THE STUDY OF SCAR VASCULARITY MEASUREMENT AND EFFECT OF CONTROLLING VASCULARITY BY PULSED DYE LASER ON MANAGING HYPERTROPHIC SCARS

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The Study of Scar Vascularity Measurement and Effect of Controlling Vascularity by Pulsed Dye Laser on Managing Hypertrophic Scars

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CERTIFICATE OF ORIGINALITY

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ABSTRACT

Background: Scar vascularity is a key indicator of scar maturation. Measurement of scar vascularity monitors scar change and guides targeted interventions to prevent excessive scarring and achieve promising outcomes. However, there is no consensus on the assessment tools for scar vascularity measurement. Given that vascularity of hypertrophic scar significantly increases at an early stage and plays an important role in hypertrophic scar development, controlling scar vascularity at an early stage might be an effective way to limit scar growth and promote scar maturation. As one type of laser therapy, pulsed dye laser (PDL) directly causes damage to scar microvascular structures and has preliminarily shown its effect on managing hypertrophic scars. However, the relationship between controlling scar vascularity by PDL and limiting scar growth is not fully understood.

Objectives: This study consists of two phases, measurement and control of scar vascularity. Phase one aims to systematically review clinical tools on scar vascularity measurement **(Chapter Two)** and validate the use of dermoscopy to measure scar vascularity **(Chapter Three)**. Phase two aims to explore the effect of controlling scar vascularity by PDL on managing hypertrophic scars **(Chapter Four)**.

Methods: Chapter two is a systematic review by searching PubMed, CINAHL, Embase and Science Direct databases. Studies, which used non-invasive measurement tools and explored their clinimetric properties, were identified and included. **Chapter three** is a longitudinal exploratory study. Patients with hypertrophic scars were recruited for scar assessments at baseline and at one-month follow-up, which consisted of the

Patient and Observer Scar Assessment Scale (POSAS), DermaLab Combo, ultrasound and dermoscopy. **Chapter Four** is a 3-month assessor-blinded experimental study. Patients with hypertrophic scars less than one year after injury were enrolled into the PDL group or the control group. Patients in the PDL group received three PDL sessions at 4-week intervals. A total of three assessments were performed, at baseline, 1 month and 3 months, consisting of the POSAS and objective measurements of scar erythema, blood perfusion and scar thickness.

Results: (Chapter Two) A total of 1458 articles were obtained, and 26 articles were finally included in this review. Subjective vascularity measurement scales include the POSAS, the Vancouver Scar Scale (VSS) and the modified Vancouver Scar Scale (mVSS), while objective vascularity measurement devices consist of the color-measuring device, the blood flow measuring device and the morphological imaging device. **(Chapter Three)** Forty hypertrophic scars at the active proliferation stage were included in this study. The dermoscopic measurements based on color significantly discriminated the hypertrophic scars from the healthy skin ($p < 0.001$). In addition, they showed moderate to strong correlations with the vascularity component of the POSAS ($r = -0.438$, $p < 0.01$; $r = -0.461$, $p < 0.01$; and $r = -0.437$, $p < 0.01$) and the erythema value as measured by DermaLab Combo ($r = -0.474$, $p < 0.01$; $r = -0.603$, $p < 0.01$; and $r = -0.498$, $p < 0.01$). For prediction of the scars with high risk of thickness change, the green value by dermoscopy was the strongest predictor (AUC = 0.738, $p = 0.034$, 95%CI = 0.570-0.906). **(Chapter Four)** A total of 45 patients were enrolled, 22 in the PDL group and 23 in the control group. After the 3-month treatment, parameters of scar vascularity ($p = 0.003$), pigmentation ($p = 0.026$), color ($p < 0.001$), thickness ($p < 0.05$) and overall scores ($p < 0.01$) on the POSAS significantly decreased in the PDL

group. Moreover, objective measurements of scar erythema and blood perfusion showed significant improvements in the PDL group (p = 0.009 and p = 0.022, respectively) but not in the control group (p = 0.296 and p = 0.115, respectively). In addition, patients in the PDL group maintained a stable scar thickness compared to the control group which significantly increased from baseline, 1 month to 3 months (p < 0.01).

