.205 at $p < 0.01$. Due to a smaller radius of curvature, all testing sites on upper limbs were found to have higher interface pressures than those on the lower limbs.

The samples were arbitrarily clustered into two types (a) scars with onset less than or equal to six months ($Scar_a$) and (b) scars with onset over six months ($Scar_b$). Although significant difference ($p < 0.01$, Table 3.6) was obtained only under the condition of no insertions underneath the PG, $Scars_a$ ($\leq$ 6 months) appeared to be subjected to higher pressure under all testing conditions when compared to the $Scar_b$ (> 6 months) as shown in Figure 3.7.

Table 3.6 One-way ANOVA statistics between $Scar_a$ ($\leq$ 6 months) and $Scar_b$ (> 6 months)

	Upper Limb			Lower Limb		
	F-value	**df**	***p*-value**	***F-value***	**df**	***p*-value**
PG[a]	13.8155[a]	10	0.005**	6.1342[a]	12	0.0327*
PG + 3mm[b]	4.2426[b]	10	0.070	0.8142[a]	12	0.3881
PG + 6mm[a]	4.8938[a]	10	0.054	4.7764[a]	12	0.0537

[a]. *Unequal variances assumed*
[b]. *Equal variances assumed*
* *The mean difference is significant at the .05 level*
** *The mean difference is significant at the .01 level*

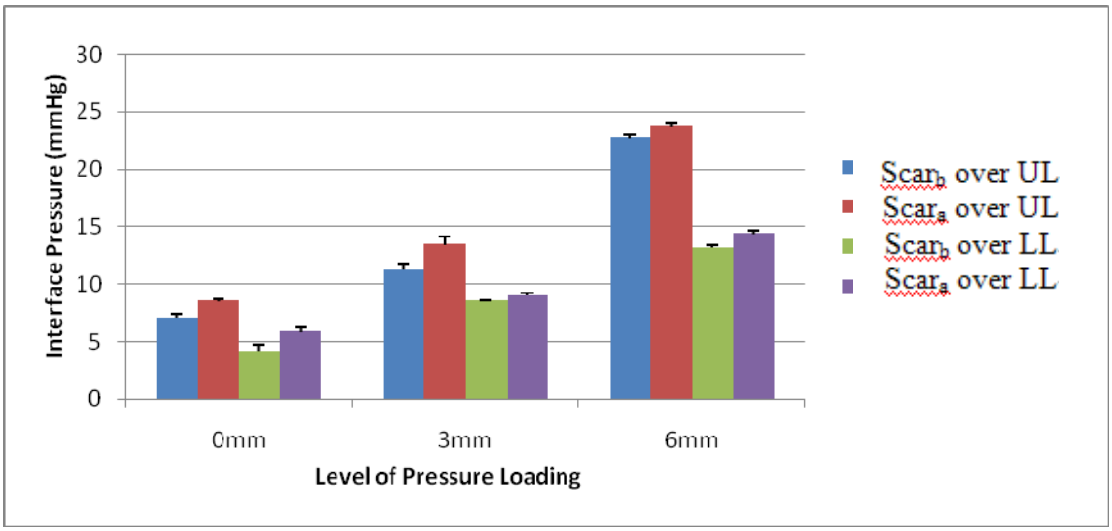


Figure 3.7 Comparisons between Scar$_a$ (≤ 6 months) and Scarb (> 6 months)

3.7 DISCUSSION

Though with much progress has been made in the pressure monitoring systems, researchers still encounter challenges in reflecting satisfactory parameters in clinical circumstances (Van den Kerckhove, et al., 2007). Great variation in optimal pressure values from 5 to 40mmHg has been reported. Persistent discrepancies by an unreliable and inaccurate sensor equipment, possibility explain the augmentative pressure dosage (Ferguson-Pell, Hagisawa, & Bain, 2000; Maklewska, Nawrocki, Kowalski, Andrzejewska, & Kuzanski, 2007; Mann, Yeong, Moore, & Engrav, 1997; Norman, 2004: Van den Kerckhove, et al., 2007). In order to maximize the therapeutic effect of pressure therapy, there is a burning need for an objective measurement of interface pressure.

The current study investigated the suitability of a newly available device – the Pliance X system for the measurement of interface pressure between pressure garment and human skin tissue. With its advantages of high sensitivity and flexibility (Bethaves, 2002) and minimal influence caused by temperature (Wolsley, & Hill, 2000) as well as the small and ultra-thin sensor, the apparatus could be a potential quantitative tool for interface pressure documentation. This study was therefore designed to examine the sensor properties of the system through a series of mechanical tests. In addition, a human study was included to testify the discriminant ability of the equipment as to find out the differences of interface pressure exerted on the normal skin and hypertrophic scar, under different layers of padding inserts, across different stages of scar maturation and scar locations.

The overall performance of the system was considered satisfactory. The values obtained by the sensor were linearly correlated with the applied pressures and good test-retest and inter-rater reliability was revealed. Less than 1mmHg pressure error was found in the sensor output with standard weights of 5g to 50g. Relatively higher coefficients of variation at lower forces (8.5% at 5g; 6.7% at 10g), however, were obtained when compared with higher forces (2.2% at 50g). Therefore, special attention should be paid when measuring pressure at lower range of forces (under 10mmHg).

The stability of sensor values was acceptable with a minimal increase after 15-minute loading. Regarding the hysteretic error, other researchers have reported data ranging from 5.4% to 61.8%, in their machines (Ferguson-Pell, Hagisawa, & Bain, 2000; Ferguson-Pell, & Cardi, 1993; Steinberg, & Cooke, 1993; Yan, 2000). The Pliance X System with minimal hysteretic errors (4.6%) obtained in this study demonstrated its potential in dynamic pressure measurements. The sensor reading was slightly higher during unloading when compared to loading; this difference might be stemmed from the reducing resistance in the sensor during the unloading phase. Dynamic measurements are viable if compensated with a minor discrepancy of sensor values. The results also indicated a reasonable accuracy of the sensor placed between human limbs and a sphygmomanometer cuff with a maximum mean difference of 6.42%.

Measurements taken on clinical cases demonstrated the feasibility of using the Pliance X system for monitoring the dosage of pressure therapy. By adding layers of padding underneath the pressure garment, pressure gradient was generated which demonstrated

the discriminant ability of the system. The sensor was found to be able to discriminate interface pressure generated under the three levels of pressure loadings ($p < 0.01$). It was also showed that an additional level of padding could induce significant pressure change to both hypertrophic scar and normal skin with $p < 0.01$. Besides the layers of pressure paddings, it was worth noting that the influence of scar location and scar maturation might need to be considered when applying pressure therapy. Statistical significance was observed when comparing the interface pressures exerted on the hypertrophic scar and normal skin. This might be due to the difference in skin tissue viability as scar tissues are generally stiffer than normal skin. The significant impact of scar location (upper limb versus lower limb) on the interface pressure obtained in the clinical measurement echoes with the Laplace Law that pressure is inversely proportional to the radius of body curvature (Hospital Authority of Hong Kong, 1998). Even though the garment fabrication has the same tensile strength, additional paddings could be added to body surface with larger radius of curvature to attain the desired pressure. Furthermore, the results showed that pressure values measured on scars with longer development ($Scar_a$) tended to be lower than those on the younger ones ($Scar_b$). During the course of scar maturation, hypertrophic scars tend to gradually become thinner and softer. This change of soft tissue viability might explain the pressure disparities recorded between scars developed less or equal to and more than six months, though no statistical significance was observed.

Interface pressure of at least 15mmHg is recommended for scar management in previous studies (Cheng, Evans, Leung, Clark, Choy, & Leung, 1983; Naismith, 1980; Van den

Kerckhove, et al., 2005), however, the PG fabricated in this experiment could not generate pressure higher than 10mmHg. To provide sufficient and uniform pressure for effective hypertrophic scar management, additional paddings with good conformation power might be considered.

In clinical measurements, the Pliance X system was user-friendly. It was easy to administer by therapists or technicians with one or two sessions of training. The readings generated could be exported to other software system for data analysis. Base-line zero pressure could be calibrated before loading was applied. Multiple measurements over various body regions are allowed with connection of additional sensors to the matrix configuration. It is convenient to carry the system to the patients because the apparatus can be connected to the computer via Bluetooth connection. The sensors are, however, too long and clumsy to handle with cables connected.

Limitations of the Study

The experiment was conducted on body regions with relatively flat skin surface without addressing the concavity or convexity of body contours. The results obtained, thus, may not apply to skin surfaces with high concavity, such as face and ear. Samples of scar tissues might also be insufficient to show variations of pressure changes. Due to the lack of quantitative assessment tools, the potential confounding variable, scar pliability, which might affect the interface pressure, could not be measured in the current investigation. Moreover, the interfacial pressure reading was currently exploited in static

means, however, physical activities and movements predictably alter the pressures significantly. Dynamic pressure effect on the sensor accuracy remains unexamined.

3.8 CONCLUSION

It is important to monitor pressure dosage in management of hypertrophic scar. Empirical observations in current clinical practice, however, are too subjective in manipulating pressure and previously available techniques to measure pressure have their limitations. In this study, a newly available device, the Pliance X System, was tested for its feasibility of measuring interface pressure between skin and pressure garment. The results demonstrated its potential as a clinical tool in measuring pressure in an objective manner. Its feasibility in low pressure measurement was supported with its superior technical performances in laboratory tests. Its clinical applicability was also revealed with its good discriminant ability under diverse loadings in static manner. Slight diminution of the sensor sensitivity, however, appeared during low pressure measurement below 10mmHg and after 15-minute loading. Further investigations were recommended to evaluate its response to concave skin surfaces and dynamic movements.

CHAPTER IV

PHASE II OF THE STUDY
BIOLOGICAL ACTIVITIES OF HYPERTROPHIC SCAR FIBROBLASTS UNDER PRESSURE INTERVENTION

4.1 INTRODUCTION

Though the entire mechanisms of pathological healing of scar have not been clearly identified, it is a common consensus that fibroblast takes a major part in this healing process (Dongmin, & Kyung, 2004). Therefore, any procedure that can down-regulate the fibroblast activities might be able to prevent hypertrophic scar formation. As mentioned in Literature Review, it has been hypothesized by researchers that pressure therapy takes effect by influencing the fibroblast activities of the hypertrophic scars (Baur, Larson, Stacey, Barratt, & Dobrkovsky, 1976; Costa, & Desmouliere, 1998; Kischer, Shetlar, & Shetlar, 1975; Linares, Larson, & Willis-Galstaun, 1993). In this present study, a series of experiments using cultured fibroblasts as an *in-vitro* model would be conducted to investigate the relationships between different pressure levels and fibroblast activities. The result obtained could hopefully help in part explain the underlying mechanisms of pressure therapy at cellular level.

4.2 REVIEW ON FIBROBLAST ACTIVITIES IN WOUND HEALING AND FIBROTIC CONDITION

4.2.1 Roles of Fibroblast in Wound Healing Process

Dermal fibroblast, a mesenchymal cell accountable for normal connective tissue production and turnover (Wong, McGrath, & Navsaria, 2007), plays a critical role in wound healing process (Eastwood, McGrouther, & Brown, 1998). The morphology of fibroblast appears in stellate structure with elongated and branching processes

(Takahashi-Iwanaga, 1994). Upon injury, inflammatory signals and growth factors released from the connective tissues and blood vessels activate dermal fibroblast to proliferate and migrate to re-populate the damaged site (Eastwood, McGrouther, & Brown, 1998) which helps re-establish functional structural integrity to the injured tissues (Diegelmann, & Evans, 2004). It also produces an enzyme, zymogen, to degrade newly synthesized collagen so as to sustain a matrix balance between synthesis and degradation processes that determines the quality of wound repair (Su, Alizadeh, Boddie, & Lee, 1998; Wong, McGrath, & Navsaria, 2007).

Under normal circumstances, a subpopulation of the fibroblasts undergoes phenotypic variation in order to facilitate expression of contractile proteins (Eichler, & Carlson, 2005). By generating isometric tension with the extracellular matrix (Desmouliere, Chaponnier, & Gabbiani, 2005), the phenotypically modulated fibroblast, termed as myofibroblast (Gabbiani, Ryan, & Majno, 1971), contracts the wound to expedite closure (Costa, & Desmouliere, 1998). Myofibroblast, the only cell parallel to line of contraction (Jensen, & Parshley, 1984), is a mesenchymal cell with features of both fibroblast and smooth muscle cell (Sappino, Schurch, & Gabbiani, 1990). On one hand, myofibroblast resembles fibroblast in spindle appearance with prominent cytoplasmic projections (Darby, & Hewitson, 2007); on the other hand, it contains the features of smooth muscle cell with longitudinal cytoplasmic bundles of microfilaments (stress fibers) and expression of α-smooth muscle actin (α-SMA), the actin isoform typical to contractile vascular smooth muscle cell (Darby, Skalli, & Gabbiani, 1990). The evolution process of myofibroblast is depicted in Figure 4.1. Under normal condition,

gradual disappearance of myofibroblasts is well-documented after re-epithelialization and wound closure (Darby, Skalli, & Gabbiani, 1990), yet much less has been known about the stimulus and underlying mechanisms of its vanishing (Desmouliere, Chaponnier, & Gabbiani, 2005; Gabbiani, 2003; Hinz, 2007). Previous researchers have suggested that this loss of myofibroblasts may result either from reversion to fibroblast phenotype (Desmouliere, Chaponnier, & Gabbiani, 2005) or from massive apoptosis, an active process of physiological and programmed cell death (Costa, & Desmouliere, 1998; Desmouliere, Redard, Darby, & Gabbiani, 1995; Gabbiani, 2003).

Depending on the type and depth of the injury, fibroblast continues its activities in the wound contraction and remodeling phase of wound healing up to six weeks. Thereafter the expanded fibroblast population, triggered by unidentified signals, stops diving (Clark, 1993; Peacock, 1984), undergoes apoptosis and regresses to a fibrocytic phenotype (Grinnell, 1994). Conversely, pathological scarring might be resulted if the mentioned processes are dys-regulated.

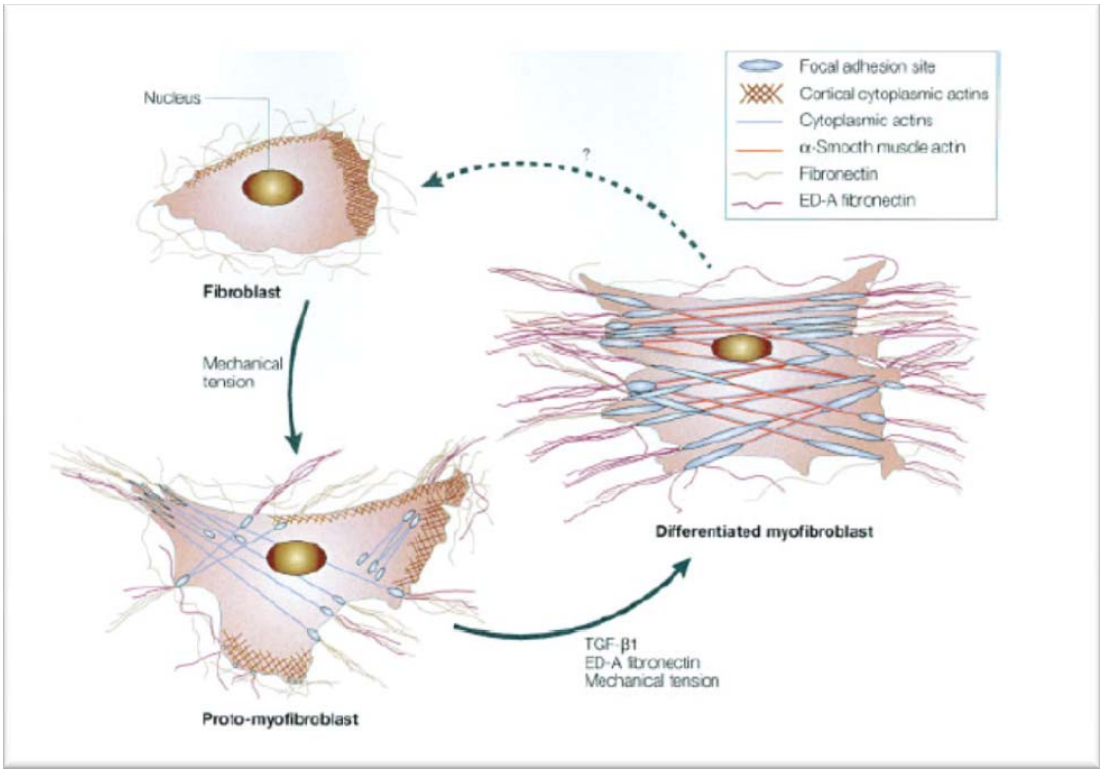


Figure 4.1 Evolution of myofibroblast phenotype in wound healing process (Gabbiani, 2003)

4.2.2 Components Involved in the Development of Hypertrophic Scar

Hypertrophic scarring and scar contracture could result from impaired wound healing process (Greenhalgh, 2005; Jensen, & Parshley, 1984; Linares, & Larson, 1976). Abnormal biological activities involving fibroblast and myofibroblast have been observed in hypertrophic scarring process.

Fibroblast in Hypertrophic Scarring

Divergences in cell biochemical responses to injury have been discovered between hypertrophic scar and normal skin fibroblasts (Thomas, & Critchley, 2006). The events differentiating normal from excessive healing start as early as the granulation tissue develops between three and five weeks post-injury (Linares, 1996). A marked difference is also found in the cyclic evolution of the granulation tissue. The structural characteristic of immature connective tissue, which normally disappears within several weeks, persists for months in hypertrophic scarring (Linares, 1996). Not only high degree of cellularity (Ehrlich, et al., 1994; Linares, 1996) spring from the augmented sensitivity to growth factors (Armour, Scott, & Tredget, 2007; Tredget, 2007), hypertrophic scar fibroblast has also been demonstrated with a substantial increase in collagen synthesis (Cohen, Keiser, & Sjoerdsma, 1971; Cohen, Beaven, Horakova, & Keiser, 1972; Kischer, 1973, 1974; Nedelec, Correa, Rachelska, Armour, & LaSalle, 2007). In spite of the four-fold increases in collagenase activity for collagen degradation in hypertrophic scar as compared to adjacent skin (Craig, 1983; McCoy, & Cohen, 1982), it is still insufficient to balance out the disproportionate raise in collagen

synthesis (Cohen, Beaven, Horakova, & Keiser, 1972; Cohen, Keiser, & Sjoerdsma, 1971; Kischer, 1973, 1974). Significantly different rates of collagen synthesis and degradation distort the matrix balance and result in overabundant collagen deposition in hypertrophic scar.

Myofibroblast in Hypertrophic Scarring

Myofibroblast, a differentiated form of fibroblast, contains microfilament bundles (stress fibers) with dense body, a well-developed rough endoplasmatic reticulum and a nucleus with indentations (Desmouliere, & Gabbiani, 1996). It contributes to connective tissue retraction (i.e. stress fibers) and is associated with deposition of extracellular matrix during wound healing process (Costa, & Desmouliere, 1998; Hinz, 2007). Sparkled by unknown signals, the myofibroblast either reverts to a quiescent form or disappears by apoptosis in normal healing (Darby, Skalli, & Gabbiani, 1990; Desmouliere, Redard, Darby, & Gabbiani, 1995). No myofibroblast could be found in wounds with contraction ceases (Jensen, & Parshley, 1984; Ketchum, Cohen, & Masters, 1974). Instead of cell vanishing after re-epithelialization and wound closure, hypercellularity of young and active myofibroblasts with expression of α-smooth muscle actin (α-SMA) perseveres in fibrocontactive scar which continues contracting the scar (Baur, Larson, & Stacey, 1975; Costa, & Desmouliere, 1998; Dongmin, & Kyung, 2004; Gabbiani, 2003; Hinz, 2007; Kose, & Waseem, 2007; Linares, 1996). Myofibroblast is unique in hypertrophic scarring which provokes scar contracture and absent in other scars (Desmouliere, 1995; Diegelmann, & Evans, 2004; Ehrlich, et al., 1994).

4.2.3 Pressure Loading on Fibroblast Behaviors

Providing the important relationship between fibroblast activities and excessive scarring, modalities that inhibit fibroblast accumulation and differentiation or induce cellular disappearance could be a way to treat the hypertrophic scar (Muir, 1998). It has been observed that the hypertrophic scar being treated by pressure was remodeling at an accelerated pace by means of modulating the fibroblast behaviors (Baur, Larson, Stacey, Barratt, & Dobrkovsky, 1976; Kischer, Shetlar, & Shetlar, 1975; Linares, 1973; Linares, Larson, & Willis-Galstaun, 1993).

Compared with the number of human studies, only a few researches have attempted to find histological evidences of pressure effect and most of those studies compared only the biopsies of pressure-treated and untreated hypertrophic scars during the scarring process. It has been reported that alterations in the mucopolysaccharide patterns in pressure-treated scars could lead to scar maturation. Reduced number of fibroblasts has been documented in pressure-treated scar biopsies as well (Baur, Larson, Stacey, Barratt, & Dobrkovsky, 1976; Kischer, Shetlar, & Shetlar, 1975). This reduction was suggested as related to the hypoxic environment brought by the vascular blanching under therapeutic pressure (Kischer, Shetlar, & Shetlar, 1975). Enzymes like collagenase and hyaluronidase are the major products in the synthetic activities of fibroblast (Armour, Scott, & Tredget, 2007). Pressure has been noted efficient to alter these enzymatic products, which favors nodule degradation (Baur, Larson, Stacey, Barratt, & Dobrkovsky, 1976; Kischer, Shetlar, & Shetlar, 1975). It has also been observed to induce the disappearance of α-smooth muscle actin-expressing myofibroblasts (Baur,

Larson, Stacey, Barratt, & Dobrkovsky, 1976; Costa, Peyrol, Porto, Foyatier, & Desmouliere, 1999; Jensen, & Parshley, 1984).

Although compression is reported effective in hypertrophic scar remodeling, most previous studies have failed to document the pressure dosages essential for the observed effects. Not until recent years have the first research team, Reno and his colleagues (2001; 2002; 2003; 2005), started to report their *in-vitro* findings together with the defined pressure dosage. By compressing a scar biopsy with 35mmHg for 24 hours, the researchers have demonstrated compression effects on some enzymes expression, and on cytokines release and cellular apoptosis; however, such high a level of pressure is practically not possible for patients to withstand. It is therefore essential to investigate the cellular responses under lower pressure magnitudes.

4.3 OBJECTIVES OF PHASE II OF THE STUDY

Based on above description, this phase of the study was designed to investigate the direct effects of pressure loading on the biological behaviours of fibroblasts extracted from hypertrophic scar tissue.

The objectives of this phase of the study were:

- to elucidate the effects of pressure loading and unloading on fibroblast proliferation;
- to investigate the effects of pressure loading and unloading on fibroblast-myofibroblast differentiation; and
- to determine if fibroblast would respond in different manners under various levels of pressure.

4.4 MATERIALS AND METHODS

4.4.1 Cell Culture

Primary cultures of human dermal hypertrophic scar fibroblasts were obtained from patients who were consented to extract the hypertrophic scar tissue post-surgical removal. The fibroblast cultures were maintained in complete Dulbecco's Modified Eagle's Medium (DMEM). It was supplemented with 10% fetal bovine serum (FBS) and antibiotic solution containing penicillin (50U/ml) and streptomycin (50g/ml). Routine sub-culturing of hypertrophic scar fibroblast of 90-100% confluency was performed by removing the old medium, washing with sterile phosphate buffered saline (PBS), and trypsinizing with 0.25% trypsin-EDTA for one minute. The activity of trypsin was then inactivated by the addition of complete medium and the trypsinized cells collected in a centrifuged tube were centrifuged at 300g for five minutes. After centrifugation, the supernatant containing the neutralized trypsin and medium was discarded and the cell pellet at the bottom of the tube was re-suspended with fresh complete DMEM and appropriate cell numbers were added into culture dishes. The cell culture was conducted at 37°C in a humidified atmosphere of 95% air and 5% CO2. All the cell culture reagents were purchased from Invitrogen.

4.4.2 Mechanical Loading Assay

Adopting the idea of pressure loading on monolayer cells in previous studies (Mitsui, Suzuki, Macno, Mayahara, Yanagisawa, & Otsuka, 2005; Mitsui, et al., 2006a; 2006b; Yanagisawa, Suzuki, Mitsui, Koyama, Otsuka, & Shimizu, 2007), a schematic diagram of the current experiment was sketched in Figure 4.2. The hypertrophic scar fibroblast

cells were plated onto a 13mm cover-slip inside 24-well plates at a density of 2 x 10^4 cells/well. After overnight incubation, the cover-slips were placed into a new culture well in an upside down position. Another clean cover-slip of the same size was placed on top of it, followed by the application of compressive force. The amount of pressure was achieved by the addition of standard weights of different masses (2g, 5g, 10g as tabulated in Table 4.1), which had been sterilized at 160^{o}C for two hours. The cells were then compressed continuously for two days. For control experiment, the cells were only covered with a cover-slip without any weights. After two-day incubation, the standard weights were carefully removed without disturbing the cells underneath. The medium was then removed and the cells were washed with PBS before fixed with 4% paraformaldehyde in PBS for 10 minutes. For unloading experiment, the medium was replaced with fresh medium and the cells were further cultured for two more days before fixation. The fixed cells on the cover-slips were stored at 4°C for further immunostaining test.

Table 4.1 Unit conversion of the weight mass into millimeter mercury (mmHg) by Equation 4.1

Weight mass	Applied pressure (mmHg)
0g	0
2g	0.833192
5g	2.08298
10g	4.165959

$$\text{Applied Pressure (mmHg)} = \frac{\text{Force}}{\text{Area}} = \frac{\text{Weight (kg) X } 9.81\text{m/s}^2}{\text{(Contact surface area) mm}^2} \text{ x } 7500.637554$$

(Equation 4.1)

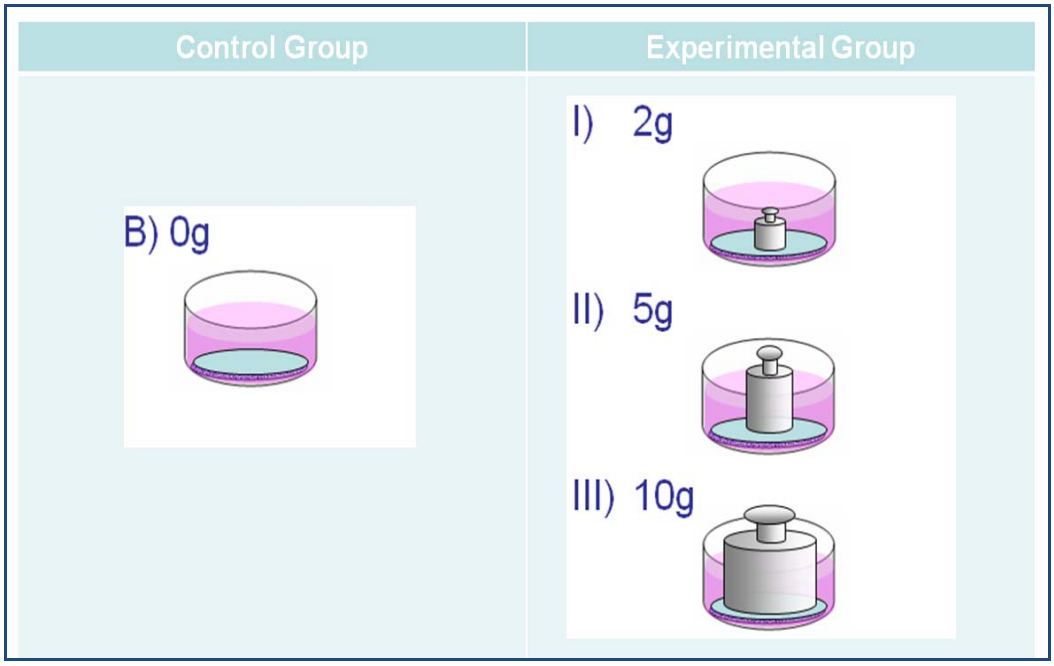


Figure 4.2 The schematic diagram of the experimental setup

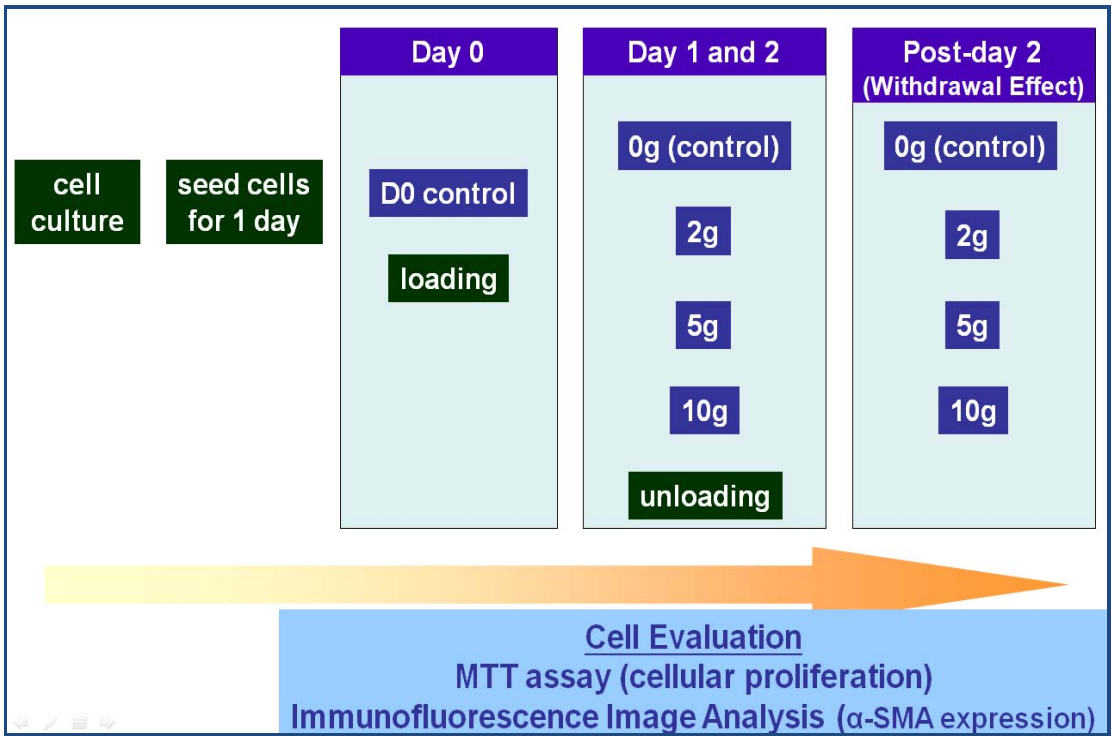


Figure 4.3 Experimental design of the *in-vitro* study

4.4.3 Fibroblast Proliferation - Cell Proliferation Assay

In order to determine the proliferation of hypertrophic scar fibroblast cells under mechanical loading, MTT-based cell proliferation assay was performed on 24 hours after initial plating (Day 0), 48 hours after mechanical loading (Day 2) and 48 hours after unloading (Post-day 2). The assay was based on the cleavage of the yellow tetrazolium salt MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) to purple formazan crystals by metabolic active cells, in which the formazan crystals formed were solubilized and the resulting colored solution was quantified using micro-plate reader. A 50µl of MTT (5mg/ml, Roche Applied Science) solution was added to each cell-containing well containing 500µl of medium (i.e. final concentration of MTT was 0.5mg/ml in complete medium) and the cells were allowed to incubate at 37°C in a 5% CO_2 incubator for two hours in dark. After replacing the MTT-containing medium with 200µl of Dimethyl Sulfoxide (DMSO), the plate was subjected to gentle shaking for 15 minutes at room temperature until the purple precipitates were dissolved. Cell viability was then assessed by measuring the absorbance at 570nm using a Victor^3 Multi-label plate reader (Perkin Elmer). Hypertrophic scar fibroblast cells under each mechanical loading condition were measured at least in triplicates.

4.4.4 Fibroblast Differentiation (Myofibroblast) - Fluorescence Immuno-cytochemistry

The fixed cells on the cover-slips were first treated with 0.2% Triton X-100 in PBS for 20 minutes to enhance permeabilization for the subsequent antibodies incubation. The cells were then washed with PBS (3 times x 5 minutes per wash) and incubated with

blocking solution containing 5% normal horse serum in PBS for at least 30 minutes to prevent non-specific binding, followed by two-hour incubation of primary antibody (monoclonal anti-α−smooth muscle actin antibody, Sigma Aldrich) at room temperature. After incubation with primary antibody, the cells were again washed with PBS and further incubated with fluorescence dye-conjugated secondary antibody (Alexa Fluor 555-conjugated donkey anti-mouse antibody, Invitrogen) for an hour at room temperature. After that, the cells underwent another washing with PBS and the cover-slips were mounted onto a glass slide with anti-fade mounting reagent containing DAPI (Invitrogen) to counterstain nucleus.

Images were captured using a fluorescence microscope (Zeiss) installed with a digital camera. A total of 10 visual fields were randomly selected and the images of both the expression of α−smooth muscle actin and DAPI staining of each single visual field were captured with 40X magnification for cell counting. The percentage of myofibroblast was expressed as the number of myofibroblasts to the total number of nuclei counted in the 10 visual fields. Only the α-smooth muscle actin (α-SMA) immunopositive cells displaying the characteristics of myofibroblast-like morphology were counted as myofibroblasts.

4.4.5 Statistical Analysis

Statistical differences in fibroblast proliferation (cellular proliferation assay) and fibroblast differentiation (fluorescence immuno-cytochemistry) were determined by analysis of variance (ANOVA). The results were considered significant when the p-

value was less than 0.05. Fisher LSD post-hoc comparison analysis was employed to compare the differences among groups. To protect against a type I error, Bonferroni correction (0.05 / 6 = 0.008) was performed for adjustment of significance level. All data were presented with the means with error bars of standard error of measurement (SEM).

4.5 RESULTS

4.5.1 Fibroblast Proliferation - Cell Proliferation Assay

Comparison of fibroblast proliferation under four pressure magnitudes was made by ANOVA analysis and the rate of cell proliferation was calculated by the slope of gradient listed below in Equation 4.2. The steeper the slope, the higher the rate of proliferation of hypertrophic scar fibroblasts.

$$m = \frac{\Delta y}{\Delta x} = \frac{y_2 - y_1}{x_2 - x_1}$$

m = slope of the cell proliferation
y = y coordinate of the graph
x = x coordinate of the graph

(Equation 4.2)

Statistical significance among the groups by ANOVA analysis was obtained on Day 2 ($F = 5.371$, $p = 0.007$). As shown in Figure 4.4, the control group (0g) demonstrated the highest level of proliferation compared with all the pressure groups after a two-day pressure loading. The control group had a slope of 0.093^{o} at Day 2 assessment, while

that for the 2g and 5g loading groups was 0.05^{o} and 0.041^{o} respectively. Fisher LSD post-hoc comparison analysis revealed a marked inhibition on fibroblast proliferation under 10g loading ($p = 0.001$). These cells underwent inhibited proliferation with an almost horizontal line (0.007^{o}). It was clear from the result that the inhibition was dose-dependent, in which higher mechanical pressure induced a more significant inhibition on the cell proliferation as compared to lower pressure levels.

The weights were removed (unloading) for another two days to investigate the withdrawal effect of pressure on fibroblast proliferation. The control group (0g) continued to proliferate as demonstrated by its highest MTT absorbance. The graph displayed in Figure 4.4 showed a gentle rate of proliferation in cells previously subjected to 2g loading (slope = 0.053^{o}). Cells previously subjected to 5g loading also proliferated steadily during the unloading period. There was no apparent difference in the rate of proliferation during unloading among the groups of 0g, 2g and 5g loading as reflected by the slopes of the MTT curves, however the slope of 10g loading appeared less steeper as compared to the other groups. Significant inhibition was consistently observed at Post-day 2 for cells subjected to 10g loading.

Table 4.2a Fibroblast proliferation under different loading magnitudes (MTT assay)

Time Interval	Weight	Mean Absorbance	SEM	Min	Max	Slope
Day 0		0.263	0.005	0.249	0.282	
Day 2	0g	0.356	0.006	0.338	0.372	0.093^{o}
	2g	0.313	0.019	0.251	0.348	0.05^{o}
	5g	0.304	0.022	0.228	0.355	0.041^{o}
	10g	0.270	0.013	0.211	0.298	0.007^{o}
Post-day 2	0g	0.412	0.006	0.385	0.426	0.056^{o}
	2g	0.366	0.010	0.340	0.400	0.053^{o}
	5g	0.352	0.009	0.324	0.380	0.048^{o}
	10g	0.294	0.020	0.231	0.343	0.031^{o}

Table 4.2b Fisher LSD post-hoc comparison for fibroblast proliferation

Time Interval	Variables	Mean Difference (I) – (J)	*p*-value
Day 2	0g (I) – 2g (J)	0.043	0.094
	0g (I) – 5g (J)	0.051	0.048
	0g (I) – 10g (J)	0.086	0.001*
	2g (I) – 5g (J)	0.008	0.736
	2g (I) – 10g (J)	0.043	0.073
	5g (I) – 10g (J)	0.034	0.143
Post-day 2	0g (I) – 2g (J)	0.046	0.016
	0g (I) – 5g (J)	0.060	0.003*
	0g (I) – 10g (J)	0.118	< 0.001*
	2g (I) – 5g (J)	0.014	0.446
	2g (I) – 10g (J)	0.072	0.001*
	5g (I) – 10g (J)	0.058	0.004*

**Statistical significance with $p \leq 0.008$*

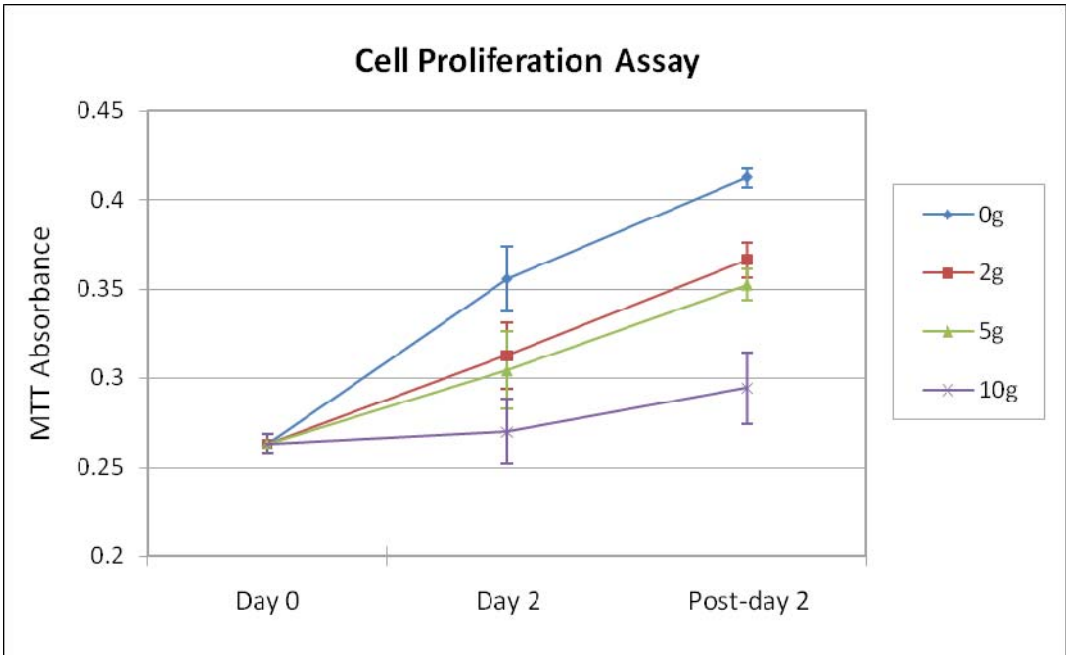


Figure 4.4 Fibroblast proliferation demonstrated by MTT assay

4.5.2 Fibroblast Differentiation (Myofibroblast) - Fluorescence Immuno-cytochemistry

Fluorescent microscopy revealed dense microfilamentous actin among the cells, however, only the cells with the characteristics of myofibroblast-like morphology were counted as myofibroblasts. Table 4.3 tabularizes the mean percentage of myofibroblasts versus normal fibroblasts. The ANOVA analysis revealed significant differences among the groups at Day 2 ($F = 17.690$, $p = 0.009$) and Post-day 2 ($F = 23.091$, $p = 0.005$). Photo representatives of cells under various conditions with 60X magnification were presented in Figures 4.5 to 4.7.

Table 4.3 Percentage of myofibroblasts expressed by α-smooth muscle actin under different loading magnitudes

Time Interval	Weight	Mean (%)	SEM (%)	Min (%)	Max (%)
Day 0		2.72	0.09	2.63	2.80
Day 2	0g	18.01	2.92	15.09	20.92
	2g	7.74	2.05	5.69	9.78
	5g	3.06	0.80	2.26	3.85
	10g	0.68	0.00	0.68	0.68
Post-day 2	0g	24.56	4.21	20.35	28.77
	2g	13.68	0.35	13.33	14.02
	5g	15.60	0.00	15.60	15.60
	10g	0.00	0.00	0.00	0.00

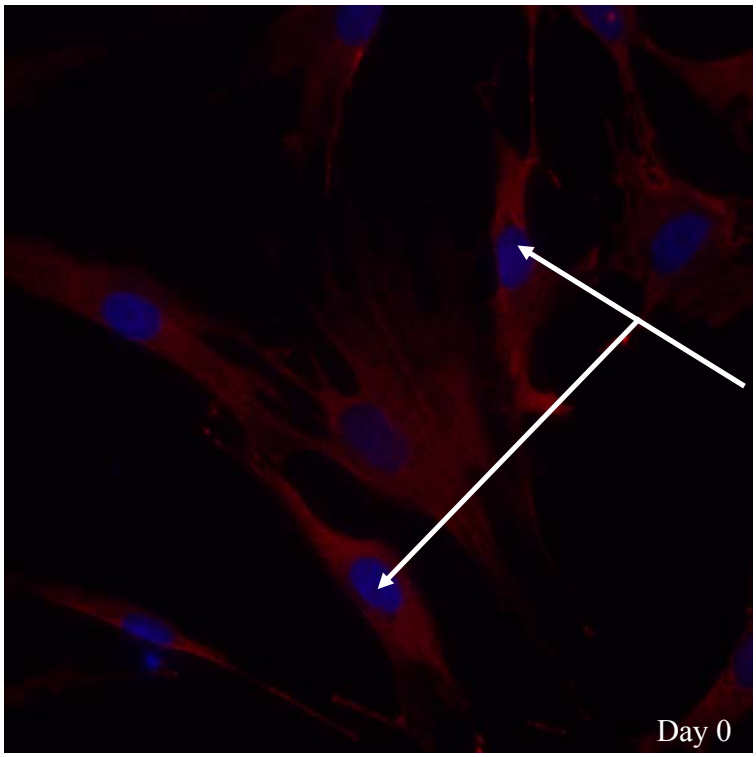


Figure 4.5 α-SMA immunofluorescence in hypertrophic scar fibroblasts (Day 0)

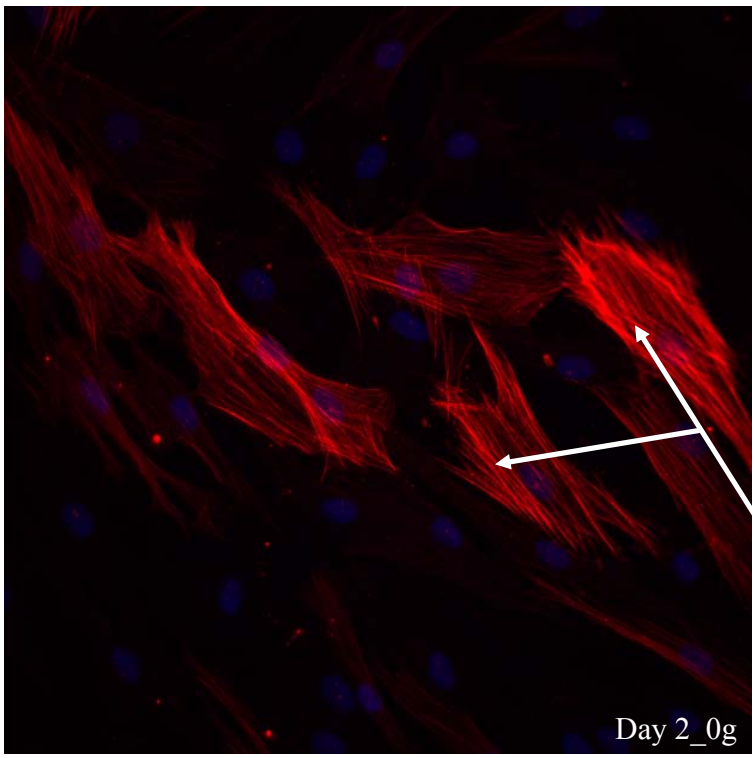


Figure 4.6a α-SMA immunofluorescence in hypertrophic scar fibroblasts (Day 2_0g)

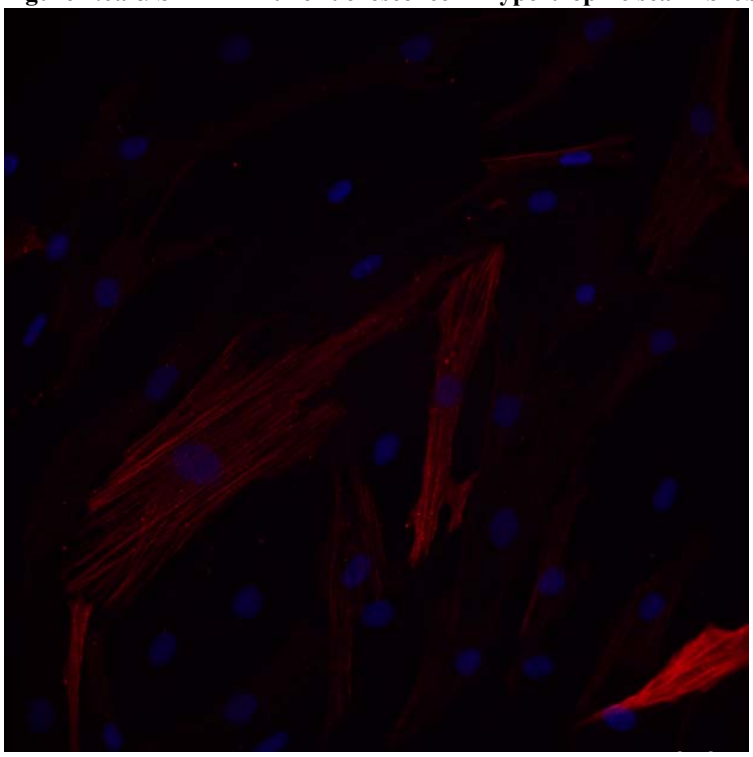


Figure 4.6b α-SMA immunofluorescence in hypertrophic scar fibroblasts (Day 2_2g)

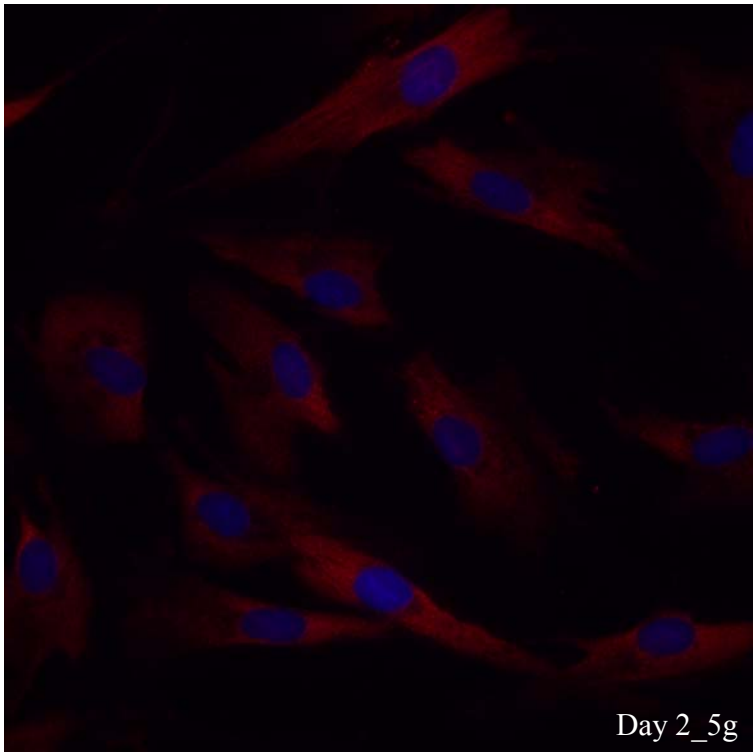


Figure 4.6c α-SMA immunofluorescence in hypertrophic scar fibroblasts (Day 2_5g)

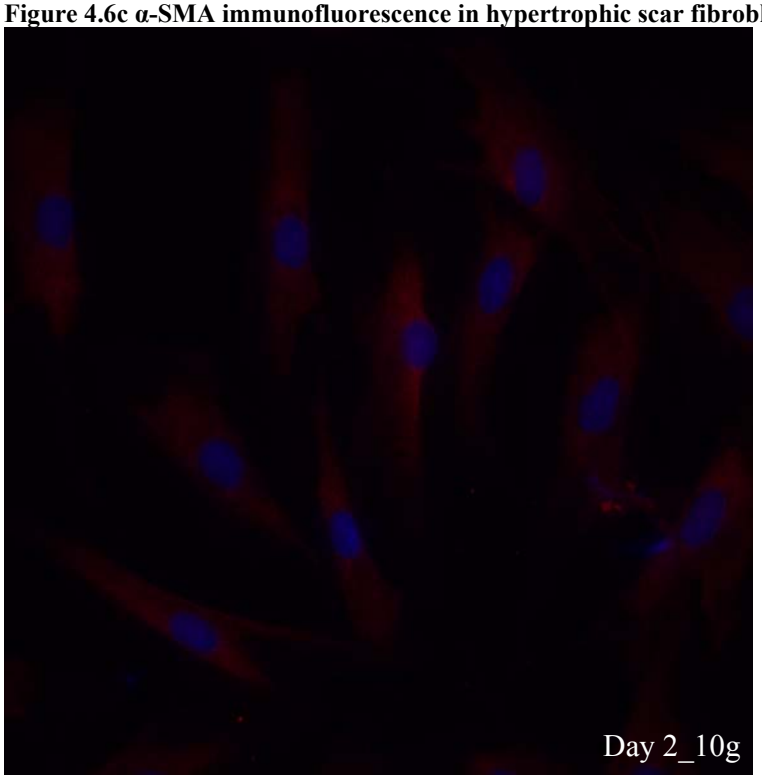


Figure 4.6d α-SMA immunofluorescence in hypertrophic scar fibroblasts (Day 2_10g)

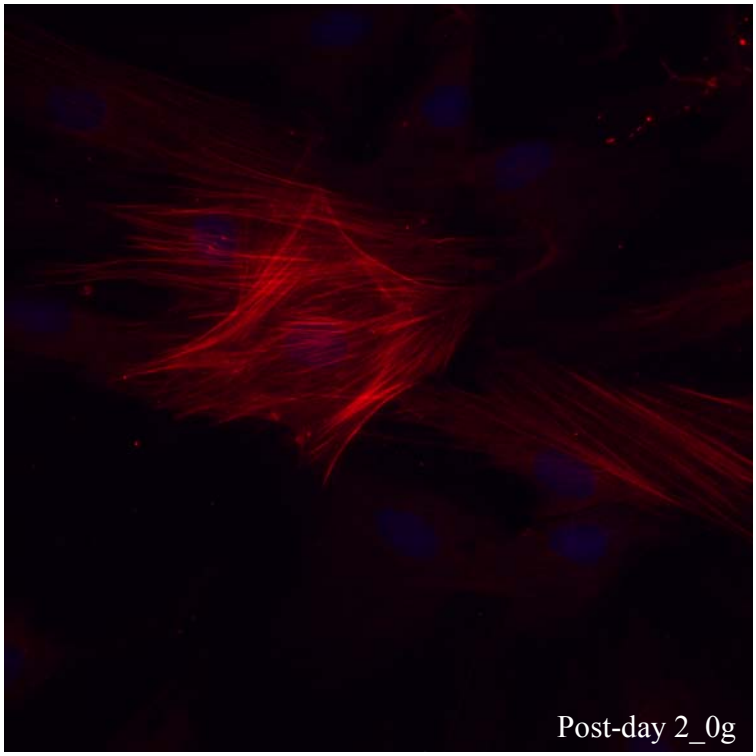


Figure 4.7a α-SMA immunofluorescence in hypertrophic scar fibroblasts (Post-day 2_0g)

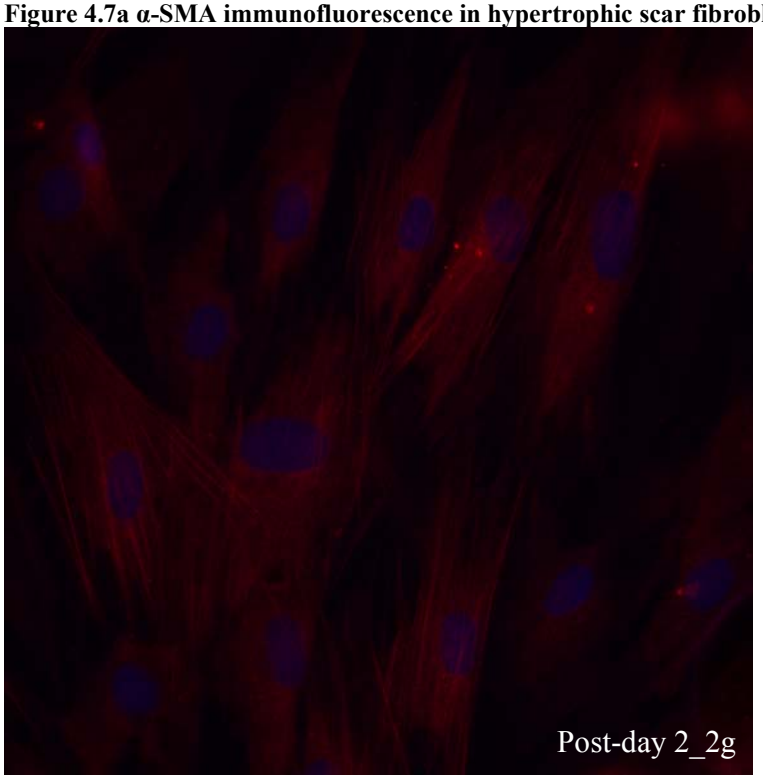


Figure 4.7b α-SMA immunofluorescence in hypertrophic scar fibroblasts (Post-day 2_2g)

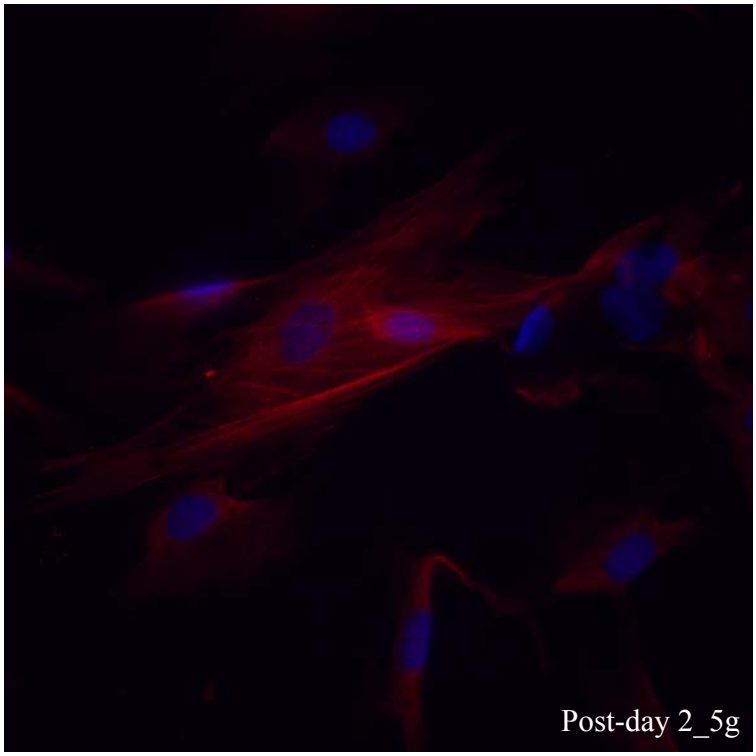


Figure 4.7c α-SMA immunofluorescence in hypertrophic scar fibroblasts (Post-day 2_5g)

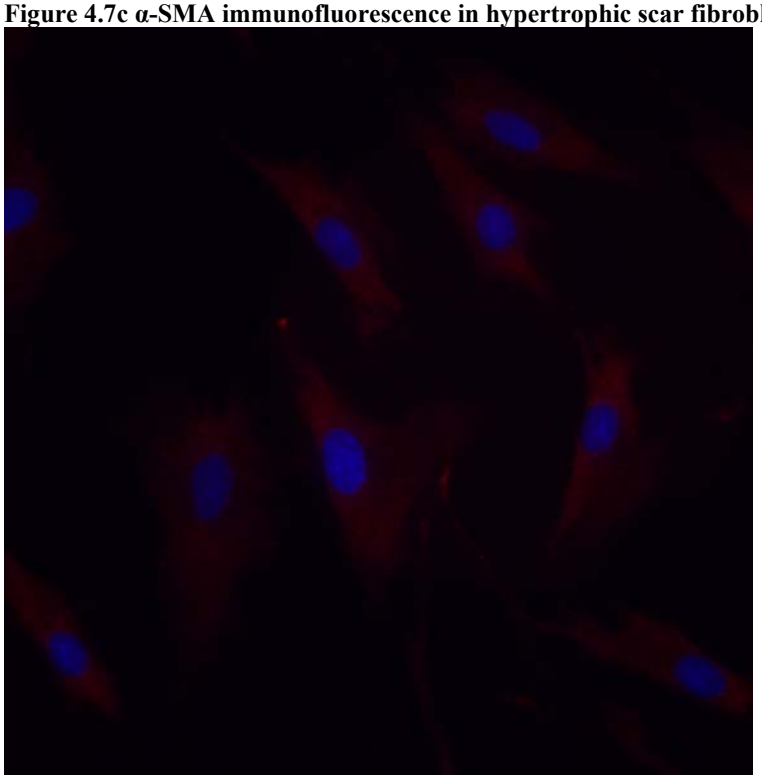


Figure 4.7d α-SMA immunofluorescence in hypertrophic scar fibroblasts (Post-day 2_10g)

The untreated hypertrophic scar fibroblasts (Figure 4.5) had only few myofibroblasts in the seeding cells (Day 0) but continued to differentiate into myofibroblasts with abundant stress fibers as observed at Day 2 and Post-day 2 assessments (Figure 4.6a and Figure 4.7a). Table 4.4 shows that pressure-treated cells exhibited a marked decrease in α-SMA expressing myofibroblasts compared with the untreated cell samples at Day 2. As displayed in Figure 4.6b, the culture subjected to 2g loading, not only showed fewer myofibroblasts, but also had weaker immune-reactivity, expressed in terms of the fluorescence intensity, when compared to the untreated cells. Limited immune-positive myofibroblasts with high intensity could be found under 5g loading (Figure 4.6c). Myofibroblasts in cells subjected to 10g loading as illustrated in Figure 4.6d were rarely observed and the immune-reactivity of the α-SMA in the cells was generally very weak.

Similar trend as illustrated in Figure 4.8 was observed after withdrawing the loadings (Post-day 2). The cells subjected to pressure loadings showed at least 10% reduction in the myofibroblast populations as compared to untreated fibroblasts at Post-day 2. The rate of fibroblast differentiation, however, was varied in different pressure loading groups. The fibroblasts under 2g loading differentiated at a constant rate after two-day loading and unloading (Figure 4.7b). Surprisingly, the differentiated myofibroblasts under 5g boosted up five times after removing the weights. In addition, the immune-positive cells as presented in Figure 4.7c tended to have stronger α-SMA immune-reactivity than those of Day 2. Figure 4.8 illustrates that no myofibroblasts were found under 10g loading at Post-day 2.

Table 4.4 Fisher LSD post-hoc comparison for fibroblast differentiation (myofibroblasts)

Time Interval	Variables	Mean Difference (I) – (J)	*p*-value
Day 2	0g (I) – 2g (J)	10.270	0.016
	0g (I) – 5g (J)	14.950	0.004 *
	0g (I) – 10g (J)	17.325	0.003 *
	2g (I) – 5g (J)	4.680	0.144
	2g (I) – 10g (J)	7.056	0.052
	5g (I) – 10g (J)	2.375	0.409
Post-day 2	0g (I) – 2g (J)	10.885	0.022
	0g (I) – 5g (J)	8.960	0.040
	0g (I) – 10g (J)	24.560	0.001 *
	2g (I) – 5g (J)	- 1.925	0.554
	2g (I) – 10g (J)	13.675	0.010
	5g (I) – 10g (J)	15.600	0.006 *

**Statistical significance with $p \leq 0.008$*

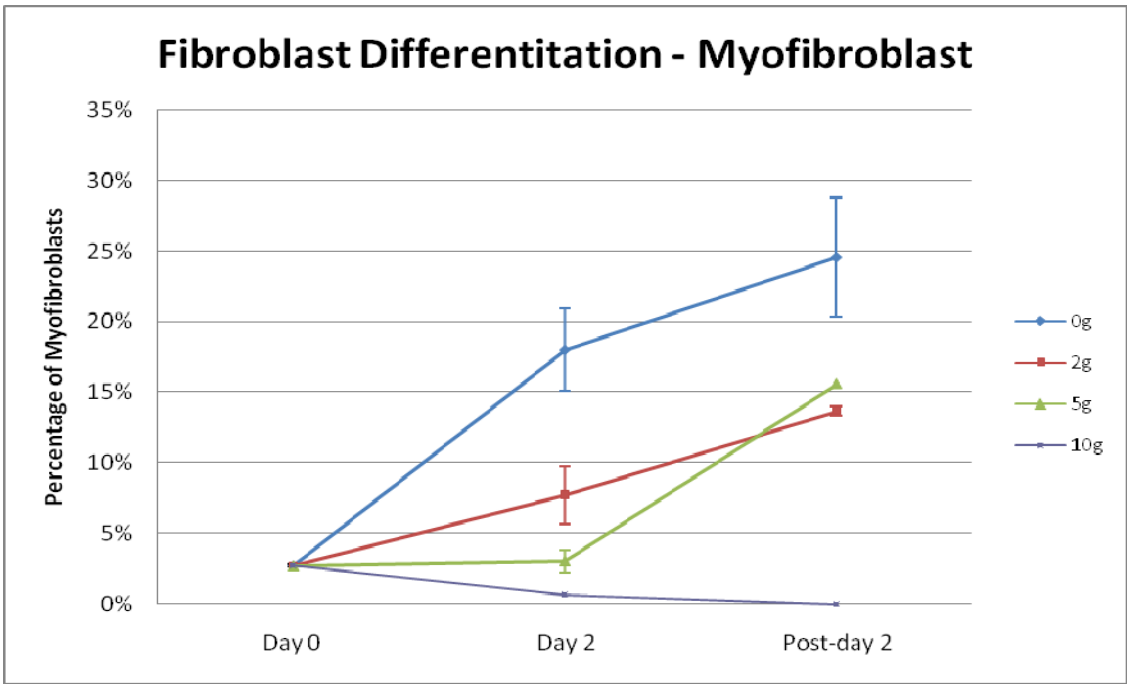


Figure 4.8 Percentage of myofibroblasts expressed by α-smooth muscle actin in various groups across time (Fluorescence immuno-cytochemistry)

4.6 DISCUSSION

Hypercellularity has been assumed as a contributing factor to hypertrophic scarring. It has been thought that any treatment modalities that either counteract the fibroblast accumulation or trigger cellular disappearance could be regarded as effective control for excessive healing. Minimizing fibroblast differentiation into myofibroblasts could help reduce the incidence of scar contracture. Though pressure therapy has been commonly practiced in most clinical settings as a therapeutic modality, much is left unexplored on its underlying mechanisms. This study was therefore conducted to investigate the potential inhibition on fibroblast activities in response to mechanical pressure loading.