Conclusion: Subjective scales are easy to use and have acceptable reliability to give a preliminary impression of scar vascularity. Three types of objective devices are not equivalent to measure scar vascularity. Dermoscopy, which measures scar color and provides a view of scar vascular structures, could be an objective tool of measuring scar vascularity. In addition, PDL, which improves scar erythema and poor perfusion, is recommended for immature and erythematous hypertrophic scars to limit scar thickness growth and promote scar maturation.

PUBLICATIONS

Journal papers:

- **Deng, H.**, Tan, T., Luo, G., Tan, J., & Li-Tsang, C. W. P. (2020). Vascularity and thickness changes in immature hypertrophic scars treated with a pulsed dye laser. *Lasers in Surgery and Medicine.* (accept)
- **Deng, H.**, Li-Tsang, C. W. P., & Li, J. (2020). Measuring vascularity of hypertrophic scars by dermoscopy: Construct validity and predictive ability of scar thickness change. *Skin Research and Technology*, 26(3), 369-375.
- **Deng, H.**, & Li-Tsang, C. W. P. (2019). Measurement of vascularity in the scar: A systematic review. *Burns*, 45(6), 1253-1265.

Conference presentations:

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LIST OF ABBREVIATIONS

5-FU	5-Fluorouracil
AHS	Active Hypertrophic Scar
AUC	Area Under the Curve
CDU	Color Doppler Ultrasound
CI	Confidence Interval
DME	Direct Magnitude Estimation
EAI	Equal Appearing Interval
ICC	Intra-class Correlation Coefficient
IPL	Intense Pulsed Light
IQR	Interquartile Range
LDI	Laser Doppler Imaging
LED	Light Emitting Diode
mVSS	modified Vancouver Scar Scale
NT	Normotrophic Scar
OCT	Optical Coherence Tomography
OR	Odds Ratio
OSAS	Observer Scar Assessment Scale
PDL	Pulsed Dye Laser
POSAS	Patient and Observer Scar Assessment Scale
PSAS	Patient Scar Assessment Scale
PWS	Port Wine Stain
RGB	Red Green Blue
RHS	Remitted Hypertrophic Scar

ROC	Receiver Operating Characteristic
SD	Standard Deviation
SDI	Standardized Digital Imaging
SpM	Spectral Modelling
TAC	Triamcinolone Acetonide
TBSA	Total Body Surface Area
TEWL	Trans-Epidermal Water Loss
VEGF	Vascular Endothelial Growth Factor
VSS	Vancouver Scar Scale

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Skin is the largest organ, which is more than 2 m^2 of surface area in adults and constitutes around 15% of total body weight. There are two layers of skin, the epidermis and the dermis. The epidermis locates on the superficial and is comprised of stratum basale, stratum spinosum, stratum granulosum, stratum lucidum and stratum corneum (Kanitakis, 2002). The dermis is underneath the epidermis and consists of superficial papillary dermis and deep reticular dermis. The papillary dermis has the greater amount of loose connective tissue while the reticular dermis has the greater amount of dense collagen fibers. There are no blood vessels in the epidermis but two vascular plexuses in the dermis for supplying oxygen and nutrition as well as disposing of metabolic waste. One vascular plexus locates between the papillary dermis and the reticular dermis while one vascular plexus lies between the dermis and the subcutaneous tissue. Skin functions include protecting body from environment damage, preventing excessive water loss, regulating body temperature and receiving sensory stimulations (Montagna, 2012).

1.1.1 Burn injury

Burn injuries, which consist of scalds, flame burns, contact burns, chemical burns and electrical burns, cause damage to skin. In the United States, a report showed that scalds were more frequent to happen in children under five-years-old while flame burns was the most common cause of burn injuries for children with older ages between 2001 to 2010 (David N. Herndon, 2018). In Australia, a review showed that 56% of burn injuries in children resulted from scalds and 31% of them was contact burns (Abeyasundara, Rajan, Lam, Harvey, & Holland, 2011). In China, a 20-year review reported that flame burns was the major cause of burn injuries (H. Li et al., 2017).