The current study was the first to use the cell culture (monolayer) model to investigate the effects of pressure on hypertrophic scar fibroblasts, and to demonstrate the responses of fibroblast behaviors to various pressure loading magnitudes. An *in-vivo* model employed in previous researches with scar biopsies and collected after a period of pressure therapy intervention, was only beneficial to examine the clinical effects of pressure (Baur, Larson, Stacey, Barratt, & Dobrkovsky, 1976; Costa, Peyrol, Porto, Foyatier, & Desmouliere, 1999; Jensen, & Parshley, 1984; Kischer, Shetlar, & Shetlar, 1975) and was insufficient to provide a clear explanation on its underlying mechanisms because of the complicated structures of the scar tissue itself. By using a monolayer culture, this experimental study could provide direct evidence on the relationships between mechanical pressure loading and fibroblast activities. To our knowledge, the present study was also the first attempt to examine the responses of the hypertrophic scar fibroblasts subjected to various pressure loadings. Early studies have mostly

focused on the pressure-induced biological changes of hypertrophic scar tissues without documenting the pressure magnitudes thereof. Only until recently have researchers started reporting the applied pressure magnitude in their studies. Reno and his colleagues (2001; 2002; 2003; 2005) have applied 35mmHg on skin biospies with hypertrophic scarring for 24 hours. Although significant alterations in cytokine release and protease expression in their studies were obtained after mechanical compression, such a high pressure dose is not clinically acceptable and the results should not be used for interpreting effects of lower pressure levels. The relationship between pressure magnitude and biological responses of hypertrophic scar tissue has never been explored in the past.

Proliferation of fibroblasts under different pressure loading

Echoed with previous research (Baur, Larson, Stacey, Barratt, & Dobrkovsky, 1976), this study confirmed the pressure-induced regulation on fibroblast proliferation. A lower rate of cell proliferation in the pressure-treated samples was clearly observed when compared to the untreated ones. More apparent inhibition effects on fibroblast proliferation were seen in cell samples subjected to higher pressure loadings (5g and 10g) in comparison with lower pressures (0g and 2g). The nature of dose-dependent pressure-induced effect on fibroblasts proliferation appeared to be affirmed in the current study (i.e. higher pressure resulted in less fibroblast proliferation). The variations of fibroblast responses under different pressure loadings indicated the requirement of careful pressure monitoring for effective scar management during pressure therapy. Previous *in-vivo* studies have reported that a few months of pressure therapy intervention could reduce

the fibroblast proliferation. Nevertheless, in the present study, even two days' loading was found to have resulted in significant changes under consistent pressure. Since the pressure magnitude was not documented in most of the early *in-vivo* studies, it was not clear whether the pressure applied was sufficient to generate any immediate effects to the cells. Even though the interface magnitude has been reported, very little has been done on the differences between interface and sub-dermal pressure magnitudes created by the pressure garments. It is therefore difficult to predict the interface pressure magnitude required for the sub-dermal modifications. Future establishment on the correlation between pressure on and underneath the skin might be useful for improving clinical protocol of pressure management on hypertrophic scarring.

Only the cells subjected to 10g still showed a slower proliferation rate at Post-day 2 assessment while those in other pressure-treated groups proliferated at a similar rate as the control samples. The upward trend of the pressure-treated samples after unloading could be a clue indicating that short-term pressure application could only result in short time inhibition on fibroblast proliferation. Further study with a more extensive period of pressure loading could provide better evidence and understanding of the correlation between pressure dosage and fibroblast activities.

Myofibroblast under pressure

Observations of the myofibroblasts in the present study were identical to those by previous studies (Baur, Larson, Stacey, Barratt, & Dobrkovsky, 1976; Costa, Peyrol, Porto, Foyatier, & Desmouliere, 1999; Kischer, Shetlar, & Shetlar, 1975). It has been

reported that subsistence of active myofibroblasts with abundant microfilament bundles after wound closure leads to scar contractures (Costa, & Desmouliere, 1998; Dongmin, & Kyung, 2004; Gabbiani, 2003; Hinz, 2007; Kose, & Waseem, 2007; Linares, 1996). Some researchers have observed as well the improvements in scar contractures resulting from pressure therapy (Baur, Larson, Stacey, Barratt, & Dobrkovsky, 1976; Costa, Peyrol, Porto, Foyatier, & Desmouliere, 1999; Jensen, & Parshley, 1984). The results of the current study also demonstrated that mechanical pressure loading inhibited the differentiation of fibroblasts into myofibroblasts. Owing to the unusual high DNA content in hypertrophic scar tissue (Hoppes, & Su, 1971) and inhibitory effect of the α-smooth muscle actin on DNase activity (Lazarides, 1974; Lindberg, & Lazarides, 1974), the reduction of α-smooth muscle actin expressing myofibroblasts after pressure loading might indicate an alteration in the inherent cellular metabolism. Therefore, mechanical pressure might be involved in modulating the DNase activity by down-regulating the expression of α-smooth muscle actin, thus the number of myofibroblasts and eventually minimizing the contractile force to the scar.

The number of myofibroblasts was found inversely related to the degrees of pressure loading; the lower the weight applied on the cell samples, the more immune-positive myofibroblasts were discovered during immunostaining. Strong immune-positive myofibroblasts were rarely observed in the cells exposed to high pressures. This study, however, could only demonstrate the reduced number of myofibroblasts after pressure loading, the pathway for the disappearance of myofibroblasts was not determined. Thus, it remains speculative whether pressure would restrict the differentiation process of

fibroblasts into myofibroblasts or it might induce massive myofibroblast apoptosis. Future studies on the pathway of myofibroblast disappearance could add further knowledge to the mechanisms of pressure therapy.

Limitations of the Study

Because of the unique etiology of hypertrophic scarring to humans, animal models have failed to produce scars analogous to human hypertrophy (Aksoy, et al., 2002; Mast, 1992; Morris, et al., 1997; Polo, Kim, Kucukcelebi, Hayward, Ko, & Robson, 1998). Scar tissues were usually obtained from patients undergoing scar revision several months after scars developed (Engrav, Garner, & Tredget, 2007). Wound repair process, however, varies time to time (Robson, Steed, & Franz, 2001) and gene expression is transient and dynamic during the early stage of wound repair (Cole, Tsou, Wallace, Gibran, & Isik, 2001). The fibroblasts extracted from an established hypertrophic scar in the present study could only address the remedial effect but not the proposed prophylactic effect of pressure therapy. The early gene expression which likely determines the pathological development of hypertrophic scarring was not investigated in the current experiment. Early investigation on gene profiling in hypertrophic scar might provide a thorough understanding of mechanical pressure on the fibrotic process.

Secondly, fibroblasts tested in the current study were extracted from a single scar tissue of an established hypertrophic scar, which limited the generalization of the results to other subjects. The experiment should, therefore, be retested on multiple scar tissues at different developmental stage to observe if heterogeneous cells would react in a

different way. In addition, genetic predisposition is generally believed also to correlate with the extent of scar formation (Aarabi, Longaker, & Gurtner, 2007; Su, Alizadeh, Boddie, & Lee, 1998). It would be interesting to investigate the cellular responses of hypertrophic scar to pressure therapy among various races. In addition, the monolayer culture adopted in this study restricted the generalization of results to human skin, a 3-D culture system may be considered in future studies.

4.7 CONCLUSION

This study, for the first time, demonstrated the inhibition effect of mechanical pressure loading on cell proliferation of hypertrophic scar at cellular level. The results also showed pressure application reduced the amount of myofibroblasts and the effect was dose-dependent, with higher pressure resulting in more significant inhibition as compared to lower pressure. These data, consistent with previous studies, proved that fibroblast proliferation and differentiation could be regulated by mechanical pressure. The differences in hypertrophic fibroblasts in response to various pressure dosages implied that the pressure level for effective scar management should be closely monitored. Although further study is necessary to characterize the phenomenon of pressure-induced effect, these data could explain, at least partially, the effectiveness of pressure therapy on scar controlling as observed in clinical practice. These results could contribute, to a certain extent, to building up the evidence of pressure therapy in hypertrophic scar management.

CHAPTER V

PHASE III OF THE STUDY
CLINICAL INVESTIGATION ON THE EFFECT OF DIFFERENT PRESSURE INTERVENTION ON MATURATION OF HYPERTROPHIC SCAR

5.1 INTRODUCTION

Further to the result of dose-dependent inhibition effect by pressure loading on hypertrophic scar fibroblasts proliferation and differentiation as presented in Chapter IV, a prospective randomized clinical trial was employed to investigate the relationship between the magnitude of pressure and scar clinical presentation was then conducted to find out the optimal pressure range for effective scar control. In this chapter, the treatment effects of low or high dosages of pressure therapy intervention would be compared. Initial assessment was conducted prior to the intervention and the scar progress was monitored on a monthly basis over a five-month intervention period through an objective assessment protocol.

5.2 REVIEW ON PRESSURE THERAPY

Despite profound clinical results of pressure therapy, many studies have failed to demonstrate statistically significant improvements in hypertrophic scarring (Anzarut, et al., 2008; Chang, et al., 1995; Groce, et al., 2001; Moore, E et al., 2000; Mustoe, et al., 2002; Stal, Cole, & Hollier, 2008). Scar tissues with different pliability of underneath tissues respond diversely to the therapy. Scars developed over body parts with more fatty tissue such as those on abdomen and buttock require much higher dosage of pressure than those over bony prominence (Hospital Authority of Hong Kong, 1998). The unavailability of objective instruments for scar progression assessment could be another reason for the disparity. The commonly used Vancouver Scar Scale (VSS) attempts to quantify most of the physical parameters, however, it is criticized as

insensitive to report significant statistical changes of scar progress. By using this subjective measurement (Greenhalgh, 2005; Li-Tsang, Lau, & Liu, 2003; McOwan, Machermid, & Wilton, 2001;), clinicians and researchers might be biased by their treatment outcomes.

Variations in therapeutic effects in previous studies could also be explained by different pressure dose prescriptions. General lack of documentating pressure magnitude in numerous studies hinders research (Atiyeh, 2007; Chang, et al., 1995; Zurada, Kriegel, & Davis, 2006). This often leads to variations in the quality of pressure garments provided to patients, which would cause unexpected outcomes such as complications of body deformity and poor compliance of the patients to garments if excessive pressure is applied (Silfen, et al., 2001) and failure to control scarring when pressure is inadequate (Mustoe, et al., 2002). The lack of frequent checkup of the pressure prescribed also limits the treatment effect because of the deterioration of fabric elastic property (Macintyre, & Baird, 2005). A prominent reduction (50%) in elasticity has been documented after four-week wearing (Cheng, et al., 1983; Giele, et al., 1995). Providing adequate pressure with regular monitoring is hence crucial to therapeutic efficacy (Devlin-Rooney, & James, 2005; Douglas, & Way, 2007).

Even though recent attempts have been made to measure the therapeutic pressure range, the pressure determination remains a challenge probably due to the unavailability of an accurate and reliable instrument (Ferguson-Pell, Hagisawa, & Bain, 2000; Maklewska, et al., 2007; Mann, et al., 1997; Norman, 2004; Van den Kerckhove, et al., 2007).

Theoretically, pressure of at least 24mmHg (Baur, et al., 1976; Larson, et al., 1971) to overcome capillary pressure, is considered crucial for effective therapy. Positive clinical results, however, have been reported with levels as low as 5 to 15mmHg pressure (Cheng, et al., 1983; Reid, et al., 1987; Ward, 1991; Wu, et al., 1996). Giele and his colleagues (1998) reported that 15mmHg is already sufficient to produce a positive scar response. While other studies showed that at least 15mmHg is required to accelerate the maturation process (Reid, et al., 1987; Van den Kerckhove, et al., 2005). With pressures over 40mmHg, maceration and paresthesia may occur within 40 to 50 minutes (Naismith, 1980) while effects of pressure below 10mmHg are minimal (Reid, et al., 1987; Naismith, 1980). There is a great variation in recommended pressure values (ranging from 5 to 40mmHg) by previous studies and thus the optimal level remains inconclusive.

5.3 OBJECTIVES OF PHASE III OF THE STUDY

This phase of the study aimed to determine the relationships of mechanical pressure and the maturation progress of post traumatic hypertrophic scar.

To achieve the aim, the corresponding objectives were:

- to examine the pressure effects on the physiological features of hypertrophic scar;
- to define the optimal range of pressure for effective scar control; and
- to assess the pressure loss of garments over time.

5.4 MATERIALS AND METHODS

5.4.1 Research Design

A randomized clinical trial (RCT) design was adopted to evaluate the therapeutic effects of pressure therapy on hypertrophic scar and to identify the optimal pressure for sound scar management. To reduce the bias on treatment, a double-blinded method was employed, with the rater and individual participants both blinded to the participants' intervention. Informed written consent was obtained from all the participants. Randomization of subjects into groups was achieved by drawing lots.

5.4.2 Selection of Subjects

Convenient sampling was adopted for subject recruitment from Longgang Central Hospital in Shenzhen, China. To achieve better distribution of interface pressure over body parts with less variance in body contour (flat surface) only those with hypertrophic scar developed over extremities were included in this study. Due to the fact that a subject might have multiple scars over the extremities, the number of scar samples, instead of number of participants, was used as the unit during the sample size estimation. Assuming $\alpha = 0.05$, power = 0.8 and effect size = 0.5, a total of 34 scar samples should be involved in this RCT study (Portney, & Watkins, 2000). Taking into account the dropouts, 53 scars which met with the following criteria were recruited in this study:

Inclusion Criteria

- subject must be co-operative and comply well with medical intervention
- formation of hypertrophic scar over extremities (upper or lower extremities)
- size of hypertrophic scar should be at least 4 x 4 cm^2

- active hypertrophic scar fulfilling the scoring criteria of the screening test:
    - total score of Vancouver Scar Scale equal to or higher than 4
    - score of each item of the Vancouver Scar Scale equal to or higher than 1

Exclusion Criteria

- hypertrophic scar with open wound
- scar developed over small joints (such as fingers, chest, back of body or facial regions)
- hypertrophic scar being treated with steroid injections, ultrasound or LASER prior to the study
- subjects with medical conditions that affect wound healing, for instance diabetes mellitus

5.4.3 Treatment

Interface Pressure Magnitudes

To date, optimal pressure range for hypertrophic scar is still unstipulated. To identify the effective pressure range for hypertrophic scar control, two pressure magnitudes were testified in this study together with objective documentation of scar maturation. Considering pressure fluctuations due to movements, a range of 5mmHg rather than an exact value of static pressure were prescribed. Following the protocol of local hospitals, pressure padding was applied for all scars to adjust the pressure level. Based on the result obtained in Chapter III that the means of static pressure for extremities was 10.75mmHg (SD = 2.68), the range of 10 to 15mmHg was set as low pressure at static while 20 to 25mmHg was set as high pressure which reached the capillary pressure. A

maximum static pressure of 25mmHg was designated since pressure could shoot up drastically five times as much as static pressure (Lam, 2008) and numbness was reported within five minutes for arm tube with 27.82mmHg (Li-Tsang, et al., 2005).

Garment Fabric and Padding Materials

Pressure garment used in the current study was made from Lycra, a fabric material containing 60% Nylon and 40% Spandex. Since the type of fabric such as the yarn and knitting, might have potential influence on the acceptance of participants and their compliance (Amsler, & Blattler, 2008), the fabric used in this study was the best fabric selected from the previous study in terms of dimensional stability, air permeability and colorfastness to light (Li-Tsang, Yu, & Lai, 2008). Given that Lycra is a visco-elastic material which might yield with prolonged stretching, the pressure garments were renewed in three months.

Plastazote is one of the most commonly used padding materials in local hospitals for increasing the radius of curvature so as to create localized pressure over the scar (Hospital Authority of Hong Kong, 1998). It is a low density foam material that conforms to heat and pressure (Figure 5.1a). The padding was applied on the scars, and to have a better conformity to the shape of the scar, it was moulded under a heat air gun by a therapist (Figure 5.1b). Considering the poor perspiration of the padding, participants were advised to place cotton clothing in between padding and skin surface to minimize skin irritation.

Figure 5.1a A sample of pressure padding

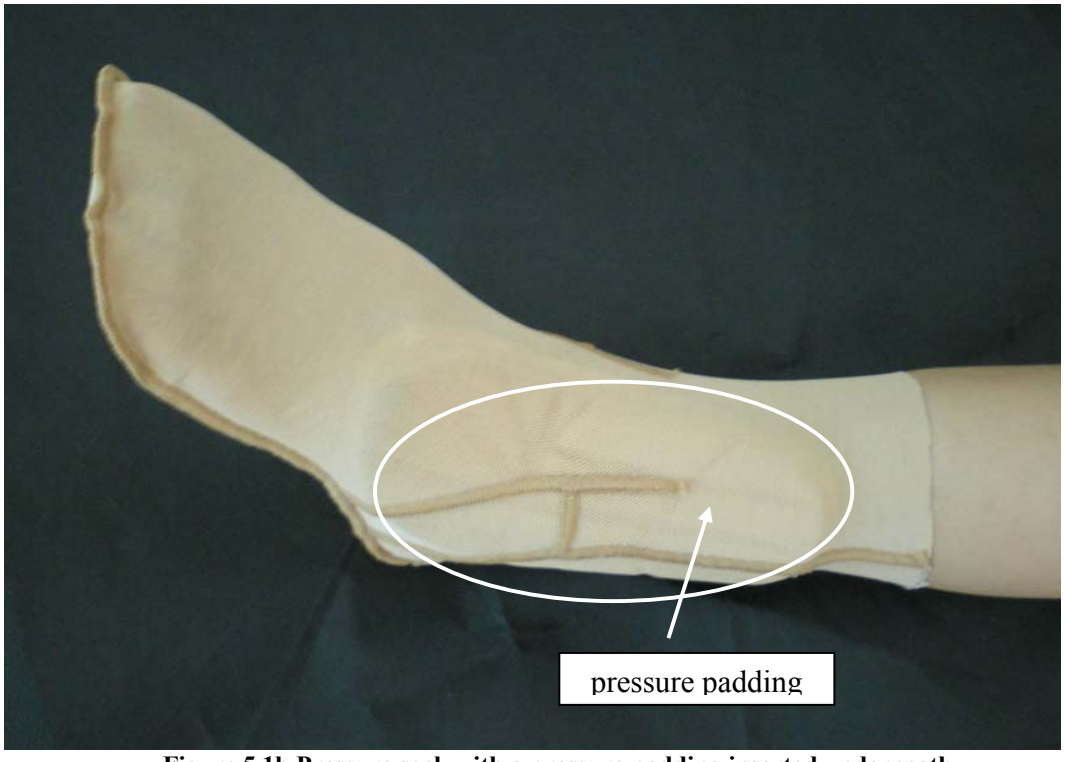


Figure 5.1b Pressure sock with a pressure padding inserted underneath

Treatment Protocol

All garments were fabricated by a therapist in this study with a 5% strain of the circumference of the limb. Every participant was prescribed with two sets of garments and padding for wearing on alternate days. They were instructed to wear the product 23 hours per day except for hygienic measures within participant's tolerance (Puzey, 2002; Su, et al., 1998) and the garments should be washed by hand with soapy water. To differentiate the groups between low (10 to 15mmHg) and high (20 to 25mmHg) pressures, paddings with different thickness were inserted underneath the garment to generate localized pressure. Minor trimming of garment was conducted to attain the assigned pressure magnitude upon fitting. All garments and paddings were renewed after three months of intervention. After fitting the garments, the participants were asked to wear the garment for 15 minutes to check for any complications before they left the department.

5.4.4 Assessment Protocol

Six monthly assessments (pre-test and post-tests) were conducted throughout the five-month intervention. The boundaries of the scar were traced onto clear transparent sheets and then used as templates. These templates were referred to during each assessment to provide measurement consistency. Several measurement points proportionate to the scar area (4 x 4 cm^2 scar area per measurement point) were randomly chosen and the mean value of three measurements per point was used for analysis. In order to stabilize the cutaneous blood flow which might influence the scar presentation, the participants were asked to rest for 15 minutes before the measurements.

Instrumentation

Listed in Table 5.1 were the instruments administered in the current clinical trial. Interface pressure was monitored by the Pliance X System for group differentiation while others were exploited for documentation of the scar remodeling.

Table 5.1 Instruments used in the study

	Instrument	Parameter assessed
Objective measurements	The Pliance X System	▪ Interface pressure
	The Tissue Ultrasound Palpation System (TUPS)	▪ Scar thickness
	The Miniscan XE Plus – Spectrocolorimeter	▪ Scar color in terms of: • lightness • redness • yellowness
Subjective measurements	Vancouver Scar Scale (VSS) (Therapist's measurements)	▪ Scar pigmentation ▪ Scar vascularity ▪ Scar pliability ▪ Scar height ▪ Total score
	Visual Analogue Scale (VAS) (Patient's feedback)	▪ Pain intensity ▪ Pruritus
Others	Digital camera	▪ Scar image
	Measuring tape	▪ Scar width and length

Measurement of Interface Pressure – the Pliance X System

The pressure monitoring system as described in Chapter III was found to be satisfactory for measuring interface pressures between the scar and the garment/padding. Monthly in-situ measurement using the Pliance X System was conducted to ensure attainment of assigned pressure dosage on the scar tissue (Figure 5.2 a & b).

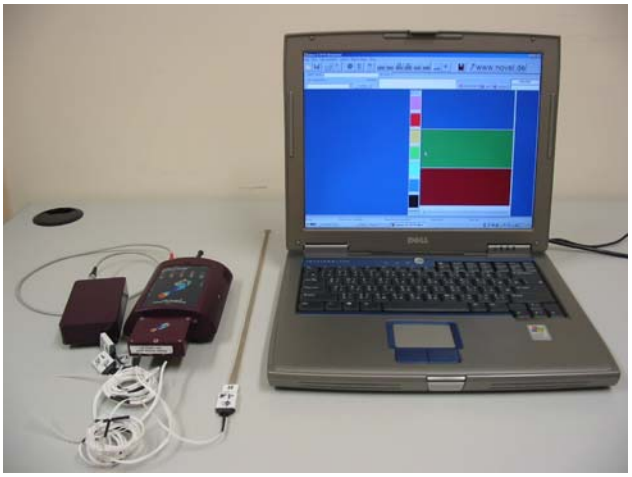


Figure 5.2a The Pliance X System for interface pressure monitoring

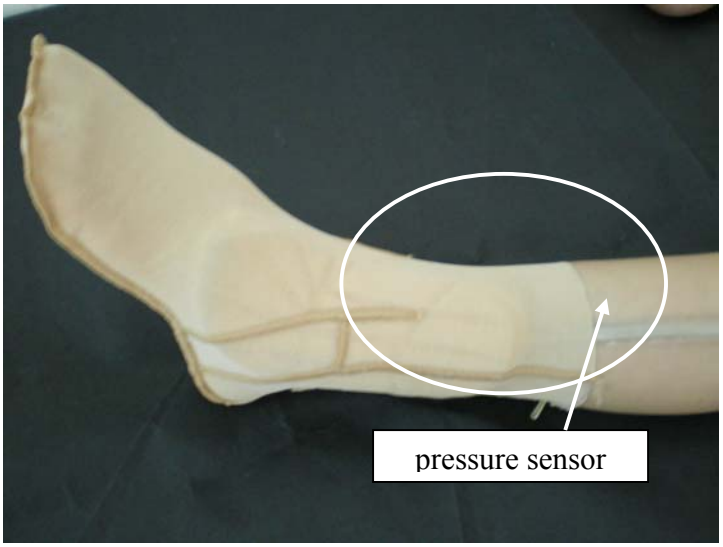


Figure 5.2b Measurement of the interface pressure with the Pliance X System Sensor inserted underneath the pressure garment and padding

Scar Thickness – the Tissue Ultrasound Palpation System (TUPS)

The Tissue Ultrasound Palpation System (TUPS) as displayed in Figure 5.3 was exploited for the monthly measurements of scar thickness. Instead of assessing by subjective hand palpations which might only reflect the superficial layer of scar (elevation of the area of scarring), the TUPS has been recommended for quantitatively documenting the whole scar thickness underneath skin surface (Zheng, Leung, & Mak, 2000; Zheng, & Mak, 1996). The TUPS consists of a micro-processor and pen-size palpation probe with an ultrasound transducer and an in-series load cell. By emitting ultrasound pulse into the scar tissue and receiving the echoed ultrasound and force signals during indentation through the probe, tissue response (deformation) is recorded for calculation of scar thickness. Its high inter-rater (ICC = 0.84), test-retest reliability (ICC = 0.98) and differentiating power in scar assessments have been reported in a previous study (Lau, Li-Tsang, & Zheng, 2005).

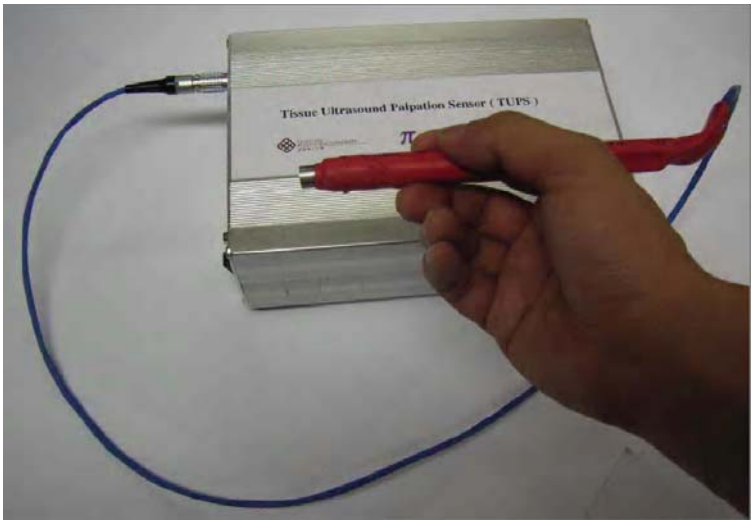


Figure 5.3 The Tissue Ultrasound Palpation System for scar thickness

Scar Pigmentation – the Miniscan XE Plus Spectrocolorimeter

The instrument shown in Figure 5.4 was administrated to quantify the scar pigmentation in terms of lightness, redness and yellowness by comparing with adjacent normal skin. The scoring of scar vascularity and pigmentation by the Vancouver Scar Scale (VSS), however, has been questioned with its poor reliability and low sensitivity to scar progression in many studies (Baryza, & Baryza, 1995; Powers, et al., 1999; Sullivan, et al., 1990; Tyack, Pegg, & Ziviani, 1997). In this study, the spectrocolorimeter was advised for objective documentation for scar color because previous study has demonstrated its strong discriminating ability and satisfactory test-retest and inter-rater reliability with ICC ranging from 0.95 to 0.99 and 0.50 to 0.99 respectively (Li-Tsang, Lau, & Liu, 2003).

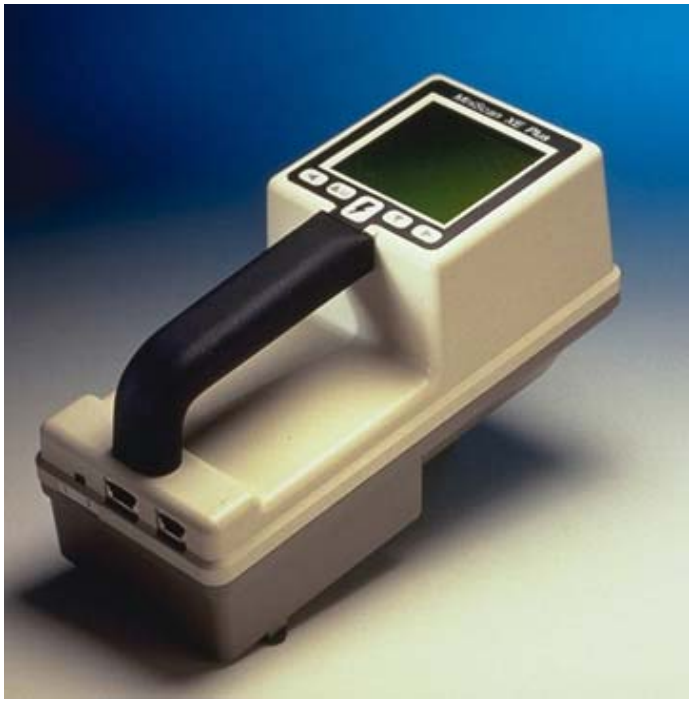


Figure 5.4 Miniscan XE Plus Spectrocolorimeter for scar color evaluation

The difference of color between hypertrophic scar and normal skin, as shown below in Equation 5.1, has been recommended to report as a result of an intervention (Schmidt, et al., 2001).

$$\Delta L^* \text{ (lightness)} = \frac{(L^* \text{ of normal skin} - L^* \text{ of hypertrophic scar})}{L^* \text{ of normal skin}} \times 100\%$$

$$\Delta a^* \text{ (redness)} = \frac{(a^* \text{ of normal skin} - a^* \text{ of hypertrophic scar})}{a^* \text{ of normal skin}} \times 100\%$$

$$\Delta b^* \text{ (yellowness)} = \frac{(b^* \text{ of normal skin} - b^* \text{ of hypertrophic scar})}{b^* \text{ of normal skin}} \times 100\%$$

(Equation 5.1)

Clinical Presentation of Scar – the Vancouver Scar Scale

The Vancouver Scar Scale (VSS), tabulated in Table 5.2, is commonly exercised in clinical settings for assessing scar characteristics in terms of pigmentation, vascularity, thickness and pliability according to rater's perception (Baryza, & Baryza, 1995; Mustoe, 2004). The score is proportionate to the severity of the scar, the higher the score rated, the more severe the scar is. The scale, however, has been complained for its fair inter-rater reliability with Cohen's $\kappa = 0.4$ to 0.5 (Baryza, & Baryza, 1995; Sullivan, et al., 1990), low internal consistency with Cronbach's alpha < 0.5 (Lieneke, et al., 2004), and restricted sensitivity with its descriptive and limited ordinal scorings for each parameter (Nguyen, Potokar, & Price, 2008). It has also been described by many researchers as a subjective guidance since the rating depends wholly on visual inspection and palpation by the rater (Greenhalgh, 2005; Li-Tsang, Lau, & Liu, 2003; McOwan, Machermid, & Wilton, 2001).

Table 5.2 The Vancouver Scar Scale (Baryza, & Baryza, 1995; Mustoe, 2004)

Pigmentation	Vascularity	Pliability	Height
0 = normal	0 = normal	0 = normal	0 = flat
1 = hypopigmented	1 = pink	1 = supple/flexible	1 = 0 – 1mm
2 = mixed	2 = red	2 = yielding to pressure	2 = 1 – 2mm
3 = hyperpigmented	3 = purple	3 = firm/inflexible	3 = 2 – 4mm
		4 = banding/rope like	4 = > 4mm
		5 = contracture	

Intensity of Pain and Pruritus – Visual Analogue Scale (VAS)

Visual Analogue Scale (VAS), a psychometric response scale to measure subject's perspective, was used to measure the intensity of pain and pruritus. The scale is a horizontal line with 100mm in length anchoring by word descriptors at each end (Gould, et al., 2001; Wewers, & Lowe, 1990). Participants in this study were asked to mark on the lines corresponding to the amount of pain and pruritus they perceived of their current status. The score was determined in millimeter by measuring the distance between the left anchor and the marked point. The more discomfort the participant experienced, the longer the distance and thus the higher the scoring was documented.

Scar Image – Digital Recording

A digital camera was used to capture the scar images over the five-month intervention. Photographic evaluation of scar has been questioned with its reliability because of variations in background color and color standard of the images (Davey, Sprod, & Neild, 1999). To minimize these variations, the distance of shooting, the intensity of lighting and background were fixed throughout the study.

Collection of Demographic Data

A record sheet, attached in Appendix III, was used to document the participant's background information including demographic data and medical history, such as age, sex, occupation, cause of injury, location of scar and scar onset. A soft measuring tape was applied to record the scar length and width during the initial assessment for screening purpose.

5.4.5 Statistical Analysis

Descriptive statistics was used to show the results of demographic data and the physiological characteristics of the hypertrophic scar. Comparison of color between hypertrophic scar and adjacent normal skin was made with paired sample t-test while the baseline differences were testified between the groups with Independent sample t-test or Mann-Whitney U test.

Two-way repeated measures analysis of variance (ANOVA) was adopted to compare the effects of the two groups on the changes of scar clinical presentation in the five-month intervention period. Friedman analysis of variance by ranks, a non-parametric test, was operated to evaluate group differences for nominal data. Independent sample t-test or Kruskal-Wallis test was performed to testify the differences between groups at all time intervals. Paired t-test or Wilcoxon signed ranks test was further executed as a post-hoc follow-up test to detect the scar progress of individual groups. To protect against a type I error, Bonferroni correction was performed for adjustment of significance level.

5.5 RESULTS

5.5.1 Demographic Data

Fifty-eight post-traumatic scars in 19 Chinese participants, aged from 15 to 43 years old were successfully recruited. Most of the scars resulted from burns (55.6%), followed by trauma (24.5%), surgery (15.1%) and scald (3.8%) as shown in Table 5.3. The scars had developed for three to nine months (5.21 ± 1.91 months). At last 17 participants (aged 26.23 ± 7.78 years; eight males and nine females) with 53 scar samples finished all the assessments over the five-month intervention period, thus the dropout rate was 8.62%. The 53 samples were randomly assigned into low pressure group (n = 25, pressure = 14.53 ± 1.05mmHg) and high pressure group (n=28, pressure = 23.23 ± 1.11mmHg). Statistical analysis revealed that there were no significant differences (all $p > 0.05$) in the demographic factors between the two groups (Table 5.4).

Table 5.3 Types of injury among the participants

	Participant		Scar Sample	
Type of Injury	**Frequency**	**Percentage**	**Frequency**	**Percentage**
Burns				
• flame burns	1	5.88%	2	3.77%
• explosion	4	23.53%	18	33.96%
• chemical burns	4	23.53%	10	18.87%
Scald	1	5.88%	2	3.8%
Trauma	5	29.41%	13	24.5%
Surgery	3	17.65%	8	15.1%
Total	17	100%	53	100%

Table 5.4 Demographic differences between the two groups (calculated based on scar sample)

	Low Pressure Group	High Pressure Group	Total	*p*-value
Number of samples (%)	25 (46.3%)	28 (51.9%)	53 (100%)	
Age of participants (years)	28.24 ± 8.22	24.43 ± 7.02		0.075[a] *(t-test)*
Scar onset (months)	5.48 ± 2.02	4.96 ± 1.80		0.330[a] *(t-test)*
Gender (%)				0.506 *(Chi-square)*
Male	13 (24.5%)	12 (22.6%)	25 (47.2%)	
Female	15 (28.3%)	13 (24.5%)	28 (52.8%)	
Location of scar (%)				0.884 *(Chi-square)*
Upper limb	14 (26.4%)	12 (22.6%)	26 (49.1%)	
Lower limb	14 (26.4%)	13 (24.5%)	27 (50.9%)	
Cause of injury (%)				0.985 *(Chi-square)*
Burn	15 (28.3%)	14 (26.4%)	29 (54.7%)	
Scald	1 (1.9%)	1 (1.9%)	2 (3.8%)	
Trauma	8 (15.1%)	6 (11.3%)	14 (26.4%)	
Surgery	4 (7.5%)	4 (7.5%)	8 (15.1%)	

[a] *Equal variances assumed*

5.5.2 Physiological Characteristics of Hypertrophic Scar

The results of subjective measurements are tabulated in Table 5.5a. Minimal pain intensity (11.84 ± 9.84mm) from most scars but extreme itchiness with VAS over 70mm from eight scar samples were found.

Objective assessments of scar conditions were conducted with TUPS (scar thickness) and the spectrocolorimeter (scar color). The average scar thickness assessed by TUPS was 5.02mm (SD = 0.98). Paired sample t-test was performed to evaluate the color

differences between adjacent normal skin and hypertrophic scar. As indicated in Table 5.5b, significant differences were obtained in all color parameters including redness, yellowness and lightness (all $p < 0.01$). Hypertrophic scar appeared more reddish but with lower values of lightness and yellowness compared with the adjacent normal skin.

Table 5.5a Subjective measurements of hypertrophic scar

Parameter	Total score (sub-score)	
Vancouver Scar Scale	Pigmentation (3)	2.53 ± 0.70
	Vascularity (3)	2.53 ± 0.64
	Pliability (5)	3.11 ± 0.75
	Height (4)	3.42 ± 0.75
	Total Score (15)	**11.58 ± 1.91**
Visual Analogue Scale (mm)	Pain	11.84 ± 9.84
	Pruritus	48.35 ± 21.25

Table 5.5b Color differences between hypertrophic scar and adjacent normal skin

	Hypertrophic scar	Adjacent normal skin	*t*	*p*-value [a]
L* (Lightness)	44.23 ± 5.39	59.74 ± 4.76	-18.857	<0.01**
a* (Redness)	7.47 ± 1.24	3.86 ± 1.01	19.854	<0.01**
b* (Yellowness)	11.08 ± 2.08	15.58 ± 1.44	-17.472	<0.01**

[a] *Equal variances assumed*

***Statistical significance with p<0.01*

Age Effects of the Scars

Differences in the parameters were noted among scars with different ages, though they were still in an active proliferating stage. The samples were arbitrarily clustered into two types (a) scars with onset less than or equal to six months ($Scar_a$) and (b) scars with

onset over six months ($Scar_b$). In the total of 53 samples, 17 scars (32.1%) were labeled as $Scar_b$ while the other 36 scars (67.9%) belonged to $Scar_a$. Statistical analysis by one-way ANOVA (Table 5.6a) revealed significant differences in scar thickness measured by TUPS ($t_{52} = 2.311$, $p = 0.025$), Δ b* ($t_{52} = -2.544$, $p = 0.014$) and intensity of pruritus ($t_{52} = -6.126$, $p < 0.01$). The result of a non-parametric test Mann-Whitney U Test, indicated no significant differences between the two groups of scars in terms of the VSS score (Table 5.6b).

Table 5.6a Comparison on scar thickness, color and VAS scores between $Scar_a$ and $Scar_b$

Parameter		Mean ± SD		*t*	*p*-value [a]
		$Scar_a$ (≤ 6 months)	**$Scar_b$ (> 6 months)**		
TUPS	Scar thickness	4.82 ± 0.98	5.47 ± 0.85	2.311	0.025 *
Spectrocolorimeter	Δ L*	24.49 ± 9.57	28.65 ± 6.30	1.597	0.116
	Δ a *	96.35 ± 56.54	121.63 ± 54.19	1.513	0.136
	Δ b *	11.54 ± 2.12	10.03 ± 1.58	-2.544	0.014 *
VAS	Pain	12.17 ± 8.81	11.17 ± 12.20	-0.368	0.715
	Pruritus	57.37 ± 17.78	27.50 ± 11.97	-6.126	< 0.01 **

[a] *Equal variances assumed*

* *Statistical significance with p<0.05*

** *Statistical significance with p<0.01*

Table 5.6b Comparison on the parameters by Vancouver Scar Scale between $Scar_a$ and $Scar_b$

VSS	Mean ± SD		*U*	*p*-value
	$Scar_a$ (≤ 6 months)	**$Scar_b$ (> 6 months)**		
Pigmentation	2.51 ± 0.73	2.56 ± 0.63	-0.247	0.805
Vascularity	2.57 ± 0.69	2.44 ± 0.51	-0.926	0.354
Pliability	3.08 ± 0.76	3.19 ± 0.75	-0.399	0.690
Height	3.38 ± 0.76	3.50 ± 0.73	-0.803	0.422
Total Score	11.54 ± 2.06	11.69 ± 1.54	-0.174	0.862

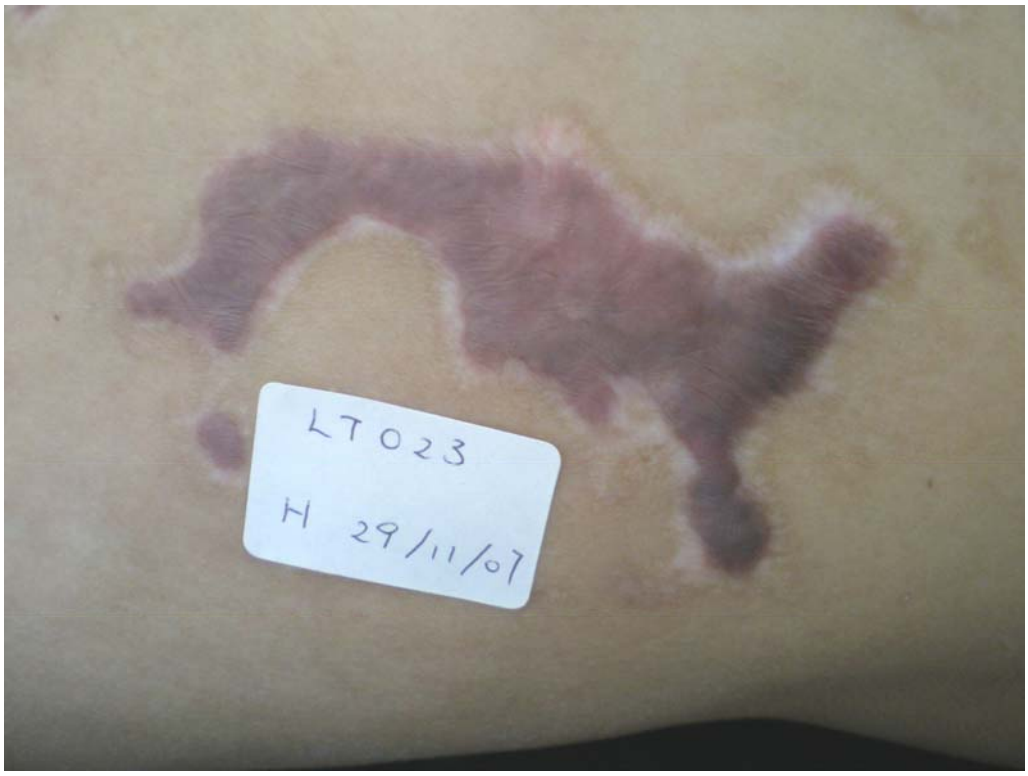


Figure 5.5a Hypertrophic scar with onset ≤ 6 months ($Scar_a$)

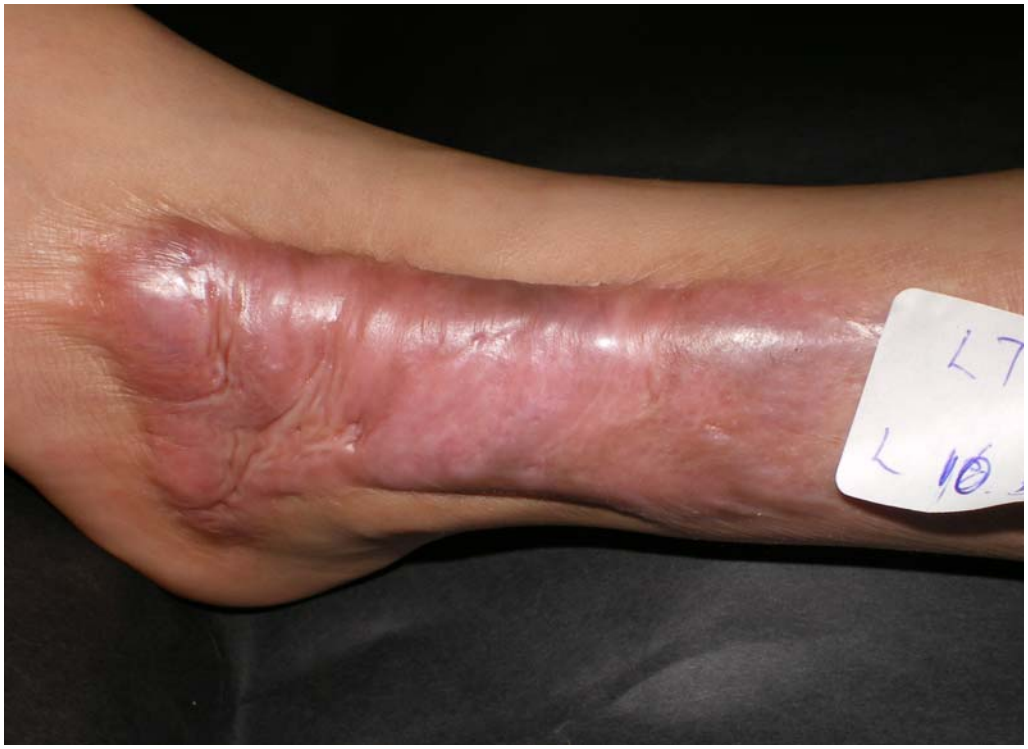


Figure 5.5b Hypertrophic scar with onset > 6 months ($Scar_b$)

5.5.3 Baseline Differences between the Two Groups

The normality assumption of the continuous parameters between the two groups was confirmed with Kolmogrov-Smirnov Normality Test. Parameters with continuous data, namely scar thickness by TUPS, scar color (Δ L*, Δ a* and Δ b*), intensity of pain and pruritus by VAS, were tested under Levene's Test and all continuous parameters passed the homogeneous concept with observed significance level ($p > 0.05$). Independent sample t-test (Table 5.7a) and Mann-Whitney U test (Table 5.7b) showed no statistical differences between the groups in all continuous and categorical parameters.

Table 5.7a Baseline comparisons in the parameters between the groups by Independent Sample T-Test

Parameter	Group	Mean ± SD (mm)	Min	Max	*t*	*p*-value [a]
Scar thickness by TUPS						
	Low pressure	5.10 ± 1.07	3.45	6.88	- 0.592	0.556
	High pressure	4.94 ± 0.90	3.46	6.85		
Scar color by the spectrocolorimeter						
Δ L* (Lightness)	Low pressure	26.71 ± 7.55	8.87	43.78	- 0.750	0.456
	High pressure	24.88 ± 9.89	5.48	44.80		
Δ a * (Redness)	Low pressure	110.68 ± 63.96	5.30	279.64	- 0.812	0.420
	High pressure	98.00 ± 49.38	40.12	199.48		
Δ b * (Yellowness)	Low pressure	10.91 ± 1.90	7.85	14.74	0.575	0.568
	High pressure	11.24 ± 2.25	7.45	15.99		
Visual Analogue Scale (100mm)						
Pain	Low pressure	10.24 ± 7.91	0.4	27.5	1.441	0.156
	High pressure	13.27 ± 11.24	0.8	39.2		
Pruritus	Low pressure	46.13 ± 20.83	17.1	89.7	0.503	0.617
	High pressure	50.34 ± 21.81	11.7	98.5		

[a] *Equal variances assumed*

Table 5.7b Baseline comparisons in Vancouver Scar Scale (VSS) scores between the groups by Mann-Whitney U Test

VSS score (max. score)	Group	Mean ± SD	Min	Max	*Z*	*p*-value
Pigmentation (3)	Low pressure	2.36 ± 0.76	1	3	-1.732	0.083
	High pressure	2.68 ± 0.61	1	3		
Vascularity (3)	Low pressure	2.52 ± 0.65	1	3	-0.072	0.942
	High pressure	2.54 ± 0.64	1	3		
Pliability (5)	Low pressure	2.96 ± 0.74	2	4	-1.425	0.154
	High pressure	3.25 ± 0.75	2	4		
Thickness (4)	Low pressure	3.36 ± 0.81	2	4	-0.350	0.726
	High pressure	3.46 ± 0.69	2	4		
Total score (15)	Low pressure	11.20 ± 1.94	6	14	-1.440	0.150
	High pressure	11.93 ± 1.84	8	14		

5.5.4 Degradation of Interface Pressure

The mean static pressures measured for low and high pressure groups in the initial session were 14.61mmHg (SD = 0.30) and 24.69mmHg (SD = 0.24) respectively. The monthly interface pressure degrading for the two groups was depicted in Figure 5.6 and Table 5.8a. The average pressure decreased over the five-month intervention period for low pressure group was 1.69mmHg (SD = 0.26) while that for high pressure group was 3.53mmHg (SD = 0.38). Minor trimming of garment was provided every month in order to maintain the pressure dose and the garment was renewed at third month. Repeated measures ANOVA revealed statistical significance in the main effect of group ($F = 411.063$, $p < 0.01$) but not in the main effect of time ($F = 1.169$, $p = 0.329$). With significance detected in Mauchly's Test of Sphericity ($p = 0.067$) of the Independent sample t-test as listed in Table 5.8b, the interface pressure degradation for both groups

was found to be significantly different ($p < 0.01$) at all time intervals. Post-hoc paired sample t-test for each group also demonstrated significant pressure change over time (Table 5.8b).

Table 5.8a Statistics for interface pressure changes over time (Independent sample t-test)

	Low Pressure Group		High Pressure Group			
	Mean (mmHg)	SD	Mean (mmHg)	SD	*t*	*p*-value
Post-1 month	- 1.47	0.25	- 3.27	0.36	- 21.110[a]	< 0.01*
Post-2 month	- 1.77	0.22	- 3.64	0.29	- 25.836[a]	< 0.01*
Post-3 month	- 2.07	0.28	- 4.09	0.35	- 22.905[a]	< 0.01*
Post-4 month	- 1.41	0.20	- 3.09	0.29	- 24.238[a]	< 0.01*
Post-5 month	- 1.73	0.15	- 3.58	0.20	- 38.296[b]	< 0.01*

[a] *Equal variances assumed*
[b] *Unequal variances assumed*
** Statistical significance with p<0.01*

Table 5.8b Post-hoc comparison for interface pressure changes over time (paired sample t-test)

	Low Pressure Group		High Pressure Group	
	t_{24}	*p*-value	t_{27}	*p*-value
Post-1 VS Post-2	- 7.525	0.000*	- 7.533	0.000*
Post-2 VS Post-3	- 6.557	0.000*	- 6.822	0.000*
Post-3 VS Post-4	9.949	0.000*	13.426	0.000*
Post-4 VS Post-5	- 7.278	0.000*	- 10.579	0.000*

** Statistical significance with p<0.01*

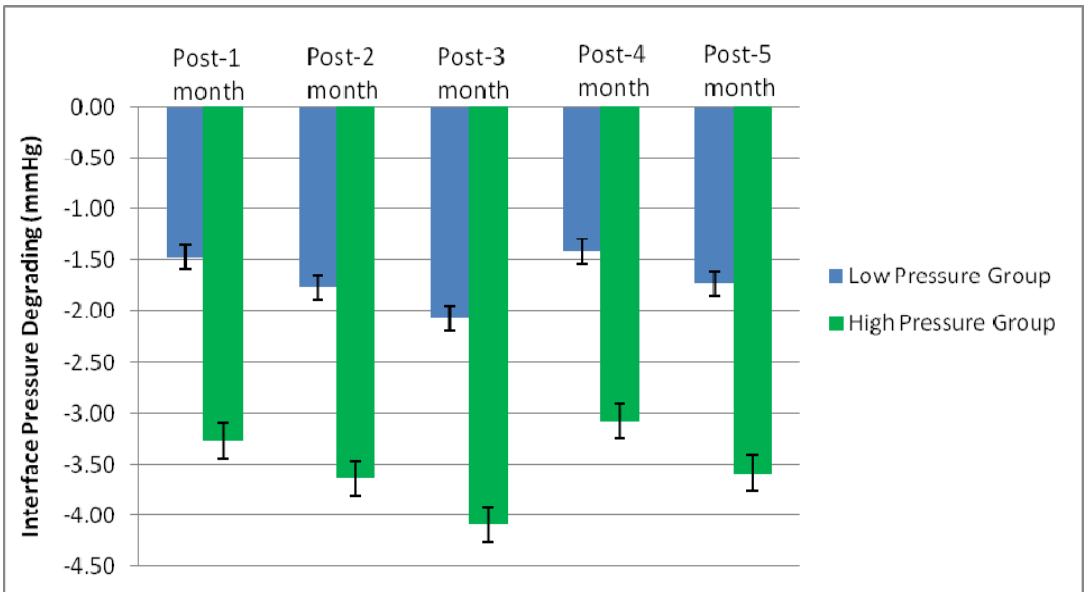


Figure 5.6 Degradation of interface pressure over time

5.5.5 Intervention Effects on Hypertrophic Scar Remodeling

Scar Thickness after Intervention (TUPS)

Two-way repeated measures ANOVA analysis was performed to compare the scar thickness between the groups with high and low pressure magnitudes. Because violation of the assumption of sphericity was indicated in Mauchly's test for time $p < 0.01$, degree of freedom was corrected using Greenhouse-Geisser estimates of sphericity with Epsilon = 0.226.

Overall significant group ($F = 9.44$, $p = 0.003$) and time ($F = 344.362$, $p < 0.01$) effects were obtained. As shown in Table 5.9a, independent sample t-test revealed that high pressure magnitude had superior effects on reducing scar thickness and the most apparent improvement was achieved at the first month of intervention (15.35% of thickness reduction). The overall diminution of scar thickness under high pressure magnitude was 40.05% after the whole session of treatment. The low pressure group also showed significant results, but with a smaller thickness reduction over time and also a lower overall decrease (19.79%).

Graphical illustration of scar progression over time was shown in Figure 5.7. Post-hoc comparisons of the five-month intervention were adopted for individual group evaluation, using multiple paired t-test (Table 5.9b) with the Bonferroni correction used on alpha (0.05/5 = 0.01). Both the groups obtained statistically significant differences for all the measurements ($p < 0.01$).

Table 5.9a Statistics for scar thickness over time (Independent sample t-test)

	Low Pressure Group		High Pressure Group			
	Mean ± SD (mm)	A.C (%)	Mean ± SD (mm)	A.C. (%)	*t*	*p*-value[a]
Initial	5.10 ± 1.07		4.94 ± 0.90		- 0.592	0.556
Post-1 month	4.85 ± 1.04	- 4.89	4.18 ± 0.95	- 15.35	- 2.452	0.018*
Post-2 month	4.64 ± 1.02	- 9.12	3.72 ± 0.98	- 24.77	- 3.340	0.002**
Post-3 month	4.44 ± 1.00	- 12.92	3.39 ± 1.02	- 31.36	- 3.769	< 0.001**
Post-4 month	4.26 ± 1.00	- 16.45	3.14 ± 1.07	- 36.51	- 3.927	< 0.001**
Post-5 month	4.09 ± 1.01	- 19.79	2.96 ± 1.10	- 40.05	- 3.869	< 0.001**

AC: Accumulative Change

[a] Equal variances assumed

**Statistical significance with p<0.05*

*** Statistical significance with p<0.01*

Table 5.9b Post-hoc comparison for scar thickness over time (paired sample t-test)

	Low Pressure Group		High Pressure Group	
	t_{24}	p-value	t_{27}	*p*-value
Initial VS Post-1	9.516	0.000*	20.614	0.000*
Post-1 VS Post-2	10.083	0.000*	16.446	0.000*
Post-2 VS Post-3	10.300	0.000*	10.847	0.000*
Post-3 VS Post-4	9.885	0.000*	10.070	0.000*
Post-4 VS Post-5	10.097	0.000*	11.705	0.000*

**Statistical significance with p<0.01*

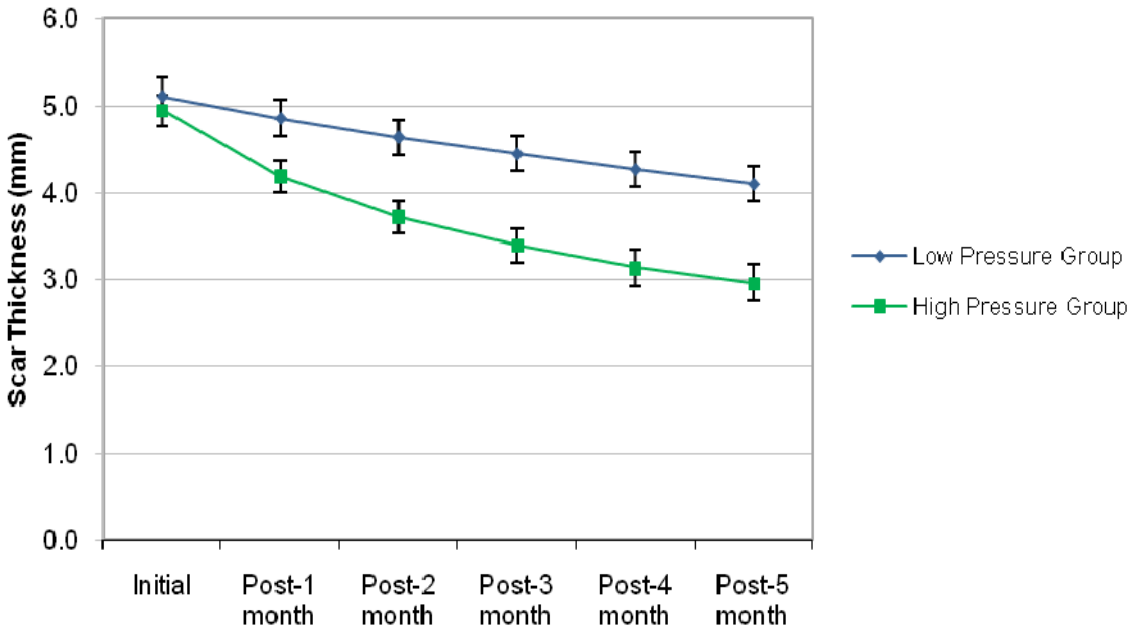


Figure 5.7 Scar thickness by TUPS over the five-month intervention

Scar Color: Differences in Lightness (ΔL), Redness (Δa*) and Yellowness (Δb*)*

a) Lightness (ΔL*)

Differences in scar lightness (ΔL*) under two pressure magnitudes were examined by two-way repeated measures ANOVA analysis with fulfillment of the assumption of sphericity indicated in Mauchly's test at p = 0.689. Significant difference was obtained across time (F = 21.766, p <0.01), but not in group effect (F = 0.583, p = 0.449).

Descriptive data of low and high pressure groups at different assessments are presented in Table 5.10a and demonstrated in Figure 5.8. Independent sample t-test did not show any significance between the groups at all time intervals. However, as shown in Table 5.10b, statistically significant difference was observed in the low pressure group at third month (t_{24} = -2.865, p = 0.009) by using post-hoc paired t-test with Bonferroni corrected alpha (0.05/5 = 0.01).

Table 5.10a Result of scar lightness (ΔL*) over time for the two groups (Independent sample t-test)

	Low Pressure Group		High Pressure Group			
	Mean ± SD (%)	A.C (%)	Mean ± SD (%)	A.C. (%)	*t*	*p*-value [a]
Initial	26.71 ± 7.55		24.88 ± 9.89		- 0.750	0.456
Post-1 month	28.92 ± 11.98	8.25	25.04 ± 13.40	0.63	- 0.100	0.920
Post-2 month	27.61 ± 10.69	3.36	25.96 ± 11.98	4.34	- 0.941	0.351
Post-3 month	30.34 ± 9.88	13.59	28.35 ± 13.84	13.93	- 0.768	0.446
Post-4 month	32.05 ± 10.54	19.96	29.55 ± 15.00	18.74	- 0.749	0.578
Post-5 month	32.11 ± 11.24	20.18	29.50 ± 15.08	18.54	- 0.573	0.599

AC: Accumulative Change

[a] *Equal variances assumed*

Table 5.10b Post-hoc comparison for scar lightness (ΔL*) over time (paired sample t-test)

	Low Pressure Group		High Pressure Group	
	t_{24}	*p*-value	t_{27}	*p*-value
Initial VS Post-1	- 2.364	0.026	- 0.168	0.868
Post-1 VS Post-2	1.506	0.145	- 1.540	0.135
Post-2 VS Post-3	- 2.865	0.009*	- 2.708	0.012
Post-3 VS Post-4	- 2.033	0.053	- 1.578	0.126
Post-4 VS Post-5	- 0.078	0.938	0.072	0.943

**Statistical significance with p<0.01*

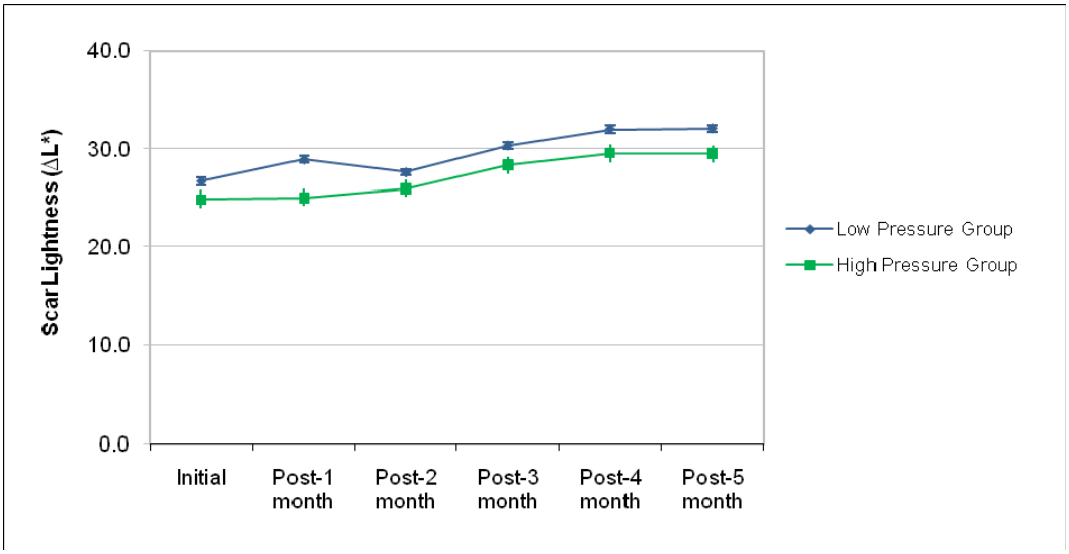


Figure 5.8 Changes of scar lightness (ΔL*) over the five-month intervention for the two groups

b) Redness (Δa*)

Corrected degree of freedom of time factor using Greenhouse-Geisser estimates of sphericity with Epsilon = 0.241 was exercised in repeated measures ANOVA analysis to examine the scar progress in terms of Δa* under two pressure magnitudes. The analysis yielded statistically significant differences in overall time ($F = 195.833$, $p < 0.01$) and group ($F = 4.710$, $p = 0.035$) effect.

The result of scar redness change across the treatment period for the two groups was shown in Table 5.11a and demonstrated in Figure 5.9. Obvious lessening in scar redness (Δa*) was observed in low pressure group at a steady rate from 110.68% to 80.04% with an overall 27.68% deduction. The value in the high pressure group decreased drastically from 98.00% (initial session) to 42.59% (post-5 month session) with an overall 56.54% drop. Table 5.11b illustrates the significant differences between the groups at all times ($p \leq 0.01$) by post-hoc paired t-test with Bonferroni corrected alpha (0.05/5 = 0.01).

Table 5.11a Result of scar redness (Δa*) over time for the two groups (Independent sample t-test)

	Low Pressure Group		High Pressure Group			
	Mean ± SD (%)	A.C (%)	Mean ± SD (%)	A.C. (%)	*t*	*p*-value [a]
Initial	110.68 ± 63.96		98.00 ± 49.38		- 0.812	0.420
Post-1 month	103.34 ± 60.77	- 6.63	76.72 ± 46.86	- 21.72	- 1.796	0.078
Post-2 month	97.02 ± 58.21	- 12.33	63.40 ± 46.07	- 35.30	- 2.344	0.023*
Post-3 month	91.46 ± 56.05	- 17.36	54.35 ± 46.31	- 44.54	- 2.638	0.011*
Post-4 month	86.27 ± 54.09	- 22.05	47.32 ± 47.03	- 51.71	- 2.804	0.007*
Post-5 month	80.04 ± 54.15	- 27.68	42.59 ± 47.74	- 56.54	- 2.676	0.010*

AC: Accumulative Change

[a] *Equal variances assumed*

** Statistical significance with p<0.01*

Table 5.11b Post-hoc comparison for scar redness (Δa*) over time for the two groups (paired sample t-test)

	Low Pressure Group		High Pressure Group	
	t_{24}	*p*-value	t_{27}	*p*-value
Initial VS Post-1	6.506	0.000*	14.411	0.000*
Post-1 VS Post-2	7.340	0.000*	12.264	0.000*
Post-2 VS Post-3	8.066	0.000*	9.617	0.000*
Post-3 VS Post-4	7.609	0.000*	8.655	0.000*
Post-4 VS Post-5	3.985	0.001*	9.219	0.000*

**Statistical significance with p<0.01*

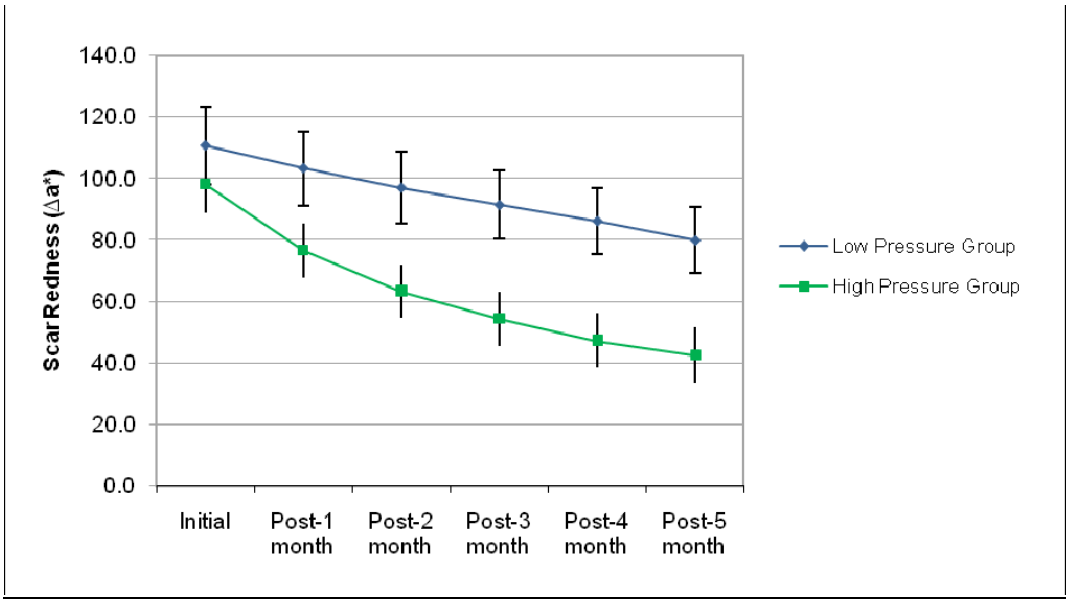


Figure 5.9 Changes of scar redness (Δa*) over the five-month intervention for the two groups

c) Yellowness (Δb*)

Greenhouse-Geisser estimates of sphericity with Epsilon = 0.583 was adopted in correcting degree of freedom during the repeated measures ANOVA analysis on Δb* over time. Statistical significance was obtained only in the main effect of time ($F = 74.444$, $p < 0.01$) but not in overall group factor ($F = 0.364$, $p = 0.549$). Independent sample t-test also revealed no significant differences between the groups at all sessions of treatment (Table 5.12a and Figure 5.10).

Further post-hoc paired t-test with a new alpha using Bonferroni correction (0.05/5 = 0.01) identified that low pressure application generated significant changes at first ($t_{24} = -5.098$, $p < 0.01$) and second ($t_{24} = -3.178$, $p = 0.004$) months while high pressure treatment produced great improvements at second ($t_{27} = -5.146$, $p < 0.01$) and third ($t_{27} = -2.845$, $p = 0.008$) months of intervention.

Table 5.12a Result of scar yellowness change (Δb*) over time for the two groups (Independent sample t-test)

	Low Pressure Group		High Pressure Group			
	Mean ± SD (%)	A.C (%)	Mean ± SD (%)	A.C. (%)	*t*	*p*-value [a]
Initial	10.91 ± 1.90		11.24 ± 2.25		0.575	0.568
Post-1 month	11.07 ± 1.94	1.53	11.31 ± 2.22	0.63	0.407	0.686
Post-2 month	11.30 ± 1.80	3.57	11.63 ± 2.31	3.47	0.329	0.566
Post-3 month	11.40 ± 1.87	4.55	11.79 ± 2.20	4.90	0.543	0.498
Post-4 month	11.49 ± 1.91	5.35	11.86 ± 2.33	5.55	0.354	0.532
Post-5 month	11.53 ± 1.91	5.67	11.95 ± 2.29	6.33	0.379	0.470

AC: Accumulative Change
[a] *Equal variances assumed*

Table 5.12b Post-hoc comparison for scar yellowness (Δb*) over time (paired sample t-test)

	Low Pressure Group		High Pressure Group	
	t_{24}	*p*-value	t_{27}	*p*-value
Initial VS Post-1	- 5.098	0.000*	- 1.994	0.056
Post-1 VS Post-2	- 3.178	0.004*	- 5.146	0.000*
Post-2 VS Post-3	- 1.706	0.101	- 2.845	0.008*
Post-3 VS Post-4	- 2.138	0.043	- 1.284	0.210
Post-4 VS Post-5	- 1.477	0.153	- 2.582	0.016

**Statistical significance with p<0.01*

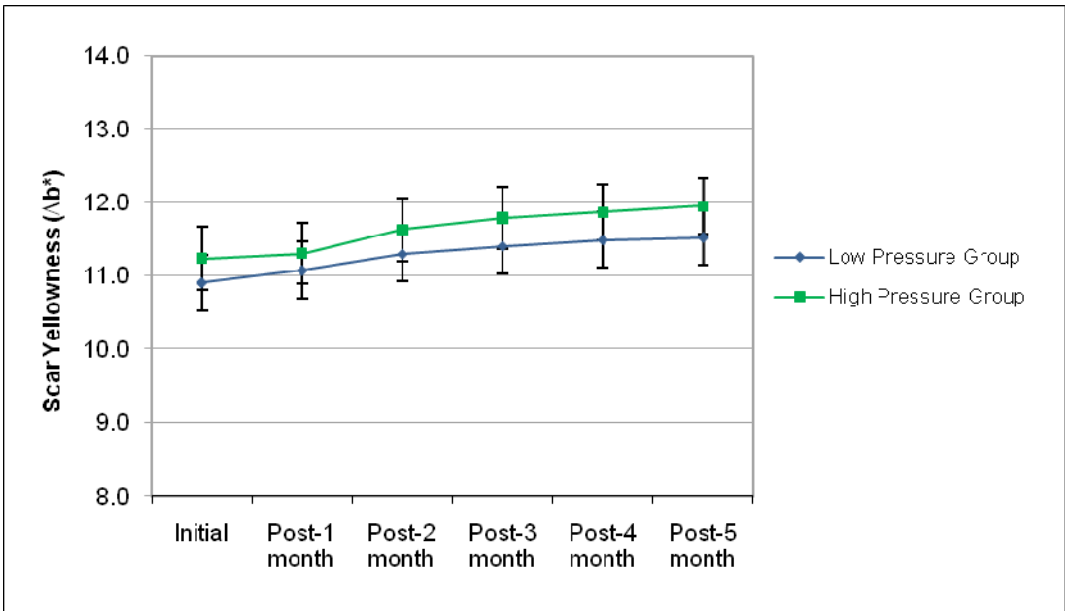


Figure 5.10 Changes of scar yellowness (Δb*) over time for the two groups

Intensity of Pain and Pruritus (Visual Analogue Scale)

a) Intensity of Pain

Repeated measures ANOVA analysis was conducted to examine the changes in pain intensity with corrected degree of freedom for time factor using Greenhouse-Geisser estimates of sphericity with Epsilon = 0.481. The results yielded significant differences only in the overall time ($F = 9.468$, $p < 0.01$) but not group ($F = 1.846$, $p = 0.180$) effects.

As shown in Table 5.13a, no statistically significant difference in pain intensity was identified between the groups at all time intervals by using Independent sample t-test. Further paired t-test revealed that there was only significant decrease in pain at second month caused by high pressure ($t_{27} = 2.779$, $p = 0.01$, Table 5.13b). But for both groups, the pain intensity showed a decreasing trend with time (Figure 5.11).

Table 5.13a Pain intensity over the intervention period for the two treatment groups (Independent sample t-test)

	Low Pressure Group		High Pressure Group			
	Mean ± SD (mm)	A.C (%)	Mean ± SD (mm)	A.C. (%)	*t*	*p*-value
Initial	9.80 ± 8.09		13.66 ± 11.00		1.441	0.156[a]
Post-1 month	8.81 ± 6.39	- 10.11	13.06 ± 10.25	- 4.37	1.788	0.080[a]
Post-2 month	8.16 ± 6.37	- 16.72	11.64 ± 9.63	- 14.77	1.533	0.132[a]
Post-3 month	8.64 ± 6.27	- 11.79	11.24 ± 9.99	- 17.72	1.117	0.269[a]
Post-4 month	8.26 ± 6.85	- 15.70	10.24 ± 10.06	- 25.04	0.827	0.412[a]
Post-5 month	7.41 ± 5.88	- 24.40	10.00 ± 9.97	- 26.82	1.165	0.250[b]

AC: Accumulative Change

[a] *Equal variances assumed*

[b] *Equal variances not assumed*

Table 5.13b Post-hoc comparison for pain over time (paired sample t-test)

	Low Pressure Group		High Pressure Group	
	t_{24}	*p*-value	t_{27}	*p*-value
Initial VS Post-1	1.230	0.231	0.615	0.544
Post-1 VS Post-2	1.393	0.176	2.779	0.010*
Post-2 VS Post-3	- 1.877	0.073	1.164	0.255
Post-3 VS Post-4	0.845	0.406	2.232	0.034
Post-4 VS Post-5	1.629	0.116	0.651	0.521

**Statistical significance with p<0.01*

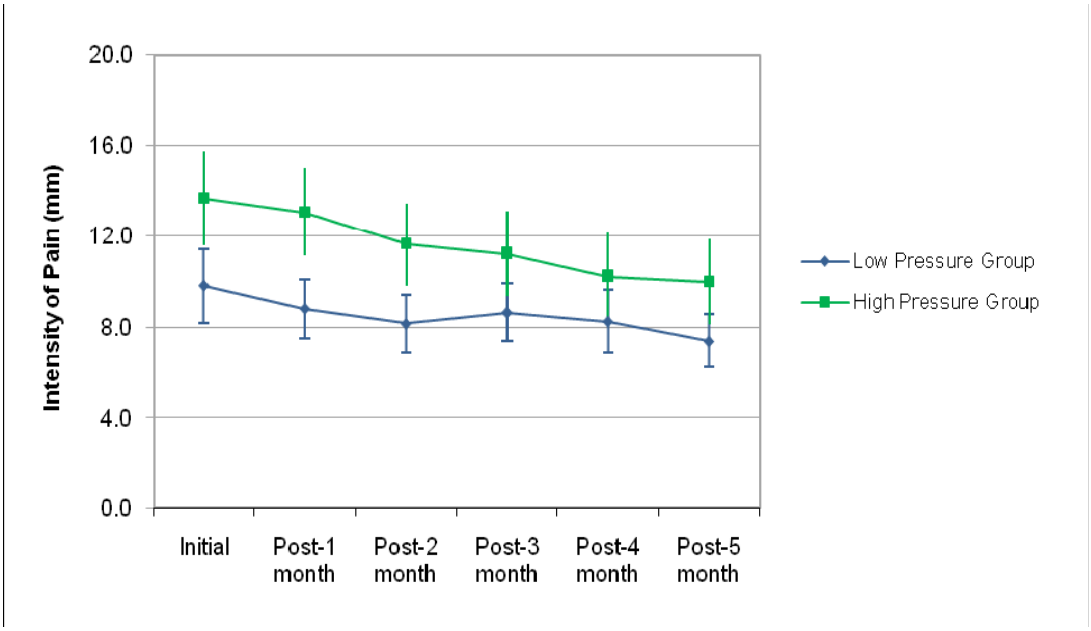


Figure 5.11 Progression of pain intensity (VAS) over time for the two treatment groups

b) Intensity of Pruritus

Greenhouse-Geisser estimates of sphericity with Epsilon = 0.491 was used to correct the degree of freedom for time factor during the repeated measures ANOVA analysis for pruritus. The main effect of time was found significant ($F = 14.398$, $p < 0.01$) but not the group effect ($F = 0.452$, $p = 0.504$).

A decreasing trend of pruritus across the treatment session by low pressure, though statistically insignificant, was noted by Independent sample t-test as shown in Table 5.14a and Figure 5.12. Two individuals in high pressure group reported severe itching over the scar areas at third and fourth months because of poor hygiene together with the hot and humid weather, resulting higher values observed for the two periods. Improved hygiene helped reduce the intensity of itching in the latter months. As displayed in Table 5.14b, post-hoc paired t-test distinguished significances for low pressure group during the fifth ($t_{24} = 3.116$, $p = 0.005$) month and for high pressure group during the first ($t_{27} = 3.863$, $p = 0.001$) and fifth ($t_{27} = 2.815$, $p = 0.009$) months of intervention after Bonferroni correction ($0.05/5 = 0.01$).

Table 5.14a Intensity of pruritus over the intervention period (Independent sample t-test)

	Low Pressure Group		High Pressure Group			
	Mean ± SD (mm)	A.C (%)	Mean ± SD (mm)	A.C. (%)	*t*	*p*-value [a]
Initial	46.81 ± 20.97		49.77 ± 21.79		0.503	0.617
Post-1 month	44.36 ± 20.84	- 5.24	43.91 ± 22.84	- 11.76	- 0.073	0.942
Post-2 month	40.19 ± 20.42	- 14.14	39.90 ± 22.02	- 19.82	- 0.049	0.961
Post-3 month	36.40 ± 16.00	- 22.23	43.30 ± 19.85	- 13.00	1.381	0.173
Post-4 month	33.86 ± 15.88	- 27.67	41.04 ± 18.51	- 17.55	1.506	0.138
Post-5 month	31.70 ± 15.04	- 32.28	34.57 ± 17.91	- 30.54	0.627	0.534

AC: Accumulative Change

[a] *Equal variances assumed*

Table 5.14b Post-hoc comparison for pruritus over time between the two groups (paired sample t-test)

	Low Pressure Group		High Pressure Group	
	t_{24}	*p*-value	t_{27}	*p*-value
Initial VS Post-1	1.048	0.305	3.863	0.001*
Post-1 VS Post-2	2.201	0.038	2.394	0.024
Post-2 VS Post-3	2.365	0.026	- 0.955	0.348
Post-3 VS Post-4	1.345	0.191	1.677	0.105
Post-4 VS Post-5	3.116	0.005*	2.815	0.009*

**Statistical significance with p<0.01*

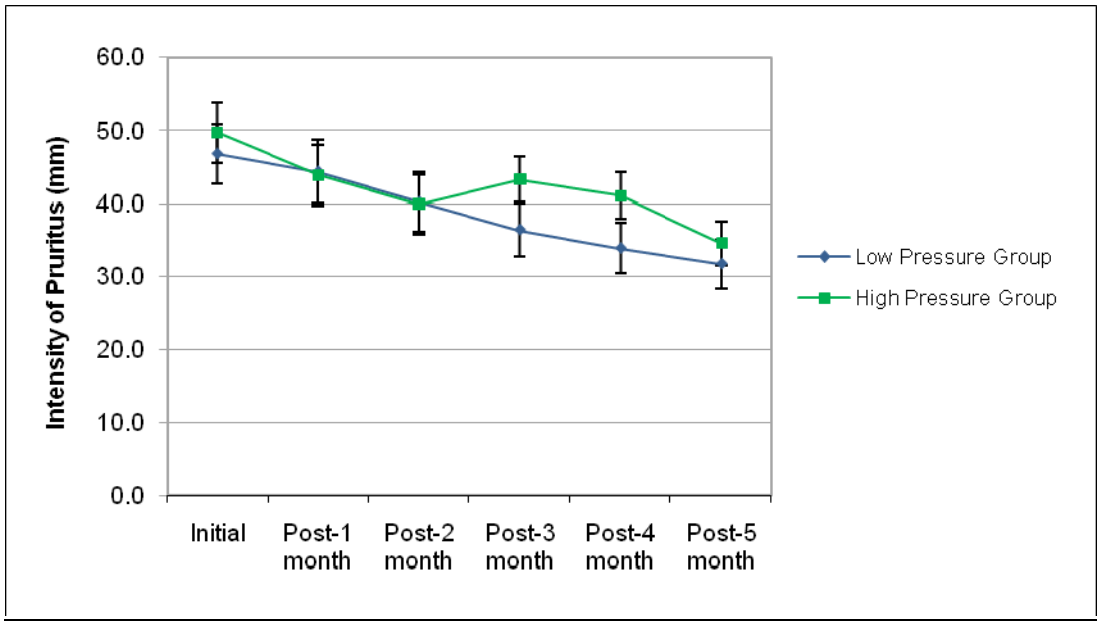


Figure 5.12 Progression of pruritus intensity (VAS) over time for the two treatment groups

Scar Clinical Presentations (Vancouver Scar Scale)

a) Scar Pigmentation by VSS

The scar pigmentation progression over time assessed using the VSS was demonstrated in Figure 5.13. Friedman test revealed a significant difference in high pressure magnitude (p = 0.01) for overall time effect. Group differences, as tabulated in Table 5.15a, were not noted in Kruskal-Wallis test at all time intervals. There was also no statistical significance detected in both groups over time by post-hoc Wilcoxon signed ranks test (Table 5.15b).

Table 5.15a Scar pigmentation over time for the two groups (Kruskal-Wallis test)

	Low Pressure Group		High Pressure Group		χ^2	*p*-value
	Mean	SD	Mean	SD		
Initial	2.36	0.76	2.68	0.61	3.001	0.083
Post-1 month	2.40	0.71	2.61	0.69	1.651	0.199
Post-2 month	2.32	0.80	2.61	0.69	2.091	0.148
Post-3 month	2.40	0.71	2.57	0.69	1.092	0.296
Post-4 month	2.32	0.75	2.25	0.89	0.016	0.899
Post-5 month	2.36	0.76	2.14	0.85	0.854	0.355

Table 5.15b Post-hoc comparison for scar pigmentation over time between the two groups (Wilicoxon signed ranks test)

	Low Pressure Group		High Pressure Group	
	Z	*p*-value	*Z*	*p*-value
Initial VS Post-1	1.000 [a]	0.317	1.414 [a]	0.157
Post-1 VS Post-2	1.414 [b]	0.157	0.000 [b]	1.000
Post-2 VS Post-3	1.414 [a]	0.157	0.378 [a]	0.705
Post-3 VS Post-4	1.414 [b]	0.157	2.111 [a]	0.035
Post-4 VS Post-5	1.000 [a]	0.317	1.732 [a]	0.083

[a] *Based on negative ranks*

[b] *Based on positive rank*

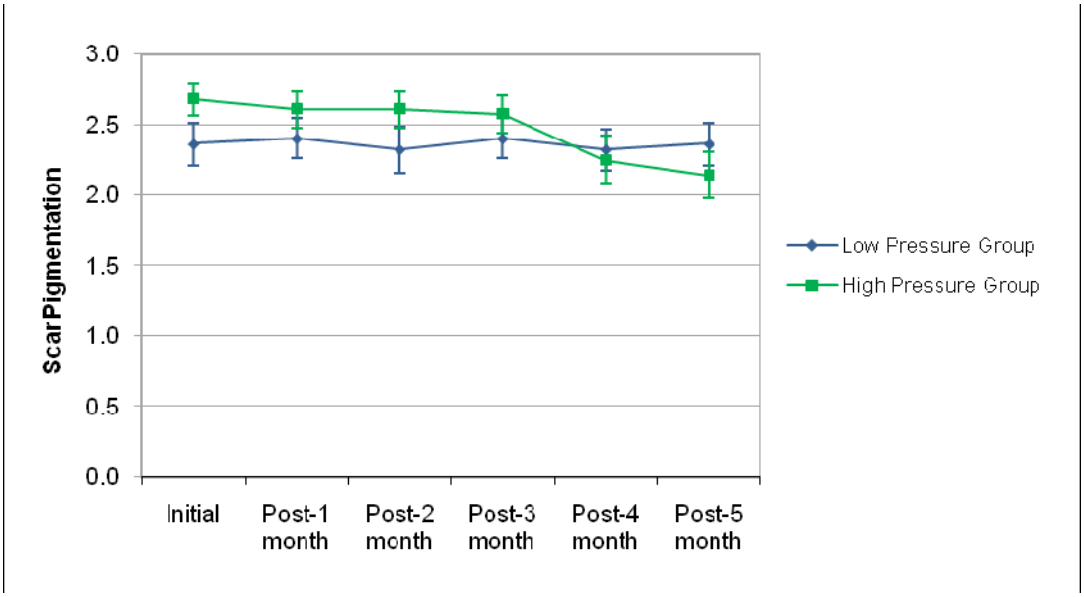


Figure 5.13 Progression of scar pigmentation (VSS) over time for the two pressure-treated groups

b) Scar Vascularity by VSS

Significant differences in scar vascularity over time were noted in both groups ($p < 0.01$) by Friedman test. Post-hoc comparison by Wilicoxon signed ranks test identified statistical significances in high pressure group between post-2 and post-3, and between post-4 and post-5 assessments with Bonferroni correction (0.05/5 = 0.01; Table 5.16b). Figure 5.14 demonstrates the progression of the scar vascularity over time.

Table 5.16a Statistics for scar vascularity over time (Kruskal-Wallis test)

	Low Pressure Group		High Pressure Group		χ^2	*p*-value
	Mean	SD	Mean	SD		
Initial	2.52	0.65	2.54	0.64	0.005	0.942
Post-1 month	2.52	0.65	2.54	0.64	0.005	0.942
Post-2 month	2.36	0.64	2.36	0.68	0.002	0.961
Post-3 month	2.36	0.64	1.93	0.66	5.342	0.021*
Post-4 month	2.16	0.69	1.75	0.52	5.355	0.021*
Post-5 month	1.96	0.61	1.50	0.51	7.270	0.007**

**Statistical significance with p<0.05*

***Statistical significance with p<0.01*

Table 5.16b Post-hoc comparison for scar vascularity over time (Wilicoxon signed ranks test)

	Low Pressure Group		High Pressure Group	
	Z	*p*-value	*Z*	*p*-value
Initial VS Post-1	0.000 [a]	1.000	0.000 [a]	1.000
Post-1 VS Post-2	- 2.000 [b]	0.046	2.236 [b]	0.025
Post-2 VS Post-3	0.000 [a]	1.000	3.464 [a]	0.001*
Post-3 VS Post-4	2.236 [b]	0.025	2.236 [b]	0.025
Post-4 VS Post-5	2.236 [b]	0.025	2.646 [a]	0.008*

[a] *Based on negative ranks*

[b] *Based on positive rank*

**Statistical significance with p<0.01*

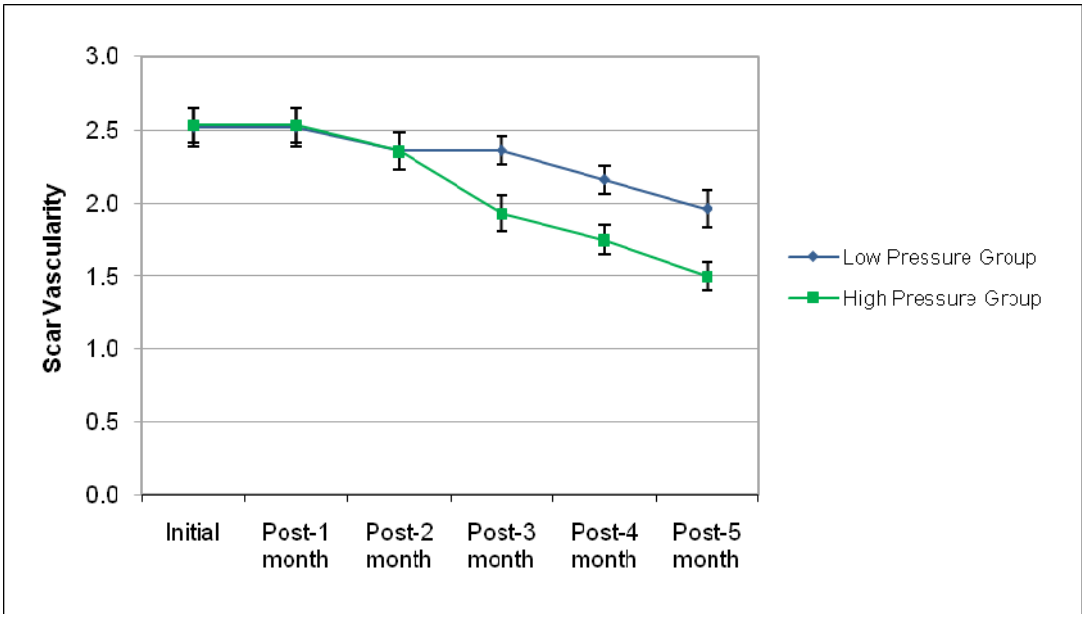


Figure 5.14 Progression of scar vascularity (VSS) over time for the two groups

c) Scar Pliability by VSS

The scar pliability measured by the VSS for the two groups was shown in Table 5.17a and demonstrated in Figure 5.15. An overall decreasing trend for both the groups was observed. A drastic drop in the scores of high pressure group at post-4 month suggested that the VSS may be insensitive to chart the scar progress. Statistical analysis revealed significance differences in the lower pressure group during the fifth month ($p = 0.03$) and in high pressure group after the fourth ($p \leq 0.05$) month of intervention (Table 5.17b).

Table 5.17a The scar pliability over time measured by the VSS for the two groups (Kruskal-Wallis test)

	Low Pressure Group		High Pressure Group		χ^2	*p*-value
	Mean	SD	Mean	SD		
Initial	2.96	0.74	3.25	0.75	2.030	0.154
Post-1 month	2.96	0.74	3.18	0.77	1.143	0.285
Post-2 month	2.92	0.70	3.07	0.77	0.562	0.453
Post-3 month	2.84	0.69	2.89	0.74	0.059	0.808
Post-4 month	2.76	0.72	2.36	0.78	3.327	0.068
Post-5 month	2.40	0.71	2.07	0.86	1.762	0.184

Table 5.17b Post-hoc comparison for scar pliability over time between the two groups (Wilicoxon signed ranks test)

	Low Pressure Group		High Pressure Group	
	Z	*p*-value	*Z*	*p*-value
Initial VS Post-1	0.000 [a]	1.000	1.414 [a]	0.157
Post-1 VS Post-2	- 1.000 [b]	0.317	- 1.732 [a]	0.083
Post-2 VS Post-3	1.414 [b]	0.157	2.236 [a]	0.025
Post-3 VS Post-4	1.414 [b]	0.157	3.873 [a]	0.000*
Post-4 VS Post-5	3.000 [b]	0.003*	2.828 [a]	0.005*

[a] *Based on negative ranks*

[b] *Based on positive rank*

**Statistical significance with p<0.01*

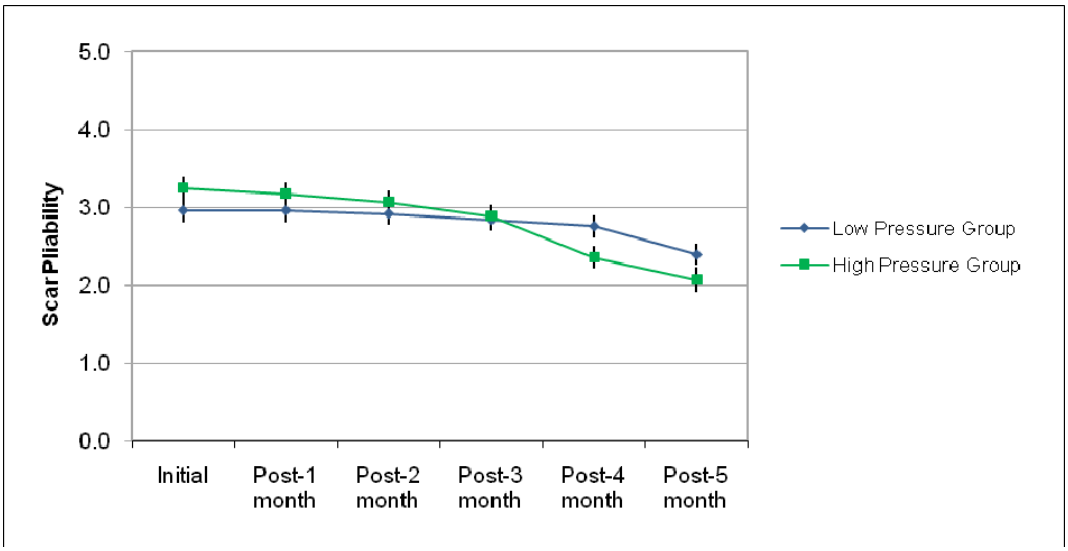


Figure 5.15 Progression of scar pliability (VSS) over time for the two groups

d) Scar Height by VSS

Friedman Test demonstrated significant differences over time for both groups ($p < 0.01$). As demonstrated in Figure 5.16, the scar height score for high pressure group dropped prominently at the first month from 3.46 (SD = 0.69) to 2.11 (SD = 0.42) then decreased steadily while steady reduction rate was observed in low pressure group over the five-month intervention. These patterns were similar to those of the scar thickness measured by TUPS. Significant group differences were noted (Table 5.18a) from first to fourth months of intervention. Post-hoc comparison using Wilicoxon signed ranks test as shown in Table 5.18b also demonstrated significant differences for low pressure group at second and fourth months and at first month of intervention for the high pressure group with Bonferroni correction (0.05/5 = 0.01).

Table 5.18a Scar height over time by VSS for the two groups (Kruskal-Wallis test)

	Low Pressure Group		High Pressure Group		χ^2	*p*-value
	Mean	SD	Mean	SD		
Initial	3.36	0.81	3.46	0.69	0.123	0.726
Post-1 month	3.16	0.75	2.54	0.58	9.273	0.002**
Post-2 month	2.84	0.62	2.39	0.57	7.002	0.008**
Post-3 month	2.72	0.61	2.29	0.54	7.619	0.006**
Post-4 month	2.40	0.65	2.11	0.42	4.525	0.033*
Post-5 month	2.28	0.74	2.11	0.42	1.713	0.191

**Statistical significance with p<0.05*
***Statistical significance with p<0.01*

Table 5.18b Post-hoc comparison for scar height over time between the two pressure-treated groups (Wilicoxon signed ranks test)

	Low Pressure Group		High Pressure Group	
	Z	*p*-value	*Z*	*p*-value
Initial VS Post-1	2.236 [a]	0.025	3.839 [a]	0.000*
Post-1 VS Post-2	- 2.828 [a]	0.005*	- 2.000 [a]	0.046
Post-2 VS Post-3	- 1.732 [a]	0.083	1.732 [a]	0.083
Post-3 VS Post-4	2.828 [a]	0.005*	2.236 [a]	0.025
Post-4 VS Post-5	- 1.732 [a]	0.083	0.000 [b]	1.000

[a] *Based on negative ranks*

[b] *Based on positive rank*

**Statistical significance with p<0.01*

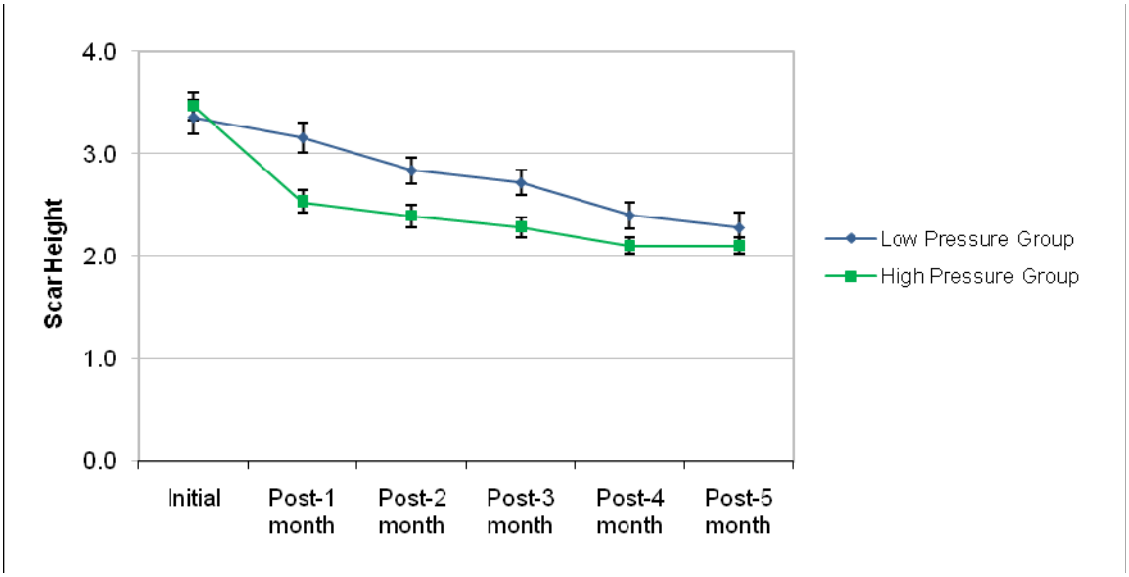


Figure 5.16 Progression of scar height (VSS) over time for the two groups

e) Total Score of VSS

As presented in Figure 5.17, the total score of VSS was found to decrease for both the low and high pressure groups. Both the groups exhibited significant differences over time with Friedman test ($p < 0.01$). Kruskal-Wallis test demonstrated significant differences between the groups after the fourth month treatment (Table 5.19a). After Bonferroni correction ($0.05/5 = 0.01$), only the fifth month under low pressure but all intervals under high pressure caused statistical significance with the post-hoc Wilicoxon signed ranks test as shown in Table 5.19b.

Table 5.19a The total score by VSS over time for the two groups (Kruskal-Wallis test)

	Low Pressure Group		High Pressure Group		χ^2	*p*-value
	Mean	SD	Mean	SD		
Initial	11.20	1.94	11.93	1.84	2.075	0.150
Post-1 month	11.04	1.72	10.86	1.63	0.249	0.618
Post-2 month	10.44	1.56	10.43	1.67	0.003	0.956
Post-3 month	10.32	1.63	9.68	1.79	2.339	0.126
Post-4 month	9.64	1.89	8.46	1.58	5.401	0.020*
Post-5 month	9.00	1.68	7.82	1.44	6.478	0.011*

**Statistical significance with p<0.05*

Table 5.19b Post-hoc comparison for total score of VSS over time between the two treatment groups (Wilicoxon signed ranks test)

	Low Pressure Group		High Pressure Group	
	Z	*p*-value	*Z*	*p*-value
Initial VS Post-1	- 1.633 [a]	1.000	4.038 [a]	0.000*
Post-1 VS Post-2	- 3.217 [a]	0.317	- 2.828 [a]	0.005*
Post-2 VS Post-3	- 1.342 [a]	0.157	3.535 [a]	0.000*
Post-3 VS Post-4	- 2.754 [a]	0.157	3.984 [a]	0.000*
Post-4 VS Post-5	- 3.557 [a]	0.003*	3.354 [a]	0.001*

[a] *Based on negative ranks*

[b] *Based on positive rank*

**Statistical significance with p<0.01*

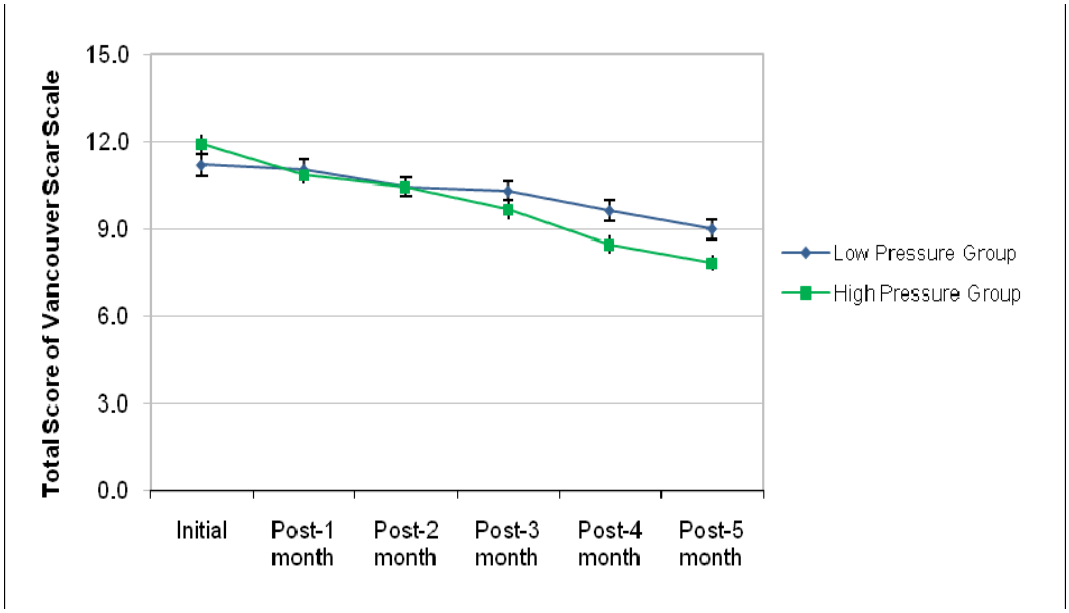


Figure 5.17 Total score of VSS over time for the two pressure-treated groups

5.6 DISCUSSION

Though pressure therapy has been widely employed for over three decades for scar control, there is still lack of scientific evidence. The therapeutic efficacy of pressure garment on hypertrophic scarring remains a subject of debate and prescription of the pressure magnitude is still mostly based on empirical observations. Recent studies to examine pressure effect on hypertrophic scarring have been conducted as prophylactic measure applied shortly after wound healing (Lau, 2006; Van den Kerckhove, et al., 2005). The effects on severe hypertrophic scar with enormous thickness, rigidity and vascularity remain unclear. This was the first RCT study attempted to substantiate pressure effect on scars that were highly hypertrophic among Chinese population using objective instruments. With quantitative documentation of interface pressure by a formerly testified apparatus at the site of scar over time, this was an important step forward to define the optimal range of pressure for hypertrophic scar treatment.

Physiological Characteristics of Hypertrophic Scar

Scar hypertrophy is typified with increasing thickness, vascularity and pliability as well as changing pattern in pigmentation. Together with the commonly used Vancouver Scar Scale (VSS), objective instruments were also employed in this study to assess the physiological features of the scar.

Compared to normal skin (less than 2mm thick) (Falkel, 1994; Johnstone, Farley, & Hendry, 2005), the hypertrophic scars in the present study (thickness by TUPS = 5.02 ± 0.98mm) were found to be much thicker. The thickness value of the thickest scar

observed in this study even reached 6.88mm. Since the protruding portion measured as the scar height by VSS (3.42 ± 0.75) was within 2 to 4mm, the findings perhaps indicated that more than 1mm scar tissue was embedded underneath the skin. As time passed, the scars appeared to increase in thickness. This could be revealed by the significant contrast of scar thickness between scars ≤ 6 months and > 6 months as found in this study.

Hypertrophic scar, on the other hand, appeared stiffer with a pliability of 3.11 ± 0.75 rated by VSS. It was more reddish as well but lighter and less yellowish in color than the adjacent normal skin. It seemed that scar color except yellowness did not change obviously across time. Since reduction of redness has been an indicator towards scar maturity, it was surprising to note that scar redness did not show great differences between younger and older scars. Due to the fact that the scars had received no interventions, those developed over six months still remained very active and it would predictably take a few more months or years for the scars to mature without treatments. Early intervention should thus be advocated to minimize the dysfunctions and cosmetic problems brought by the scar development.

A previous study has reported an itching peak before discharged (Latarjet, & Choinere, 1995); in this research, a minimal pain intensity (11.84 ± 9.84mm) was recorded since the scars had developed for at least three months. Scars ≤ 6 months (57.37 ± 17.78mm) tended to be more itching than the scars > 6 months (27.50 ± 11.97mm).

Different Pressure Effects on the Hypertrophic Scar

Pressure demonstrated in the current study its therapeutic efficacy on some physiological outcomes of hypertrophic scar. Positive effect of pressure was noted on scar thickness (TUPS) and scar height (VSS) with the decrease in the two parameters ranging from 20% to 40% after five-month intervention depending on pressure dose. Even though the exact mechanisms of pressure on scar remain unknown, based on the results obtained in Chapter IV, the pressure effect on cell proliferation could be one of the reasons. Besides, it has been postulated that pressure could realign the contorted collagen fibers and reduce the whorl collagen nodules (Costa, et al., 1999; Kischer, Shetlar, & Shetlar, 1975; Larson, et al., 1971; Longacre, 1976; Reid, et al., 1987; Shetlar, et al., 1972) which might induce thinning and softening effect on scar (Costa, et al., 1999).

Consistent with previous studies (Lau, 2006; Van den Kerckhove, et al., 2005), pressure therapy (pressure garment and padding) was also found to be effective in improving scar redness (Δa^*) as determined by the spectrocolorimeter. It has been reported that there is high correlation between scar redness and vascularity detected by Laser Doppler (Clark, et al., 1996; Hosoda, Holloway, & Heimbach, 1986). The diminishing values of scar redness might be an indicator for the occlusion of vascularity (Hosoda, Holloway, & Heimbach, 1986; Leung, et al., 1989) which limits the nutrient and oxygen supply for cellular activities (Kischer, Shetlar, & Shetlar, 1975). The hypoxic environment resulted accelerates the apoptotic process of fibroblasts (Hunt, et al., 1978; Jensen, & Parshley, 1984; Kischer, 1993; Kischer, & Shetlar, 1974; Kischer, Shetlar, & Chvapil, 1982;

Kischer, Thies, & Chvapil, 1982; Reid, et al., 1987). Reduction of blood flow, as well, favors collagenases for collagen degradation (Baur, et al., 1976; Cohen, Keiser, & Sjoerdsma, 1971) that regulates the excessive collagen deposition. The scar vascularity using the VSS rating, nonetheless, was not able to show the pressure effect as in scar redness (Δa*) by the spectrocolorimeter. Compared to the prominent improvement of scar redness (spectrocolorimeter) noted in both groups at all time intervals, the scar vascularity by VSS failed to demonstrate any statistical differences except at third and fifth months under high pressure. The results might further demonstrate the insensitivity of the VSS in detecting scar progress as stated in previous literature (Greenhalgh, 2005; Li-Tsang, Lau, & Liu, 2003; McOwan, Machermid, & Wilton, 2001).

It was interesting to note from the findings that pressure exerted limited effects on scar pigmentation (VSS). The study was unable to conclude the pressure effect on scar pigmentation because of the limitations of the VSS rating. Higher score has been assigned to hyper-pigmentation than hypo-pigmentation, however, severe scar might not always be hyper-pigmented (Nguyen, Potokar, & Price, 2008). The rating scale has been disputed with its inability to distinguish pigmentation of hypertrophic and normal scars (Oliveira, et al., 2005).

Similar problem exists in scar pliability of the VSS. Elastic scar similar to normal skin is rated as zero whilst increasing firmness of the scar is rated progressively from one to five. The VSS rating for scar pliability has been criticized inaccurate or insensitive in assessing small differences in scar stiffness (Rennekampff, et al., 2006). This became more obvious when scoring from three to five.

The result of this study showed that pressure intervention generated only diminutive effect on pain and pruritus which was consistent with the results of some previous studies (Bell, et al., 1988; Demling, & DeSanti, 2001; Lau, 2006). Pain and itchiness of hypertrophic scars could be intense and prolonged. Because of its distinctive characteristics and fluctuating patterns, not a single treatment modality could control scar pain alone (Choiniere, 2001).

Some former studies (Cheng, et al., 1983; Cheng, et al., 2001; Roques, 2002) have suggested that pressure should be at least applied for over one year to produce its effects, however, a distinct improvement in scar characteristics could be observed in the current study shortly after pressure was implemented. This could be explained by the differences noted in the treatment and assessment protocols. Foreign studies manipulate pressure magnitude by reducing the size of the garments whilst the present study employed the concept of padding inserts, commonly used in local hospitals, to generate localized pressure. As demonstrated in a previous study (Li-Tsang, et al., 2005), participants could not tolerate garments with 20% tensile strength (24.90 ± 9.14mmHg) because of numbness, the use of padding inserts may offer a better way to generate high pressure. Lower pressure dose could thus be perceived in foreign studies. In addition, vast elastic deterioration from 15% to 40% after a 30-minute to few-hour stress test (Boone, 1995; Ng, 1994) and 50% tension loss of garment after a month (Cheng, et al., 1983; Giele, et al., 1995) have been reported, without regularly measuring the interface pressure in a quantitative manner and thus trimming/renewal of garment, it could be doubtful if those studies have provided sufficient pressure to control the scarring. The

unique practice of inserting paddings underneath pressure garment in Hong Kong produces localized pressure to the scar (Hospital Authority of Hong Kong, 1998). It also allows expedient manipulation of the pressure throughout the intervention period.

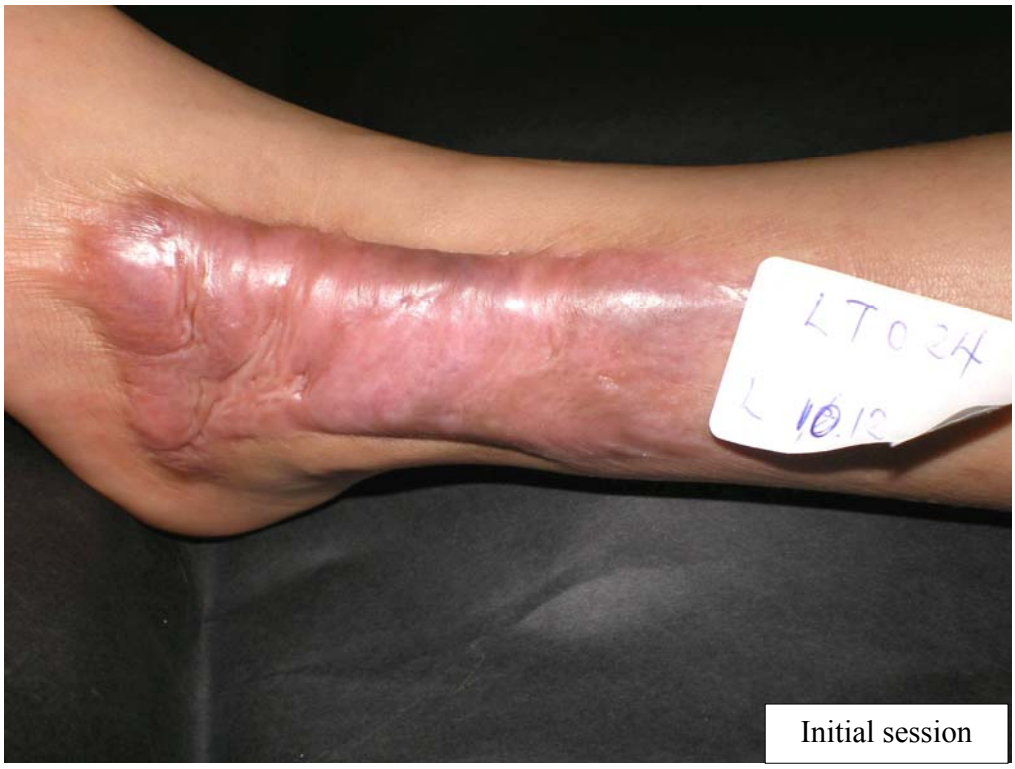
The Vancouver Scar Scale has been the most common assessment tool for scar evaluation in previous studies. The scale, however, has been criticized for its fair inter-rater reliability (Baryza, & Baryza, 1995; Sullivan, et al., 1990) and low internal consistency (Lieneke, et al., 2004). Ceiling effect of the scale has also been complained, such as on the determination of scar thickness (Lau, 2006) and volume (Nedelec, Shankowsky, & Tredget, 2000), in revealing the scar clinical presentation. Its subjective rating (Greenhalgh, 2005; Li-Tsang, Lau, & Liu, 2003; McOwan, Machermid, & Wilton, 2001; Powers, et al., 1999; Tyack, Pegg, & Ziviani, 1997) and low sensitivity (Nguyen, Potokar, & Price, 2008) to assess scar changes further urge the development of better evaluation equipments in measuring the treatment outcome. Researches employing the scale as the major evaluation tool might not be able to document the relatively subtle changes of scar resulting from a short period of treatment.

Optimal Range of Pressure for Effective Scar Control

Echoing with the findings in the *in-vitro* study (Chapter IV), later clinical study demonstrated that scar remodeling under pressure could be dose-dependent. It seemed that high pressure dosage could bring more favorable outcomes than low pressure. Representatives of scar photos are depicted in Figure 5.18a & b. Significant differences were noted in certain parameters between the low and high pressure groups. A static pressure magnitude of over 20mmHg was found to accelerate the scar remodeling

process with improved clinical presentations. High pressure demonstrated its superior effects on reducing scar thickness (overall 40.1%). Though significant results were also obtained in low pressure group, the rate was slower and the decrease in thickness (overall 19.8%) over time was much smaller as compared to high pressure. This finding was further confirmed with the rating of scar height by the VSS. Similar pattern was also seen in scar redness (Δa*). As recorded by the spectrocolorimeter, high pressure was found more effective in controlling scar redness than low pressure at all time intervals. Scar vascularity assessed by the VSS also revealed the differences between the two groups after three months of intervention. Pressure dosage was proven in this study to be a determining factor for successful scar control; lack of monitoring of the pressure dosage might lead to a variation in the treatment outcomes.

In general, the participants reported less itching for scars under low pressure. Using fewer levels of pressure padding in low pressure group might be the reason. Due to the poor thermo-regulation ability and moisture conductivity of the padding materials, some subjects in the high pressure group thus with more pressure paddings complained skin irritability especially when it was hot and humid. The condition fairly improved as the paddings were enfolded with cotton clothing. In addition, several participants in both the groups reported severe itchiness immediately after removing the garment. A possible explanation was that sudden removal of pressure might induce a flush of blood flow to the scar (Bell et al., 1988), the situation could imply that the scar was still in an active stage and would proliferate if treatment was ceased.



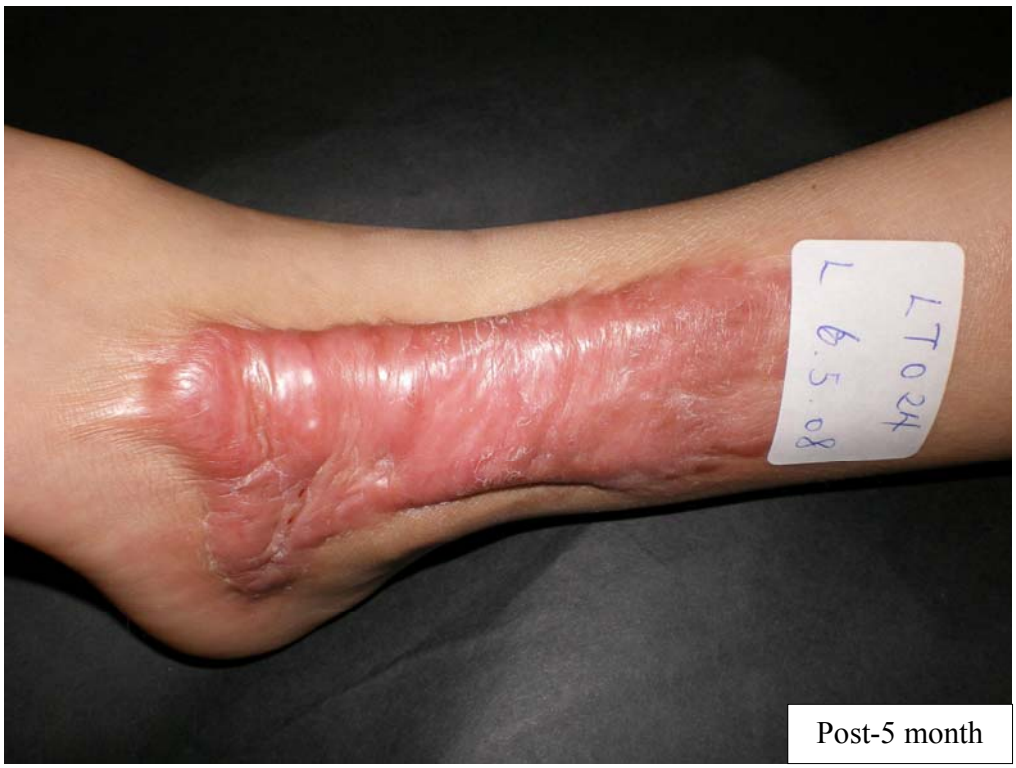
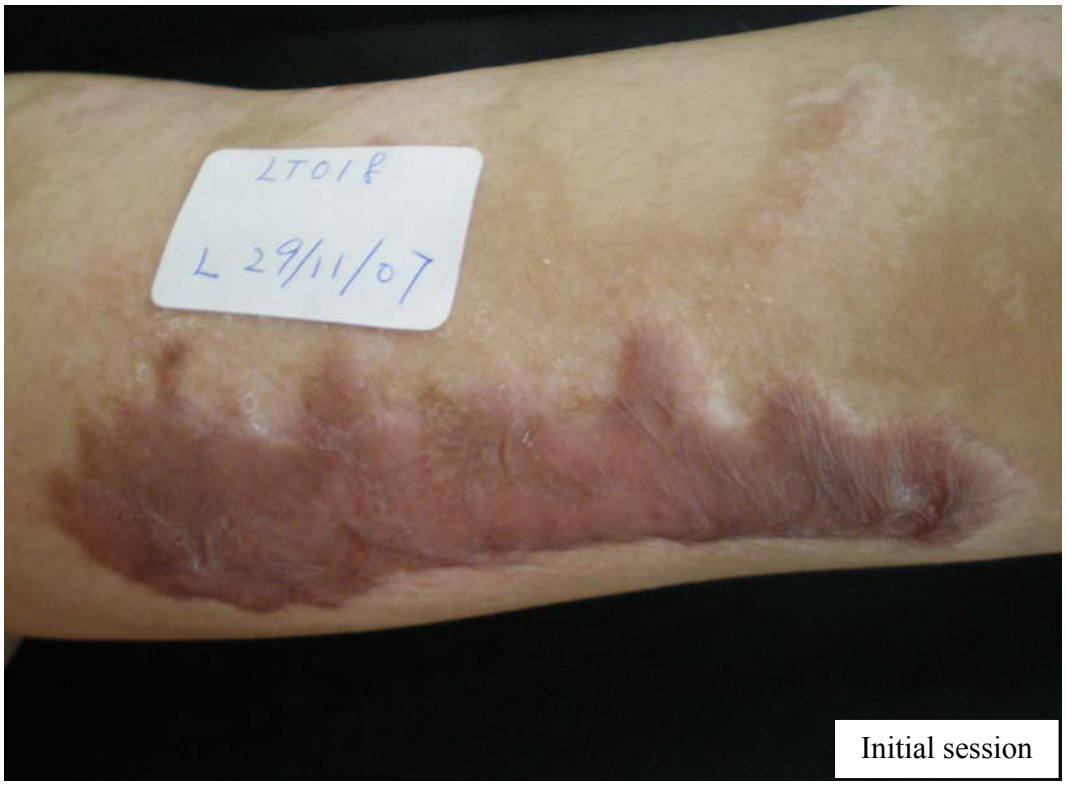


Figure 5.18a The scar over lateral side of ankle in the low pressure group (initial session and post-5 months)



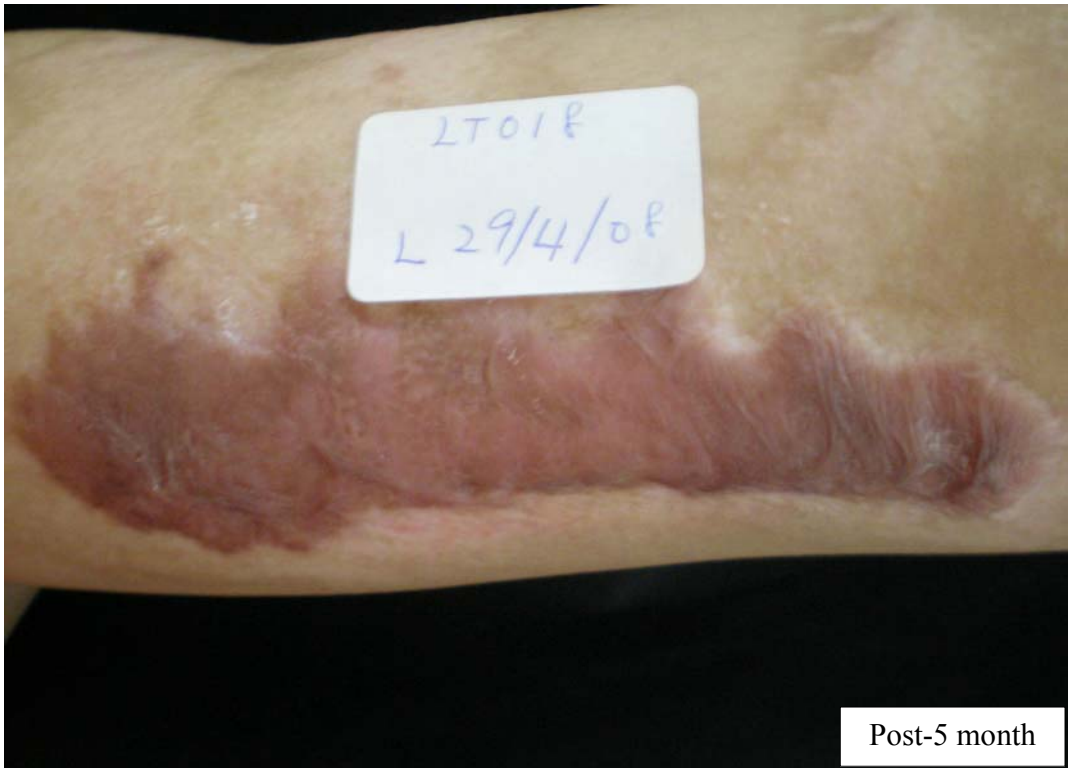


Figure 5.18bThe scar over calf in the high pressure group (initial session and post-5 months)

Pressure Loss over Time

In view of the criticism over scarce knowledge of fabric properties (Boone, 1995; Cheng, et al., 1983; Naismith, 1980; Ng, 1990, 1993, 1994), a series laboratory tests had been conducted in advance to identify the best fabric for current study (Li-Tsang, Yu, & Lai, 2008). Compared to the gigantic deterioration in fabric elasticity (20% to 50% loss over four weeks) reported in previous studies (Cheng, et al., 1983; Van den Kerckhove, et al., 2005), much lower rate of pressure loss (11.73 ± 2.22%) was found in this study, indicating that careful selection of fabric materials for garment fabrication was necessary for pressure maintenance. Frequent trimming or replacement of garment was also required for sustaining the desired pressure magnitude; therefore, it is not astounding that pressure therapy has been confronted with its cost-effectiveness (Chang, et al., 1995).

It was proposed that additional inserts, other than the reduction factor of the garment, should also be used for determining the magnitude of interface pressure. Less stress was therefore generated on the fabric during daily wearing when compared to garments with larger reduction factor. Relatively greater pressure loss, however, was observed in the high pressure group because of the additional layers of padding, suggesting that the level of pressure padding could also be a factor influencing the tension loss of the garment fabric. The level of padding prescribed, thus, should be considered when planning the schedule of trimming or replacement of the garment.

Since mobilization has been proven to have prominent effects on the interface pressure (Lam, 2008; Ramelet, 2002; Yim, & Li-Tsang, 2008), measuring pressures continuously during mobilization could provide better understanding of the real pressure effect than merely measuring static pressure at particular time intervals. Continuous measurement, however, was still a challenge because the pressure monitor was not designed for wearing or using for a long period.

Limitations of the Study

To gain better control of pressure dosage, only hypertrophic scar developed over extremities were recruited in this study. Conditions of scars developed on other body areas were left unexplored, thus the results obtained in this research might not be applicable to scars on other body parts. In addition, since the measuring sites of each scar area were randomly chosen for assessment, they might not be able to represent the whole scar area.

The limited number of participants and the single source for recruiting the participants (all the subjects were from the same centre) also limited the generalization of the study. As varying degrees of scar development among races have been reported in previous studies (Alhady, & Sivanantharajah, 1969; Bombaro, et al., 2003; Li-Tsang, Lau, & Chan, 2005), the results observed from the Chinese population as in this study might not represent the responses from other races. It would be attractive to perform a world-wide research for comparing the responses among various races.

The interface pressure reading was currently recorded in static, however, as discussed earlier, physical activities have been proven to have significant effect on the interface pressure (Lam, 2008; Yim, & Li-Tsang, 2008). Dynamic pressure effect on remodeling potential of the scar remains unexamined. Instruments that would allow real-time even 24-hour pressure measurements could help understand better the effect of pressure therapy on hypertrophic scar and help determine the optimal pressure range for the therapy.

Although effort had been made for documenting scar pliability in an objective manner, it was difficult to find such an existing apparatus. The commercially available systems, for instance, cutometer and tonometer, are designed for relatively elastic scar. Rigid scar which is more severe in terms of stiffness and inflexibility often reaches ceiling effect of the machine. Scar pliability was thus still evaluated subjectively using the Vancouver Scar Scale in the current study. Furthermore, due to the lack of objective means to measure patients' compliance to the therapy, subjective self-reported data was the only way to determine their compliance to the wearing regime. Building rapport with patients and patient education on the consequences were therefore very important to encourage their compliance.

Continual pressure has been suggested until scar becomes atrophic (Baur, et al., 1976; Leung, et al., 1984; Linares, Larson, & Willis-Galstaun, 1993). This study, however, investigated only the therapeutic effects within five months when some scar samples were still immature. A study with more extensive investigation until the scar matures

should be proposed in the future. On the other hand, although high pressure demonstrated its superior effects on scar presentations, the study gave little explanation on the underlying mechanisms of pressure therapy. Studying the scar biopsy before and after pressure application might help understand the biochemical changes.

5.7 CONCLUSION

This clinical trial attempted to examine the relationships of pressure and the scar remodeling. By comparing the therapeutic outcomes under low (10 to 15mmHg) and high (20 to 25mmHg) pressures, the study tried to define the optimal pressure for effective scar control. Although low pressure was also found effective in improving certain parameters, high pressure exhibited its prevailing effects on scar thickness and redness.

High pressure in certain extent demonstrated supremacy to low dosage, however, more intense itching and higher pressure loss over time possibly caused by additional layers of padding were reported by some participants. To minimize skin irritability, therapists could consider the use of cotton clothing to enfold the paddings. Frequent trimming and renewal of garment could be scheduled along with high pressure prescribed as to compensate the pressure loss over time. The dose-dependent effect of pressure and the pressure loss over time indicated the importance of interface pressure measurement.

CHAPTER VI

CONCLUSION

Hypertrophic scarring is a torment to patients. Not only could it bring physical annoyance with pain and pruritus (Azad, Gerrish, & Dziewulski, 2000; Beldon, 2000; Kawecki, Bernad-Wisniewska, Sakiel, Nowak, & Ansriessen, 2008; Naismith, 1980), the aberrant scar could also lead to psychosocial ordeal because of its severe cosmetic disfigurement and scar contracture (Haverstock, 2001; Reid, Evans, Naismith, Tully, & Sherwin, 1987). Whichever modality that helps minimize the harrowing nature of scarring is valuable to the patients. Nearly all patients with hypertrophic scar would be treated with pressure therapy in local settings (Cheng, Chan, Fong, Lam, Wong, & Wu, 1999), yet the treatment has been challenged with insufficient substantiation for its clinical efficiency (Anzarut, Olson, Singh, Rowe, & Tredget, 2008; Mustoe, et al., 2002; Stal, Cole, & Hollier, 2008). A dearth of objective apparatuses for scar assessment and interface pressure measurement could be a reason for the gaps between clinical practice and academic research. This three-phased study was thus designed to investigate the effects of pressure on post-traumatic hypertrophic scars, hoping with the employment of objective equipments, the optimal range of pressure for effective scar control could be identified.

Quantifying the interface pressure is the prerequisite step for defining optimal dosage of pressure. Effort was therefore made to testify the sensor performance of a lately available interface pressure monitor, the Pliance X System. The results in Chapter III demonstrated its feasibility to provide quantitative measurement of interface pressure. Its reliability was confirmed by three independent assessors. The apparatus was further scrutinized for its clinical applicability. Its discriminant ability was corroborated by

differentiating the interface pressure value between normal skin and hypertrophic scar and among different levels of pressure padding. With the sensitivity and reliability of the advancement, values of interface pressure prescribed in the latter clinical trial could be documented in a quantitative and objective manner.

Prior to the clinical trial, it was worthwhile to have an investigation on the underlying mechanism of pressure therapy on hypertrophic scar remodeling. As fibroblast has been acknowledged with its foremost position in hypertrophic scar formation, pressure therapy might be able to induce disturbances on the unwarranted fibroblast activities. An *in-vitro* study as inscribed in Chapter IV was performed to compare the biological responses between pressure-treated and untreated hypertrophic scar fibroblasts. Inhibition of fibroblast proliferation and reduction of myofibroblasts were observed in samples subjected to mechanical pressure loading. The effect on the fibroblast responses was found dose-dependent, in which the higher the pressure dosage, the larger the effect of pressure would have on the fibroblast cells. Its dose-dependent nature of the fibroblasts could be a clue to explain the divergent conclusions of research studies on the effectiveness of pressure therapy.

The dose-dependent pressure effect on fibroblasts in Chapter IV suggested the necessity of defining the optimal pressure for efficient scar management. Since randomized clinical trial (RCT) provides the best evidence of treatment efficacy by eliminating the potential bias attributable to the differences, the clinical study in Chapter V adopted a prospective RCT research design to study the treatment efficacy of pressure therapy

with lower and higher interface pressures. Nineteen Chinese subjects with 58 post-traumatic hypertrophic scars were recruited to participate in the study and randomly allocated into two groups with static pressure ranging from 10 to 15mmHg (low pressure group) and 20 to 25mmHg (high pressure group). By implementing an objective assessment protocol on scar thickness and color (Li-Tsang, Lau, Choi, Chan, & Li, 2006), the scars were assessed on a monthly basis over a five-month intervention period. The traditional assessment tool, Vancouver Scar Scale, was also applied for the scar pigmentation, vascularity, pliability and height. Visual Analogue Scale was used to assist participants to report the intensity of pain and itchiness. Superior therapeutic outcomes in terms of scar thickness and redness were noticed in subjects under high pressure dosage throughout the five-month period. Low pressure could also induce favourable alterations of scar features but at a slower rate when compared to higher dosage. High pressure, however, had its major defects in inducing itchness and maintaining pressure with time.

Instead of an one-year therapy as suggested by previous studies, it was observed in this study that sufficient pressure dosage could afford noticeable improvements in scar features swiftly after pressure was implemented. This finding further supported pressure magnitude as a major component for successive therapy. Though further investigation is obligatory for better understanding the phenomenon of pressure-induced effect, the current study could contribute, to a certain extent, in providing therapeutic insights in clinical practice.

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LIST OF APPENDICES

Appendix A

香港理工大學康復治療科學系
職業治療學部科研同意書

科研題目：壓力治療法對治療增生性瘢痕的長期研究

科研人員：李曾慧平博士，李博士為香港理工大學康復治療科學系副系主任。

科研內容：香港理工大學康復治療科學系現正進行有關增生瘢痕的研究：1）探討不同程度的壓力與增生瘢痕各項特徵的關係；2）找出最有效控制增生瘢痕生長的壓力。我們的研究結果將有助醫護人員發展增生性瘢痕之最佳治療。

我們現時正招募肢體上有增生瘢痕的人士，我們誠邀閣參加這項研究。如果閣下同意參加本計畫，你將會被邀請在瘢痕剛生長時、治療後一個月、兩個月、三個月、四個月及五個月到龍崗中心醫院接受評估。每次評估過程約二小時。整個過程約需六個月時間完成，包括六次評估/治療節數。所有測試結果將會保密及不會公開。

此項研究需要測試閣下身體上瘢痕的狀況，包括以下程式：

1. 即時檢查及量度瘢痕的情況
2. 測試瘢痕情況包括瘢痕厚度，色澤，柔韌度及充血情況
3. 在瘢痕上施行壓力治療
4. 調整壓力衣的臨床壓力
5. 治療前、治療後第一個月、第二個月、第三個月、第四個月及第五個月均需進行定期檢查壓力衣之壓力及瘢痕的狀況

如果閣下同意參加這項研究，請在以下同意書上簽名。此項研究之成功，有賴閣下之參與。謹致予衷心感謝。

潛在益處：增生瘢痕將得到有效的治療

潛在危險性：沒有

同意書

本人_____已瞭解此次研究探討壓力與增生瘢痕血液迴流、組織結構特質之關係的具體情況。本人同意參與這次研究。本人明白這次研究目的和內容，測試結果將會保密。本人有權要求一份測試撮要報告拷貝和在沒有懲罰及不公平治療情況下終止參與此項調查。如對這個計畫有疑問，本人可向李曾慧平博士(電話：852-27666715)查詢。若本人對此研究人員有任何投訴，可以聯繫梁女士（部門科研委員會秘書），電話：852-2766 5397。本人亦明白，參與此研究課題需要本人簽署一份同意書。

簽名（參與者）：_____ 日期：_____

簽名（證人）：_____ 日期：_____

Appendix B

香港理工大学康复治疗科学系
职业治疗学部科研同意书

科研题目：压力治疗法对治疗增生性瘢痕的长期研究

科研人员：李曾慧平博士，李博士为香港理工大学康复治疗科学系副系主任。

科研内容：香港理工大学康复治疗科学系现正进行有关增生瘢痕的研究：1）探讨不同程度的压力与增生瘢痕各项特征的关系；2）找出最有效控制增生瘢痕生长的压力。我们的研究结果将有助医护人员发展增生性瘢痕之最佳治疗。

我们现时正招募肢体上有增生瘢痕的人士，我们诚邀阁参加这项研究。如果阁下同意参加本计划，你将会被邀请在瘢痕刚生长时、治疗后一个月、两个月、三个月、四个月及五个月到龙岗中心医院接受评估。每次评估过程约二小时。整个过程约需六个月时间完成，包括六次评估/治疗节数。所有测试结果将会保密及不会公开。

此项研究需要测试阁下身体上瘢痕的状况，包括以下程序：

1. 实时检查及量度瘢痕的情况
2. 测试瘢痕情况包括瘢痕厚度，色泽，柔韧度及充血情况
3. 在瘢痕上施行压力治疗
4. 调整压力衣的临床压力
5. 治疗前、治疗后第一个月、第二个月、第三个月、第四个月及第五个月均需进行定期检查压力衣之压力及瘢痕的状况

如果阁下同意参加这项研究，请在以下同意书上签名。此项研究之成功，有赖阁下之参与。谨致予衷心感谢。

潜在益处：增生瘢痕将得到有效的治疗

潜在危险性：没有

同意书

本人_____已了解此次研究探讨压力与增生瘢痕血液回流、组织结构特质之关系的具体情况。本人同意参与这次研究。本人明白这次研究目的和内容，测试结果将会保密。本人有权要求一份测试撮要报告拷贝和在没有惩罚及不公平治疗情况下终止参与此项调查。如对这个计划有疑问，本人可向李曾慧平博士(电话：852-27666715)查询。若本人对此研究人员有任何投诉，可以联系梁女士（部门科研委员会秘书），电话：852-2766 5397。本人亦明白，参与此研究课题需要本人签署一份同意书。

签名（参与者）：_____ 日期：_____

签名（证人）：_____ 日期：_____

香港理工大學康復治療科學系
職業治療學部

Appendix C

增生疤痕評估表

病人編號：_____

病人姓名：_____ 評估日期：_____
治療師：_____

基本資料

性別：☐ 男性 ☐ 女性
年齡：_____ 出生日期：_____年_____月_____日
受傷日期：_____ 受傷原因：
疤痕出現時間：_____星期 / 月

疤痕出現原因：☐ 燙傷 ☐ 燒傷 ☐ 手術 ☐ 外傷
☐ 其他：_____

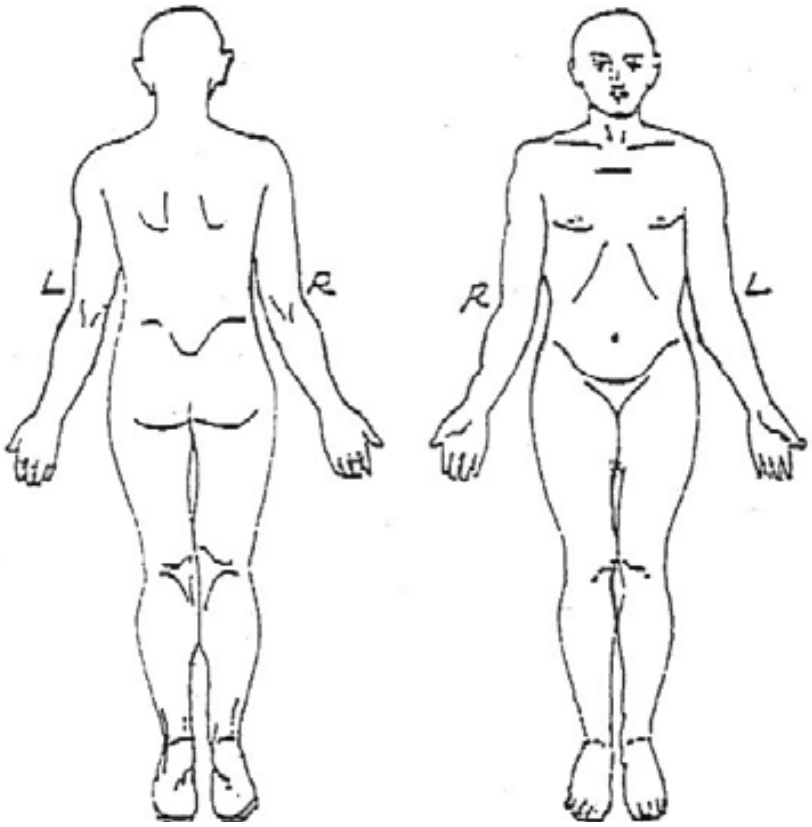
其他治療：_____

疤痕評估

1. 疤痕位置

2. 疤痕面積
   長度：_____
   闊度：_____

3. 疤痕拍照



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Appendix D

病人編號 : _____
評估日期: _____

溫哥華疤痕測試分數	分數
疤痕色澤 (Pigmentation)	/ 3
血液迴圈 (Vascularity)	/ 3
柔軟程度 (Pliability)	/ 5
疤痕厚度 (Height)	/ 4
總分	**/ 15**

視覺評估比例尺	讀數 (mm)
疤痕痛楚程度	
疤痕痕養程度	

壓力測量		讀數 (mmHg)			
		第一次	第二次	第三次	平均值
位置	一				
	二				
	三				
	四				
	五				
				總平均值	

顏色量化表										
位置		第一次			第二次			第三次		
		L*	**a***	**b***	**L***	**a***	**b***	**L***	**a***	**b***
疤痕	一									
	二									
	三									
	四									
	五									
平均值										
普通皮膚	一									
	二									
	三									
	四									
	五									
平均值										

超聲波疤痕厚度評估		讀數 (mm)							
		疤痕厚度				疤痕柔軟度			
		第一次	第二次	第三次	平均值	第一次	第二次	第三次	平均值
位置	一								
	二								
	三								
	四								
	五								

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職業治療學部

Appendix E

病人編號：_____
評估日期: _____

視覺評估比例尺

1. 疤痕痛楚程度

_____

0 **10**
完全不痛 不能忍受的痛

2. 疤痕痕養程度

_____

10
完全不痕養 不能忍受的痕養

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Appendix F

病人編號 : _____
評估日期: _____

溫哥華疤痕評估表

測試分數	分數
疤痕色澤 (Pigmentation)	/ 3
血液迴圈 (Vascularity)	/ 3
柔軟程度 (Pliability)	/ 5
疤痕厚度 (Height)	/ 4
總分	**/ 15**

檢定項目	分數	
色澤 Pigmentation	0 = 正常顏色 1 = 淺白色或淺粉紅色	2 = 深淺混集 3 = 深色
血液循環 Vascularity	0 = 正常 1 = 粉紅色	2 = 紅色 3 = 紫色
柔軟程度 Pliability	0 = 正常 1 = 柔軟 2 = 有少許拉緊 3 = 有點硬	4 = 令關節彎曲, 很難把關節伸直 5 = 已造成永久性軟組織攣縮, 例如關節畸形
疤痕厚度 Height	0 = 正常(平坦的) 1 = 0 - 1 mm 2 = 1 - 2 mm	3 = 2 - 4 mm 4 = > 4 mm

Appendix G.1

Table of Linearity and Repeatability Test

Experiment No.	Standard Weight					
	5g	**10g**	**20g**	**30g**	**40g**	**50g**
1	4.363	8.805	18.868	28.753	38.362	46.385
2	4.102	9.064	18.868	28.680	37.439	46.768
3	4.392	9.434	18.860	28.886	38.141	44.976
4	4.128	9.434	18.712	29.539	38.419	46.910
5	3.773	10.396	18.868	28.679	38.431	46.543
6	3.773	10.692	18.868	28.695	38.486	46.155
7	3.773	8.780	19.261	28.872	37.334	46.853
8	3.779	8.875	17.610	26.975	37.924	47.053
9	4.729	9.434	17.596	28.682	38.240	47.813
10	3.778	9.434	17.610	28.702	38.362	47.167
Mean	**4.059**	**9.717**	**18.512**	**28.646**	**38.114**	**46.662**
SD	**0.343**	**0.648**	**0.641**	**0.643**	**0.418**	**0.748**

* Unit = millimeter mercury (mmHg)

Appendix G.2

Table of Drift Test for 30-minute loading

Time (minutes)	Standard Weight	
	20g	**50g**
1	18.868	45.282
5	18.868	45.282
10	18.868	45.282
15	20.377	46.791
20	20.377	46.791
25	20.377	46.791
30	20.377	46.791
Mean	**19.730**	**46.144**
SD	**0.807**	**0.807**

* Unit = millimeter mercury (mmHg)

Appendix G.3

Table of Hysteresis Test

Experiment No.	Pressure (mmHg)											
	Loading						Unloading					
	0	**10**	**20**	**30**	**40**	**50**	**50**	**40**	**30**	**20**	**10**	**0**
1	0.061	10.501	20.502	30.003	39.503	49.874	50.788	39.984	30.003	18.983	10.531	0.021
2	0.045	10.982	23.500	32.380	41.241	49.791	51.750	42.263	31.486	21.622	11.321	0.257
3	0.000	10.014	22.615	31.655	40.797	50.401	51.810	42.683	33.456	20.799	12.302	0.165
4	0.042	10.544	20.650	30.233	40.062	48.234	48.810	39.266	31.874	19.619	11.311	0.154
5	0.052	10.459	21.341	30.952	40.699	49.088	50.440	41.068	32.708	19.154	10.576	0.096
6	0.078	10.177	20.650	30.197	39.803	48.664	49.764	38.479	31.169	19.610	11.311	0.135
7	0.000	10.542	21.341	30.922	41.244	50.133	51.077	39.155	32.018	19.874	10.557	0.077
8	0.000	9.589	20.457	30.197	39.750	48.282	48.777	38.465	31.851	18.869	11.137	0.174
9	0.000	10.547	21.409	31.067	41.972	50.611	51.004	40.768	31.640	19.729	11.318	0.157
10	0.025	9.786	20.486	30.489	40.684	50.798	51.067	41.684	31.784	20.165	9.987	0.165
Mean	**0.030**	**10.373**	**21.295**	**30.809**	**40.576**	**49.588**	**50.529**	**40.381**	**31.799**	**19.842**	**11.035**	**0.140**
SD	**0.029**	**0.398**	**1.024**	**0.754**	**0.789**	**0.957**	**1.087**	**1.540**	**0.905**	**0.847**	**0.643**	**0.064**

Appendix G.4a

Table of *In-vivo* Pressure Measurement with upper arm as the measuring site

Applied Pressure	Sensor Value					Mean	SD
	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5		
10	11.221	10.227	9.826	9.811	9.811	**10.179**	**0.609**
20	21.576	21.428	21.168	21.120	21.132	**21.285**	**0.206**
30	31.697	31.667	31.531	31.338	31.021	**31.451**	**0.279**
40	40.748	39.676	41.433	40.331	40.367	**40.511**	**0.644**
50	51.528	49.795	49.541	48.920	49.291	**49.815**	**1.011**

* Unit = millimeter mercury (mmHg)

Appendix G.4b

Table of *In-vivo* Pressure Measurement with forearm as the measuring site

Applied Pressure	Sensor Value					Mean	SD
	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5		
10	11.339	11.004	9.935	9.956	10.225	**10.492**	**0.642**
20	21.132	21.132	19.725	20.848	20.948	**20.757**	**0.590**
30	29.690	30.019	28.896	30.191	29.965	**29.752**	**0.511**
40	40.760	40.763	39.766	40.084	40.709	**40.416**	**0.463**
50	49.964	49.729	49.369	49.668	49.732	**49.692**	**0.213**

* Unit = millimeter mercury (mmHg)

Appendix G.4c

Table of *In-vivo* Pressure Measurement with thigh as the measuring site

Applied Pressure	Sensor Value					Mean	SD
	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5		
10	9.811	11.321	10.768	9.850	9.817	**10.314**	**0.696**
20	20.691	19.634	19.628	19.622	19.625	**19.840**	**0.476**
30	30.212	30.174	30.236	30.248	30.188	**30.212**	**0.031**
40	39.990	40.790	40.754	40.117	40.781	**40.486**	**0.398**
50	52.585	50.046	50.831	49.617	50.592	**50.734**	**1.138**

* Unit = millimeter mercury (mmHg)

Appendix G.4d

Table of *In-vivo* Pressure Measurement with calf as the measuring site

Applied Pressure	Sensor Value					Mean	SD
	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5		
10	9.941	9.811	9.811	9.811	9.802	**9.835**	**0.059**
20	21.050	19.746	19.640	20.993	21.005	**20.487**	**0.726**
30	30.310	29.382	29.823	29.814	29.998	**29.865**	**0.337**
40	39.244	40.715	39.244	40.123	40.567	**39.978**	**0.705**
50	50.206	50.052	49.855	49.825	51.211	**50.230**	**0.570**

* Unit = millimeter mercury (mmHg)

Appendix G.5a

Table to compare the interface pressure measured under different conditions in hypertrophic scar tissues

Sample	Sensor Value		
	PG	**PG + 3mm**	**PG + 6mm**
1	7.816	10.156	23.156
2	6.515	12.489	22.156
3	7.516	11.154	23.156
4	6.515	11.215	22.456
5	3.157	8.542	13.490
6	4.952	8.435	13.052
7	4.352	8.512	12.987
8	8.516	11.546	23.156
9	8.515	14.351	22.156
10	8.516	15.548	13.490
11	9.516	15.543	13.052
12	7.516	11.546	12.987
13	8.515	11.154	23.156
14	8.516	14.351	24.789
15	6.515	9.016	12.489
16	7.519	9.016	15.423
17	6.515	9.155	14.899
18	5.156	9.516	14.855
19	4.516	8.019	14.516
20	6.516	8.486	15.033
21	5.155	7.515	14.516
22	4.516	10.424	14.023
23	6.106	9.516	13.517
24	5.455	10.864	16.681
Mean	**6.600**	**10.670**	**17.050**
SD	**1.680**	**2.350**	**4.435**

* Unit = millimeter mercury (mmHg)
* PG = pressure garment
* PG + 3mm = pressure garment with a 3-millimeter pressure insert
* PG + 6mm = pressure garment with a 6-millimeter pressure insert

Appendix G.5b

Table to compare the interface pressure measured under different conditions in normal skin

Sample	Sensor Value		
	PG	**PG + 3mm**	**PG + 6mm**
1	3.157	9.516	16.547
2	4.952	8.516	17.517
3	4.352	7.547	17.868
4	3.959	8.153	18.487
5	3.157	8.517	16.847
6	3.154	7.519	17.532
7	3.486	7.816	16.850
8	3.489	8.516	18.487
9	2.154	8.515	18.487
10	2.154	8.516	18.487
11	2.110	8.515	17.378
12	1.046	4.517	11.546
13	1.154	3.156	11.154
14	2.110	4.153	11.215
15	1.216	5.456	10.156
16	2.516	5.156	11.436
17	2.154	6.514	9.016
18	2.435	4.516	9.016
19	2.146	4.848	9.516
20	1.046	4.153	13.515
21	2.102	5.517	12.489
22	2.109	4.517	10.123
23	1.154	5.517	11.435
24	2.015	6.984	12.987
Mean	**2.472**	**6.527**	**14.087**
SD	**1.056**	**1.886**	**3.566**

* Unit = millimeter mercury (mmHg)
* PG = pressure garment
* PG + 3mm = pressure garment with a 3-millimeter pressure insert
* PG + 6mm = pressure garment with a 6-millimeter pressure insert

Appendix H.1

Table of the MTT absorbance for fibroblast proliferation

Time	Weight (g)	Experimental Sample						Mean	SD
		1	2	3	4	5	6		
Day 0	0	0.257	0.254	0.277	0.282	0.260	0.249	**0.263**	**0.013**
Day 2	0	0.338	0.372	0.342	0.370	0.365	0.35	**0.356**	**0.015**
	2	0.348	0.329	0.251	0.347	0.343	0.258	**0.313**	**0.046**
	5	0.334	0.355	0.247	0.335	0.327	0.228	**0.304**	**0.053**
	10	0.297	0.279	0.264	0.298	0.271	0.211	**0.270**	**0.032**
Post-day2	0	0.410	0.416	0.421	0.426	0.385	0.415	**0.412**	**0.014**
	2	0.343	0.340	0.400	0.390	0.358	0.365	**0.366**	**0.025**
	5	0.324	0.331	0.359	0.365	0.355	0.380	**0.352**	**0.021**
	10	0.343	0.335	0.313	0.310	0.231	0.234	**0.294**	**0.050**

Appendix H.2

Table of the number of myofibroblasts for fibroblast differentiation

Time	Wgt (g)	Visual Field (mean of the experimental samples)																				Mean	SD
		1		2		3		4		5		6		7		8		9		10			
		D	α	D	α	D	α	D	α	D	α	D	α	D	α	D	α	D	α	D	α		
Day 0	0	10	0	9	0	13.5	1.5	13.5	1	13.5	0	11	0	8	0	14	0	22	0.5	14	0.5	**6.60**	**6.96**
Day 2	0	19.5	1	17	4	12	2.5	13	1	13	2.5	17	4	21.5	3	21.5	3.5	16	5.5	17	2.5	**9.85**	**7.52**
	2	23	2.5	15	0	27.5	1.5	19	2	22	2	20.5	2	23	2.5	17	1.5	23.5	1	27.5	2	**11.75**	**10.70**
	5	21.5	0.5	21.5	1	24.5	0.5	26	1	16.5	1	17	0	19.5	1.5	19.5	0	14.5	0	21	0.5	**10.38**	**10.33**
	10	34	0	30	0	41	0	39	0	30	0	19	0	23	0	26	2	30	0	23	0	**14.85**	**15.81**
Post-day 2	0	21.5	8	20.5	5.5	22	6	25.5	5.5	20.5	5	23.5	3	21	7.5	20	3.5	20	5.5	30.5	5.5	**14.00**	**9.07**
	2	9	2	14	1	7.5	0.5	11	2	7.5	1	8	1	7	0.5	8.5	1.5	9.5	1	10	2	**5.23**	**4.35**
	5	14	4	17	4	12	2	19	1	12	3	18	5	8	0	13	0	14	1	14	2	**8.15**	**6.61**
	10	28	0	22	0	23	0	13	0	12	0	21	0	24	0	24	0	22	0	19	0	**10.40**	**11.20**

D = Dapi (nucleus of the cell)

α =α-SMA positive myofibroblast

Appendix I.1a

Table of the progression of scar thickness (measured by Tissue Ultrasound Palpation System) under low pressure

Scar Sample	Scar Thickness					
	Initial	Post-1 mth	Post-2 mth	Post-3 mth	Post-4 mth	Post-5 mth
1	6.58	5.93	5.58	5.37	5.18	5.05
2	5.07	4.87	4.73	4.59	4.47	4.36
3	3.45	3.13	2.81	2.63	2.45	2.35
4	5.34	5.02	4.71	4.45	4.23	4.04
5	3.45	3.36	3.33	3.29	3.27	3.18
6	6.79	6.71	6.57	6.43	6.34	6.24
7	5.75	5.56	5.38	5.23	5.09	4.99
8	4.52	4.38	4.24	4.12	4.00	3.88
9	5.95	5.81	5.71	5.59	5.46	5.33
10	4.52	4.40	4.33	4.26	4.19	4.12
11	5.35	5.05	4.76	4.49	4.22	3.94
12	3.49	3.25	3.02	2.81	2.58	2.34
13	6.49	6.16	5.84	5.52	5.22	4.92
14	4.15	3.87	3.62	3.38	3.14	2.90
15	3.98	3.73	3.47	3.21	2.97	2.73
16	5.78	5.53	5.30	5.10	4.92	4.74
17	4.78	4.49	4.23	3.98	3.76	3.54
18	5.84	5.45	5.07	4.71	4.37	4.09
19	3.79	3.46	3.19	2.95	2.72	2.48
20	5.85	5.54	5.31	5.10	4.89	4.68
21	6.88	6.54	6.19	5.85	5.53	5.23
22	5.78	5.45	5.12	4.82	4.55	4.30
23	5.13	4.87	4.73	4.60	4.48	4.38
24	3.88	3.83	3.80	3.77	3.75	3.73
25	4.98	4.95	4.89	4.85	4.81	4.78
Mean	**5.10**	**4.85**	**4.64**	**4.44**	**4.26**	**4.09**
SD	**1.07**	**1.04**	**1.02**	**1.00**	**1.00**	**1.01**

* Unit = millimeter (mm)

Appendix I.1b

Table of the progression of scar thickness (measured by Tissue Ultrasound Palpation System) under high pressure

Scar Sample	Scar Thickness					
	Initial	Post-1 mth	Post-2 mth	Post-3 mth	Post-4 mth	Post-5 mth
1	5.61	5.01	4.69	4.40	4.16	3.98
2	5.62	4.63	4.28	4.03	3.89	3.79
3	5.95	4.99	4.45	4.07	3.71	3.53
4	3.92	3.06	2.65	2.30	2.01	1.81
5	4.95	4.70	4.46	4.23	4.00	3.82
6	5.26	4.61	4.29	4.05	3.75	3.53
7	4.57	3.92	3.71	3.57	3.47	3.37
8	4.78	4.30	4.00	3.82	3.70	3.60
9	6.85	6.35	6.05	5.91	5.90	5.80
10	5.78	5.27	4.93	4.78	4.65	4.55
11	5.52	5.02	4.63	4.42	4.29	4.22
12	5.85	5.34	5.02	4.84	4.69	4.59
13	4.29	3.41	2.73	2.26	1.84	1.54
14	3.85	2.98	2.38	2.04	1.84	1.70
15	5.45	4.46	3.75	3.37	3.20	3.03
16	3.58	2.73	2.32	2.20	2.10	2.03
17	3.46	2.71	2.30	2.17	2.03	1.95
18	4.55	3.60	3.07	2.83	2.55	2.42
19	5.76	4.86	4.24	3.79	3.43	3.11
20	5.15	4.31	3.65	3.40	3.18	3.06
21	5.35	4.46	3.86	3.35	2.93	2.65
22	5.78	4.80	4.24	3.71	3.37	3.15
23	4.88	4.03	3.48	2.97	2.49	2.17
24	3.95	3.16	2.75	2.46	2.24	2.03
25	3.55	2.86	2.44	2.09	1.77	1.56
26	5.48	4.70	4.02	3.33	2.82	2.50
27	5.16	4.21	3.53	2.88	2.37	2.16
28	3.48	2.64	2.18	1.71	1.46	1.32
Mean	**4.94**	**4.18**	**3.72**	**3.39**	**3.14**	**2.96**
SD	**0.90**	**0.95**	**0.98**	**1.02**	**1.07**	**1.10**

* Unit = millimeter (mm)

Appendix I.2a

Table of the progression of scar color – Δ lightness (measured by the spectrocolorimeter) under low pressure

Scar Sample	Δ L*					
	Initial	**Post-1 mth**	**Post-2 mth**	**Post-3 mth**	**Post-4 mth**	**Post-5 mth**
1	22.54	25.7341	20.7307	21.74	22.541	19.737
2	34.23	33.8216	36.11461	33.1114	38.2252	36.1006
3	8.87	2.065	7.3851	4.2898	4.20161	5.2117
4	30	32.65451	31.466	37.646	34.66	38.4707
5	19.71	19.0633	21.0606	23.0741	14.7852	17.0731
6	31.05	32.754	29.44606	37.6636	36.73537	32.62453
7	31.61	27.8411	34.58451	31.6907	34.8741	40.861
8	26.92	33.506	28.4851	30.3161	34.4851	39.5938
9	22.88	20.41396	22.4141	24.415	27.4141	21.5241
10	17.55	19.8652	14.7541	16.7531	21.7541	14.7742
11	28.08	26.92462	26.8351	27.9251	34.9251	33.80505
12	43.78	55.3251	58.3251	52.3251	58.3251	57.3251
13	29.64	27.1507	23.81077	35.0451	34.03604	41.9331
14	23.43	25.5057	17.70168	22.3166	24.5136	26.3365
15	18.88	24.40518	20.1666	22.4131	25.3247	25.402
16	25.29	24.9365	24.234	23.22565	30.23457	27.10455
17	28.46	31.26556	26.5604	28.5666	39.4555	34.6842
18	20.61	28.6875	21.8692	29.9841	27.572	27.98542
19	20.1	24.96492	20.8541	30.8751	29.9652	28.3636
20	34.07	35.70466	31.9051	40.6836	38.79361	39.7946
21	32.12	31.8751	29.0051	33.89465	37.775	36.8941
22	41.65	44.97457	52.877	46.7315	43.93457	47.0467
23	26.25	38.0246	33.2407	36.26591	40.1741	42.23161
24	25.74	34.2452	31.2262	39.2217	40.9452	38.2447
25	24.4	21.2678	25.26506	28.4468	25.54168	29.5407
Mean	**26.71**	**28.92**	**27.61**	**30.34**	**32.05**	**32.11**
SD	**7.55**	**9.82**	**10.69**	**9.87**	**10.54**	**11.24**

* Unit = percentage (%)

Appendix I.2b

Table of the progression of scar color – Δ lightness (measured by the spectrocolorimeter) under high pressure

Scar Sample	Δ L*					
	Initial	Post-1 mth	Post-2 mth	Post-3 mth	Post-4 mth	Post-5 mth
1	26.69	25.035	32.837	36.0251	35.1442	29.8406
2	30.68	27.921	26.9066	35.1251	34.1161	36.1251
3	25.15	24.0651	27.95357	37.0642	37.7762	34.0477
4	17.95	11.546	15.55341	24.26106	16.6642	19.66568
5	5.48	-4.40394	0.6141	-2.39882	-3.2983	-2.2881
6	31.94	34.5341	35.5347	42.5342	38.627	41.64414
7	29.62	31.565	32.8941	38.8941	40.8941	37.89404
8	26.58	25.8341	23.031	32.721	35.7906	32.7232
9	44.8	57.2541	53.94504	55.0671	53.366	58.0168
10	31.89	34.4341	37.5207	39.4106	45.5447	38.5451
11	34.83	34.2921	37.5396	31.3141	32.42581	37.311
12	30.42	27.984	29.48541	32.4941	33.4931	31.4941
13	12.23	11.92542	14.11451	9.2251	10.9251	12.1071
14	9.6	8.9653	8.966	8.9652	8.8432	5.94192
15	26.2	28.1641	30.0451	26.0741	32.0541	27.1652
16	6.28	4.3252	7.23571	5.211	2.3251	0.0362
17	28.92	35.49471	32.38761	37.7161	45.5861	43.6042
18	20.27	22.3051	21.1941	22.30452	24.3041	30.3042
19	12.43	11.5051	13.5051	11.4951	13.3104	16.6241
20	25.22	32.09451	26.091	37.091	39.2041	38.0041
21	17.27	16.3041	16.3051	17.3041	21.9761	24.3041
22	38.29	44.19504	40.235	46.232	44.31055	41.1235
23	18.14	11.0268	16.0451	17.5256	13.7452	13.7561
24	19.48	19.5918	18.61408	16.275	14.7247	15.524
25	24.47	22.7047	24.591	27.408	26.61477	29.4165
26	38.7	48.05454	45.0351	47.03536	51.533	59.16492
27	31.45	26.3655	27.6641	31.4355	43.44655	39.5577
28	31.78	28.0365	31.12407	30.01675	33.91646	34.307
Mean	**24.88**	**25.04**	**25.96**	**28.35**	**29.55**	**29.50**
SD	**9.89**	**13.40**	**11.98**	**13.84**	**15.00**	**15.08**

* Unit = percentage (%)

Appendix I.2c

Table of the progression of scar color – Δ redness (measured by the spectrocolorimeter) under low pressure

Scar Sample	Δ a*					
	Initial	**Post-1 mth**	**Post-2 mth**	**Post-3 mth**	**Post-4 mth**	**Post-5 mth**
1	184.98	157.92	143.05	134.29	126.40	120.12
2	80.93	73.97	69.52	65.17	61.00	57.17
3	54.88	48.96	43.49	40.22	37.02	35.20
4	132.78	122.24	112.16	103.93	96.61	90.83
5	5.30	3.75	3.36	2.82	2.53	1.16
6	191.74	189.02	186.13	182.47	180.11	176.61
7	152.18	145.88	140.32	135.72	131.70	129.17
8	80.35	77.84	75.12	72.60	70.51	68.45
9	191.40	186.84	183.33	178.84	174.67	170.52
10	64.68	62.27	60.14	58.10	55.94	53.96
11	141.28	133.35	125.50	117.97	110.64	102.79
12	57.47	53.72	50.07	46.57	42.55	1.70
13	187.22	176.45	166.21	155.98	145.74	136.10
14	58.42	53.34	48.64	44.14	39.65	35.17
15	57.45	52.45	47.74	42.63	38.11	33.91
16	100.52	92.30	83.72	77.07	70.88	64.97
17	60.21	54.33	48.16	42.11	36.44	30.66
18	153.81	143.34	130.65	119.39	108.56	101.99
19	51.42	43.41	35.86	29.71	24.36	19.01
20	137.41	127.30	119.48	112.49	105.60	98.88
21	279.64	263.98	249.09	235.66	222.22	208.92
22	140.42	126.70	114.11	103.43	93.64	85.15
23	77.32	70.71	68.19	65.17	62.60	60.22
24	55.04	54.18	53.56	52.95	52.69	52.42
25	70.01	69.15	68.02	67.16	66.54	65.81
Mean	**110.68**	**103.34**	**97.02**	**91.46**	**86.27**	**80.04**
SD	**63.96**	**60.77**	**58.21**	**56.05**	**54.09**	**54.15**

* Unit = percentage (%)

Appendix I.2d

Table of the progression of scar color – Δ redness (measured by the spectrocolorimeter) under high pressure

Scar Sample	Δ a*					
	Initial	**Post-1 mth**	**Post-2 mth**	**Post-3 mth**	**Post-4 mth**	**Post-5 mth**
1	155.33	138.16	128.56	121.24	113.73	108.51
2	141.20	106.93	94.16	85.36	80.27	76.66
3	186.58	152.54	133.47	119.17	105.94	101.92
4	98.18	73.17	61.44	51.83	43.30	37.56
5	64.08	58.18	51.84	46.15	41.01	36.27
6	81.48	62.25	52.28	45.67	37.74	32.29
7	59.29	40.74	35.01	31.12	28.67	27.05
8	68.21	59.02	53.40	49.91	47.56	45.59
9	196.91	180.20	170.77	166.02	165.70	165.30
10	100.60	90.22	82.47	79.74	76.82	75.40
11	99.43	86.09	78.25	71.87	69.22	67.18
12	176.96	158.77	145.83	139.06	132.69	128.80
13	62.30	42.71	26.01	15.86	7.10	0.89
14	65.35	37.82	20.73	10.04	4.24	0.28
15	87.08	53.02	25.04	12.46	6.33	0.32
16	40.12	23.53	15.16	13.22	11.33	9.51
17	45.11	31.51	24.13	22.36	19.70	18.14
18	67.38	46.96	33.49	28.03	22.15	18.68
19	199.48	170.14	147.58	138.89	132.30	124.72
20	71.96	47.24	30.89	19.56	10.42	2.60
21	82.97	54.80	35.98	20.22	9.03	0.48
22	167.68	135.39	116.32	99.56	89.72	82.85
23	98.64	74.78	56.49	39.10	25.04	16.25
24	52.83	32.62	20.91	13.25	7.22	2.05
25	47.95	35.92	27.58	21.04	15.13	11.17
26	97.84	71.75	51.40	32.27	13.63	1.45
27	81.89	55.50	38.02	20.53	6.22	0.08
28	47.17	28.09	18.09	8.23	2.89	0.42
Mean	**98.00**	**76.72**	**63.40**	**54.35**	**47.32**	**42.59**
SD	**49.38**	**46.86**	**46.07**	**46.31**	**47.03**	**47.74**

* Unit = percentage (%)

Appendix I.2e

Table of the progression of scar color – Δ yellowness (measured by the spectrocolorimeter) under low pressure

Scar Sample	Δ b*					
	Initial	**Post-1 mth**	**Post-2 mth**	**Post-3 mth**	**Post-4 mth**	**Post-5 mth**
1	7.86	8.09	8.52	8.39	8.46	8.41
2	11.69	11.92	12.33	12.47	12.46	12.45
3	10.38	10.25	10.81	11.15	11.53	11.68
4	12.72	12.59	12.83	13.16	13.54	13.29
5	13.95	14.19	13.71	14.06	14.26	14.37
6	10.93	11.10	10.90	11.00	11.06	11.06
7	12.02	12.19	12.58	12.12	12.18	12.18
8	7.85	7.98	8.38	8.33	8.43	8.49
9	10.96	11.00	11.16	11.39	11.44	11.43
10	8.00	7.68	8.33	8.52	8.47	8.64
11	12.01	12.23	11.84	12.11	12.33	12.23
12	8.01	7.98	8.57	8.22	8.38	8.47
13	11.86	12.12	12.44	12.88	12.57	12.67
14	8.32	8.66	9.26	9.04	9.07	9.07
15	14.74	14.86	15.25	14.72	14.92	14.82
16	12.06	12.39	12.98	13.38	13.58	13.74
17	11.87	12.20	11.96	12.46	12.26	12.41
18	11.04	11.30	11.29	11.54	11.70	11.57
19	12.98	13.23	12.80	13.26	13.80	14.08
20	10.11	10.43	10.53	10.16	9.93	10.03
21	11.77	11.93	12.19	12.40	12.50	12.63
22	10.22	10.45	10.78	11.33	11.23	11.20
23	12.07	12.32	12.82	12.68	12.44	12.59
24	9.98	10.13	9.99	10.19	10.39	10.38
25	9.30	9.63	10.14	10.12	10.32	10.23
Mean	**10.91**	**11.07**	**11.30**	**11.40**	**11.49**	**11.53**
SD	**1.90**	**1.94**	**1.80**	**1.87**	**1.91**	**1.91**

* Unit = percentage (%)

Appendix I.2f

Table of the progression of scar color – Δ yellowness (measured by the spectrocolorimeter) under high pressure

Scar Sample	Δ b*					
	Initial	Post-1 mth	Post-2 mth	Post-3 mth	Post-4 mth	Post-5 mth
1	10.44	10.21	10.79	10.94	10.82	10.71
2	14.69	15.03	15.38	15.15	15.36	15.35
3	12.24	12.47	12.80	13.01	12.53	12.66
4	11.93	12.06	12.63	12.39	12.74	12.99
5	11.84	12.05	11.91	12.07	12.51	12.65
6	15.99	16.11	16.71	16.85	17.28	16.92
7	9.80	9.71	10.11	10.60	10.36	10.62
8	10.74	10.97	11.27	11.55	11.37	11.40
9	9.01	9.00	9.10	8.90	9.06	9.07
10	7.45	7.58	8.07	8.24	8.31	8.21
11	10.90	11.13	11.52	11.77	11.97	12.07
12	11.31	11.18	11.45	11.75	11.60	11.50
13	13.77	13.54	13.95	14.28	14.77	14.41
14	8.79	8.64	8.91	9.34	9.05	9.24
15	8.98	9.01	10.08	10.51	10.19	10.38
16	15.88	15.61	16.26	15.86	16.29	16.49
17	10.55	10.69	11.12	10.67	11.04	11.31
18	12.15	12.21	12.62	13.12	13.37	13.56
19	11.86	12.21	11.83	12.07	12.33	12.64
20	9.22	9.38	9.51	9.98	9.74	9.87
21	13.65	13.51	13.73	14.06	13.77	13.98
22	11.84	11.85	12.33	12.54	12.81	12.61
23	11.99	12.12	12.41	12.57	12.44	12.71
24	9.66	10.09	10.43	10.81	10.57	10.70
25	13.40	13.28	13.68	13.42	13.88	14.06
26	9.98	10.02	10.72	10.39	10.63	10.82
27	8.70	8.84	8.45	9.01	9.30	9.42
28	7.92	8.15	7.79	8.25	8.04	8.25
Mean	**11.24**	**11.31**	**11.63**	**11.79**	**11.86**	**11.95**
SD	**2.25**	**2.22**	**2.31**	**2.20**	**2.33**	**2.29**

* Unit = percentage (%)

Appendix I.3a

Table of the progression of pain intensity (measured by the Visual Analogue Scale) under low pressure

Scar Sample	Pain Intensity					
	Initial	Post-1 mth	Post-2 mth	Post-3 mth	Post-4 mth	Post-5 mth
1	1.40	1.20	1.20	1.40	1.30	0.60
2	1.20	2.80	2.50	2.20	0.40	1.40
3	3.70	8.40	2.30	3.30	1.50	0.50
4	14.70	12.40	12.40	14.40	16.50	7.30
5	1.70	1.80	0.30	0.20	1.30	0.40
6	2.10	0.00	1.40	0.40	0.80	1.40
7	8.60	5.60	4.40	7.50	6.20	7.30
8	1.60	0.00	0.20	1.50	0.80	0.70
9	1.80	0.00	0.60	0.20	0.60	0.20
10	22.50	10.70	11.60	13.40	15.50	14.50
11	14.30	10.70	9.50	11.30	12.60	8.40
12	0.40	2.50	0.60	1.40	0.60	2.40
13	15.30	12.50	15.30	14.30	17.40	12.60
14	19.70	12.70	16.30	15.60	12.40	14.40
15	0.70	7.80	5.30	6.20	4.30	6.20
16	20.78	15.50	16.30	17.30	12.60	15.20
17	16.85	20.40	14.60	12.60	10.80	11.70
18	27.51	22.50	19.30	19.60	21.80	18.60
19	13.87	14.40	11.80	12.50	8.30	7.30
20	12.97	15.30	14.50	16.20	15.20	12.30
21	9.65	10.50	11.30	10.60	8.30	9.20
22	8.62	10.60	12.60	13.60	18.50	16.30
23	16.81	12.50	13.60	11.60	12.60	10.60
24	4.65	6.90	4.60	5.20	3.60	2.10
25	3.56	2.50	1.50	3.60	2.60	3.60
Mean	**9.80**	**8.81**	**8.16**	**8.64**	**8.26**	**7.41**
SD	**8.09**	**6.39**	**6.37**	**6.27**	**6.85**	**5.88**

* Unit = millimeter (mm)

Appendix I.3b

Table of the progression of pain intensity (measured by the Visual Analogue Scale) under high pressure

Scar Sample	Pain Intensity					
	Initial	Post-1 mth	Post-2 mth	Post-3 mth	Post-4 mth	Post-5 mth
1	2.60	2.40	2.70	1.60	1.40	0.50
2	2.40	3.80	2.30	1.50	2.60	0.30
3	1.80	5.70	3.40	1.30	2.40	3.30
4	11.70	9.40	6.50	7.20	4.40	3.40
5	12.40	10.80	5.20	6.30	4.30	2.50
6	12.60	13.40	6.20	5.60	7.30	3.40
7	12.10	4.80	5.10	3.40	2.20	0.60
8	35.40	37.20	38.30	38.20	40.30	39.40
9	39.20	29.40	22.50	19.50	20.40	18.30
10	5.70	3.40	3.50	2.40	1.30	1.50
11	15.80	19.40	18.60	19.30	17.20	18.30
12	14.30	10.70	8.30	9.20	6.20	7.80
13	31.50	15.40	16.30	12.60	16.30	17.20
14	11.90	16.70	16.30	14.60	9.30	8.30
15	15.20	18.90	20.30	19.20	17.30	21.30
16	2.40	0.00	0.90	0.30	0.60	0.20
17	2.80	0.00	0.40	1.40	0.60	1.40
18	6.70	8.50	6.50	4.80	5.30	2.40
19	15.70	16.60	16.50	17.30	15.30	14.70
20	17.30	19.40	17.30	19.40	18.30	19.30
21	12.90	15.60	15.80	14.60	16.20	18.30
22	10.60	19.60	17.30	16.40	8.60	9.20
23	0.90	1.60	0.80	0.50	0.80	0.80
24	18.60	10.70	9.60	10.60	7.20	8.30
25	19.35	14.50	16.30	11.60	8.30	9.10
26	38.95	40.70	32.30	36.20	34.80	29.40
27	0.84	3.60	1.60	2.40	1.60	3.60
28	10.85	13.60	15.20	17.30	16.20	17.10
Mean	**13.66**	**13.06**	**11.64**	**11.24**	**10.24**	**10.00**
SD	**11.00**	**10.25**	**9.63**	**9.99**	**10.06**	**9.97**

* Unit = millimeter (mm)

Appendix I.3c

Table of the progression of pruritus intensity (measured by the Visual Analogue Scale) under low pressure

Scar Sample	Pruritus Intensity					
	Initial	Post-1 mth	Post-2 mth	Post-3 mth	Post-4 mth	Post-5 mth
1	58.60	34.90	36.50	49.50	34.30	32.30
2	17.10	18.20	20.80	23.50	6.30	4.20
3	54.00	42.60	5.20	6.70	7.10	4.20
4	37.50	32.00	26.00	11.60	15.20	14.20
5	30.50	64.00	70.50	59.60	22.60	25.20
6	19.10	10.60	13.60	12.50	15.40	12.30
7	46.70	36.20	22.30	24.50	25.20	20.60
8	29.70	20.40	19.30	20.40	21.40	17.20
9	20.50	21.30	22.30	21.50	24.20	25.20
10	26.70	25.30	27.30	23.30	23.20	22.30
11	87.60	63.50	42.50	32.50	33.30	29.20
12	51.20	68.60	53.00	48.20	32.60	38.20
13	58.70	60.90	62.90	52.20	56.40	49.20
14	38.90	32.80	29.40	35.30	38.10	36.20
15	89.70	77.80	75.20	52.80	58.20	48.20
16	35.70	43.00	38.20	32.60	31.60	28.40
17	49.90	42.80	44.90	48.30	42.70	43.30
18	50.60	47.10	43.90	42.60	45.20	42.30
19	53.70	52.80	50.80	46.20	45.20	48.30
20	49.70	43.70	42.70	40.20	36.20	36.20
21	83.70	88.90	79.70	62.50	60.20	59.20
22	80.20	82.90	77.40	62.50	67.20	58.30
23	29.40	34.90	35.80	35.50	38.20	36.40
24	34.70	32.90	36.90	37.30	37.20	35.20
25	36.10	30.80	27.60	28.30	29.20	26.20
Mean	**46.81**	**44.36**	**40.19**	**36.40**	**33.86**	**31.70**
SD	**20.97**	**20.84**	**20.42**	**16.00**	**15.88**	**15.04**

* Unit = millimeter (mm)

Appendix I.3d

Table of the progression of pruritus intensity (measured by the Visual Analogue Scale) under high pressure

Scar Sample	Pruritus Intensity					
	Initial	Post-1 mth	Post-2 mth	Post-3 mth	Post-4 mth	Post-5 mth
1	26.70	32.40	52.20	51.20	32.60	33.20
2	62.00	58.00	42.70	54.80	36.40	22.60
3	71.50	64.00	49.00	32.50	34.60	24.50
4	68.50	58.20	53.70	57.30	49.30	42.30
5	42.20	32.40	10.20	12.50	14.20	13.30
6	32.90	22.50	10.20	52.10	51.10	32.40
7	29.90	11.50	10.20	79.90	73.40	22.30
8	24.60	12.50	10.20	53.00	52.30	15.20
9	21.60	10.20	11.20	14.20	13.50	12.30
10	11.70	8.50	9.60	5.20	6.20	2.50
11	20.10	18.30	19.60	12.50	11.30	15.30
12	17.20	4.30	5.20	2.30	3.40	5.20
13	45.10	20.20	19.30	21.50	22.20	18.30
14	35.60	32.30	29.40	28.30	30.20	27.30
15	46.60	48.20	47.20	43.30	45.30	42.20
16	98.50	79.50	55.20	48.90	46.30	49.20
17	84.90	69.50	72.30	52.30	62.40	69.60
18	64.60	72.30	64.00	59.30	42.40	32.30
19	49.70	52.90	55.80	57.50	56.50	62.10
20	60.50	57.90	60.50	63.20	66.40	63.10
21	57.90	60.90	54.30	56.20	58.30	52.30
22	62.70	58.20	48.60	53.50	56.30	52.20
23	80.50	77.00	82.20	68.40	52.60	50.20
24	53.50	58.90	52.30	42.30	43.60	40.50
25	64.50	56.00	48.20	52.80	48.30	38.20
26	46.70	47.80	38.90	42.40	40.30	38.20
27	62.40	57.90	56.20	52.50	54.40	50.60
28	50.90	47.30	48.90	42.40	45.20	40.50
Mean	**49.77**	**43.91**	**39.90**	**43.30**	**41.04**	**34.57**
SD	**21.79**	**22.84**	**22.02**	**19.85**	**18.51**	**17.91**

* Unit = millimeter (mm)

Appendix I.4a

Table of the progression of scar pigmentation (measured by the Vancouver Scar Scale) under low pressure

Scar Sample	Scar Pigmentation					
	Initial	Post-1 mth	Post-2 mth	Post-3 mth	Post-4 mth	Post-5 mth
1	3	3	3	3	3	3
2	3	3	3	3	3	3
3	3	3	3	3	3	3
4	3	3	3	3	2	3
5	1	2	1	1	1	1
6	1	1	1	2	1	1
7	3	3	3	3	3	3
8	3	3	3	3	3	3
9	2	2	1	2	2	2
10	2	2	2	2	2	2
11	1	1	1	1	1	1
12	3	3	3	3	3	3
13	3	3	3	3	3	3
14	2	2	2	2	2	2
15	3	3	3	3	3	3
16	2	2	2	2	2	2
17	1	1	1	1	1	1
18	3	3	3	3	3	3
19	2	2	2	2	2	2
20	3	3	3	3	3	3
21	3	3	3	3	3	3
22	2	2	2	2	2	2
23	2	2	2	2	2	2
24	3	3	3	3	3	3
25	2	2	2	2	2	2
Mean	**2.36**	**2.40**	**2.32**	**2.40**	**2.32**	**2.36**
SD	**0.76**	**0.71**	**0.80**	**0.71**	**0.75**	**0.76**

* The highest score of scar pigmentation in VSS is 3 and the lowest is 0

Appendix I.4b

Table of the progression of scar pigmentation (measured by the Vancouver Scar Scale) under high pressure

Scar Sample	Scar Pigmentation					
	Initial	Post-1 mth	Post-2 mth	Post-3 mth	Post-4 mth	Post-5 mth
1	3	3	3	3	3	3
2	3	3	3	3	1	1
3	3	3	3	3	3	3
4	1	1	1	1	1	1
5	3	3	3	3	3	2
6	3	3	3	2	3	2
7	3	3	3	3	3	3
8	3	3	3	3	3	2
9	2	2	1	2	2	2
10	3	3	3	3	1	1
11	3	2	3	3	3	3
12	3	3	3	3	3	3
13	2	2	2	2	2	2
14	3	3	3	3	3	3
15	3	3	3	3	3	3
16	3	3	3	3	1	1
17	1	1	1	1	1	1
18	2	2	2	2	2	2
19	3	3	2	3	1	1
20	3	3	3	3	3	3
21	3	3	3	3	3	3
22	3	3	3	3	3	3
23	2	1	2	2	2	2
24	3	3	3	1	1	1
25	3	3	3	3	3	3
26	3	3	3	3	3	3
27	3	3	3	3	1	1
28	2	2	2	2	2	2
Mean	**2.68**	**2.61**	**2.61**	**2.57**	**2.25**	**2.14**
SD	**0.61**	**0.69**	**0.69**	**0.69**	**0.89**	**0.85**

* The highest score of scar pigmentation in VSS is 3 and the lowest is 0

Appendix I.4c

Table of the progression of scar vascularity (measured by the Vancouver Scar Scale) under low pressure

Scar Sample	Scar Vascularity					
	Initial	Post-1 mth	Post-2 mth	Post-3 mth	Post-4 mth	Post-5 mth
1	2	2	2	2	2	2
2	2	2	2	2	2	2
3	3	3	2	2	2	1
4	2	2	2	2	1	1
5	1	1	1	1	1	1
6	2	2	2	2	2	2
7	3	3	3	3	3	2
8	2	2	2	2	2	2
9	3	3	3	3	3	2
10	3	3	3	3	3	3
11	3	3	3	3	2	2
12	3	3	3	3	2	2
13	2	2	2	2	2	2
14	3	3	3	3	2	2
15	2	2	2	2	2	2
16	3	3	2	2	1	1
17	3	3	3	3	3	3
18	3	3	3	3	3	3
19	1	1	1	1	1	1
20	3	3	2	2	2	2
21	3	3	2	2	2	2
22	3	3	3	3	3	2
23	3	3	3	3	3	3
24	2	2	2	2	2	2
25	3	3	3	3	3	2
Mean	**2.52**	**2.52**	**2.36**	**2.36**	**2.16**	**1.96**
SD	**0.65**	**0.65**	**0.64**	**0.64**	**0.69**	**0.61**

* The highest score of scar vascularity in VSS is 3 and the lowest is 0

Appendix I.4d

Table of the progression of scar vascularity (measured by the Vancouver Scar Scale) under high pressure

Scar Sample	Scar Vascularity					
	Initial	Post-1 mth	Post-2 mth	Post-3 mth	Post-4 mth	Post-5 mth
1	2	2	2	2	2	2
2	3	3	3	3	3	2
3	2	2	2	2	1	1
4	1	1	1	1	1	1
5	3	3	2	2	2	1
6	3	3	2	2	2	1
7	3	3	2	1	1	1
8	2	2	2	1	1	1
9	2	2	2	1	1	1
10	2	2	1	1	1	1
11	3	3	3	3	2	1
12	3	3	3	3	2	2
13	3	3	3	2	2	2
14	2	2	2	1	1	1
15	3	3	3	2	2	1
16	3	3	2	2	2	2
17	2	2	2	2	2	1
18	3	3	3	2	2	2
19	3	3	3	2	2	1
20	3	3	3	2	2	2
21	3	3	3	3	2	2
22	3	3	3	2	2	2
23	2	2	2	2	2	2
24	3	3	3	2	2	2
25	1	1	1	1	1	1
26	3	3	3	2	2	2
27	2	2	2	2	2	2
28	3	3	3	3	2	2
Mean	**2.54**	**2.54**	**2.36**	**1.93**	**1.75**	**1.50**
SD	**0.64**	**0.64**	**0.68**	**0.66**	**0.52**	**0.51**

* The highest score of scar vascularity in VSS is 3 and the lowest is 0

Appendix I.4e

Table of the progression of scar pliability (measured by the Vancouver Scar Scale) under low pressure

Scar Sample	Scar Pliability					
	Initial	Post-1 mth	Post-2 mth	Post-3 mth	Post-4 mth	Post-5 mth
1	4	4	4	4	4	3
2	3	3	3	3	3	2
3	3	3	3	3	3	3
4	3	3	3	3	3	2
5	2	2	2	2	2	2
6	4	4	4	3	2	2
7	4	4	4	4	4	4
8	2	2	2	2	2	2
9	3	3	3	3	3	3
10	3	3	3	3	3	3
11	3	3	3	3	3	2
12	2	2	2	2	2	2
13	4	4	3	2	2	2
14	3	3	3	3	3	2
15	3	3	3	3	2	2
16	2	2	2	2	2	2
17	2	2	2	2	2	2
18	3	3	3	3	3	2
19	2	2	2	2	2	2
20	3	3	3	3	3	2
21	4	4	4	4	4	4
22	4	4	4	4	4	4
23	3	3	3	3	3	2
24	2	2	2	2	2	2
25	3	3	3	3	3	2
Mean	**2.96**	**2.96**	**2.92**	**2.84**	**2.76**	**2.40**
SD	**0.73**	**0.73**	**0.70**	**0.69**	**0.72**	**0.71**

* The highest score of scar pliability in VSS is 5 and the lowest is 0

Appendix I.4f

Table of the progression of scar pliability (measured by the Vancouver Scar Scale) under high pressure

Scar Sample	Scar Pliability					
	Initial	Post-1 mth	Post-2 mth	Post-3 mth	Post-4 mth	Post-5 mth
1	4	4	4	4	3	3
2	4	4	4	4	3	3
3	4	4	4	3	2	2
4	4	4	4	3	2	2
5	4	4	4	4	4	3
6	3	3	3	3	3	2
7	2	2	2	2	2	1
8	3	3	2	2	2	1
9	2	2	2	2	2	1
10	4	3	3	3	3	3
11	4	4	4	4	4	4
12	3	3	3	3	3	2
13	3	3	3	2	2	1
14	3	3	3	3	2	1
15	4	4	4	3	3	3
16	4	4	4	4	3	3
17	3	2	2	2	1	1
18	2	2	2	2	2	2
19	3	3	3	3	2	2
20	4	4	3	3	3	3
21	4	4	3	3	2	2
22	3	3	3	2	2	2
23	3	3	3	3	2	2
24	3	3	3	3	2	2
25	2	2	2	2	1	1
26	3	3	3	3	2	2
27	4	4	4	4	3	3
28	2	2	2	2	1	1
Mean	**3.25**	**3.18**	**3.07**	**2.89**	**2.36**	**2.07**
SD	**0.75**	**0.77**	**0.77**	**0.74**	**0.78**	**0.86**

* The highest score of scar pliability in VSS is 5 and the lowest is 0

Appendix I.4g

Table of the progression of scar height (measured by the Vancouver Scar Scale) under low pressure

Scar Sample	Scar Height					
	Initial	Post-1 mth	Post-2 mth	Post-3 mth	Post-4 mth	Post-5 mth
1	4	3	3	3	3	3
2	4	4	3	3	3	3
3	3	3	2	2	2	2
4	4	4	3	3	2	2
5	2	2	2	2	2	1
6	4	3	2	2	2	2
7	4	3	3	3	3	2
8	2	2	2	2	2	2
9	4	4	3	3	3	3
10	3	3	3	3	3	3
11	4	4	3	3	2	2
12	2	2	2	2	1	1
13	4	4	4	4	3	3
14	3	3	3	3	2	2
15	2	2	2	2	1	1
16	4	4	4	4	3	3
17	3	3	3	2	2	1
18	4	3	3	3	2	2
19	3	3	3	2	2	2
20	4	4	4	3	3	3
21	4	4	3	3	3	3
22	4	4	3	3	3	3
23	4	3	3	3	3	3
24	2	2	2	2	2	2
25	3	3	3	3	3	3
Mean	**3.36**	**3.16**	**2.84**	**2.72**	**2.40**	**2.28**
SD	**0.81**	**0.75**	**0.62**	**0.61**	**0.65**	**0.74**

* The highest score of scar height in VSS is 4 and the lowest is 0

Appendix I.4h

Table of the progression of scar height (measured by the Vancouver Scar Scale) under high pressure

Scar Sample	Scar Height					
	Initial	Post-1 mth	Post-2 mth	Post-3 mth	Post-4 mth	Post-5 mth
1	4	2	2	2	2	2
2	4	3	3	3	3	3
3	4	2	2	2	2	2
4	3	3	2	2	2	2
5	4	4	4	4	3	3
6	4	3	3	3	3	3
7	2	2	2	2	2	2
8	3	2	2	2	1	1
9	4	2	2	2	2	2
10	3	3	2	2	2	2
11	4	3	3	2	2	2
12	4	3	3	3	2	2
13	3	2	2	2	2	2
14	3	3	3	2	2	2
15	4	2	2	2	2	2
16	3	2	2	2	2	2
17	2	2	2	2	2	2
18	2	2	2	2	2	2
19	4	2	2	2	2	2
20	4	3	3	3	2	2
21	4	3	3	3	2	2
22	4	2	2	2	2	2
23	4	3	3	3	3	3
24	3	3	2	2	2	2
25	3	3	3	2	2	2
26	4	2	2	2	2	2
27	4	2	2	2	2	2
28	3	3	2	2	2	2
Mean	**3.46**	**2.54**	**2.39**	**2.29**	**2.11**	**2.11**
SD	**0.69**	**0.58**	**0.57**	**0.53**	**0.42**	**0.42**

* The highest score of scar height in VSS is 4 and the lowest is 0

Appendix I.4i

Table of the progression of the total score of the Vancouver Scar Scale under low pressure

Scar Sample	Total Score					
	Initial	**Post-1 mth**	**Post-2 mth**	**Post-3 mth**	**Post-4 mth**	**Post-5 mth**
1	13	12	12	12	12	11
2	12	12	11	11	11	10
3	12	12	10	10	10	9
4	12	12	11	11	8	8
5	6	7	6	6	6	5
6	11	10	9	9	7	7
7	14	13	13	13	13	11
8	9	9	9	9	9	9
9	12	12	10	11	11	10
10	11	11	11	11	11	11
11	11	11	10	10	8	7
12	10	10	10	10	8	8
13	13	13	12	11	10	10
14	11	11	11	11	9	8
15	10	10	10	10	8	8
16	11	11	10	10	8	8
17	9	9	9	8	8	7
18	13	12	12	12	11	10
19	8	8	8	7	7	7
20	13	13	12	11	11	10
21	14	14	12	12	12	12
22	13	13	12	12	12	11
23	12	11	11	11	11	10
24	9	9	9	9	9	9
25	11	11	11	11	11	9
Mean	**11.20**	**11.04**	**10.44**	**10.32**	**9.64**	**9.00**
SD	**1.94**	**1.72**	**1.56**	**1.63**	**1.89**	**1.68**

* The highest score of total score in VSS is 15 and the lowest is 0

Appendix I.4j

Table of the progression of the total score of the Vancouver Scar Scale under high pressure

Scar Sample	Total Score					
	Initial	Post-1 mth	Post-2 mth	Post-3 mth	Post-4 mth	Post-5 mth
1	13	11	11	11	10	10
2	14	13	13	13	10	9
3	13	11	11	10	8	8
4	9	9	8	7	6	6
5	14	14	13	13	12	9
6	13	12	11	10	11	8
7	10	10	9	8	8	7
8	11	10	9	8	7	5
9	10	8	7	7	7	6
10	12	11	9	9	7	7
11	14	12	13	12	11	10
12	13	12	12	12	10	9
13	11	10	10	8	8	7
14	11	11	11	9	8	7
15	14	12	12	10	10	9
16	13	12	11	11	8	8
17	8	7	7	7	6	5
18	9	9	9	8	8	8
19	13	11	10	10	7	6
20	14	13	12	11	10	10
21	14	13	12	12	9	9
22	13	11	11	9	9	9
23	11	9	10	10	9	9
24	12	12	11	8	7	7
25	9	9	9	8	7	7
26	13	11	11	10	9	9
27	13	11	11	11	8	8
28	10	10	9	9	7	7
Mean	**11.93**	**10.86**	**10.43**	**9.68**	**8.46**	**7.82**
SD	**1.84**	**1.63**	**1.67**	**1.79**	**1.57**	**1.44**

* The highest score of total score in VSS is 15 and the lowest is 0