Workplace was one of the most common places where burn injuries happened (Clouatre, Gomez, Banfield, & Jeschke, 2013). Meanwhile, ignition of alcohol, cooking oil and other flammable liquids, automobiles, motorcycle exhaust pipes, fireworks, explosion and fishing related burn injuries were reported. It was estimated that 322,000 patients died from burn injuries in 2002 (Peck, Kruger, van der Merwe, Godakumbura, & Ahuja, 2008). Comparing with developed countries, the incidence and mortality rate of burn injuries were higher in developing countries due to limited protection and medical resources (Ahuja & Bhattacharya, 2004). Burn injuries not only affect burn survivors and their family but also increase financial burden for the government.

Depth of burn injury is used to describe injury severity and is divided into four degrees. First degree of burn injury affects the epidermis only with a red and dry injured site. Second degree of burn injury involves part of the dermis with blisters and painful feelings. Third degree of burn injury causes damage to the whole layer of skin and the injured skin might be white, while fourth degree of burn injury affects till the underlying tissue such as muscle or tendon. After a burn injury, the injured skin follows a healing process which consists of inflammation phase, proliferation phase and maturation phase (Gurtner, Werner, Barrandon, & Longaker, 2008). The inflammation phase usually takes two to three days. The proliferation phase lasts three to six weeks and gradually transfers to the maturation phase. Scar formation takes place during the wound healing process. Generally, first degree of burn injury does not leave a scar for burn survivors, while no less than second degree of burn injury heals with a scar. A burn wound with healing time more than three weeks is at high risk of developing a hypertrophic scar (Monstrey, Hoeksema, Verbelen, Pirayesh, & Blondeel, 2008).

1.1.2 Hypertrophic scar

A study showed 38% of scar prevalence after burn injuries and 26% of scar prevalence in a single anatomical area based on analyzing 100 patients (Deitch, Wheelahan, Rose, Clothier, & Cotter, 1983). Another study evaluated more than 700 children with burn injuries and reported a more than 32% of scar prevalence (Dedovic, Koupilova, & Brychta, 1999). In addition, a study, which recruited and included Chinese patients, reported a 74.7% of hypertrophic scar prevalence after one month post-injury (Li-Tsang, Lau, & Chan, 2005). Recent studies showed that more than 16% of patients developed hypertrophic scars after burn injuries (Brown & Bayat, 2009; Ud-Din & Bayat, 2014).

Some risk factors, which are closely related to developing hypertrophic scars, have been reported such as wound healing time and number of surgeries (Gangemi et al., 2008). Increasing studies also showed that injured sites with experiencing more internal or external mechanical forces such as scars over joints were more prone to developing hypertrophic scars (Hsu et al., 2018). Internal and external mechanical forces in skin might trigger bio-chemical responses and further stimulate scar growth (Eyckmans, Boudou, Yu, & Chen, 2011).

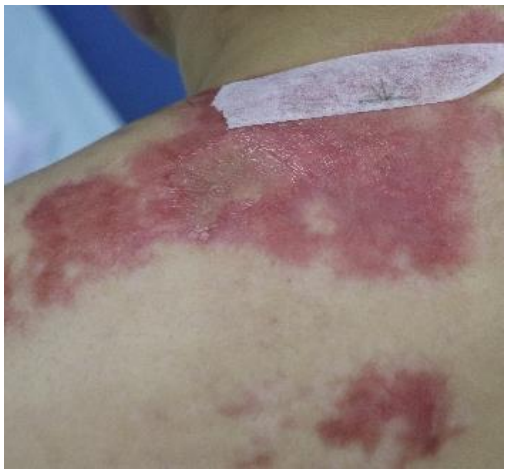
Hypertrophic scar is the outcome of abnormal wound healing and is featured with the excessive scar formation. Histological studies show that hypertrophic scar develops within the original wound border, improves with time and mainly contains type III collagen (Slemp & Kirschner, 2006). Clinically, hypertrophic scar is featured with red and uneven appearance, poor pliability and increased thickness (Figure 1.1). Some

patients with hypertrophic scars also experience pruritus and pain symptoms. Moreover, hypertrophic scars around joints commonly limit range of joint motion resulting in functional problems and affecting survivors' participation in daily living, and severe cases might develop permanent joint deformities (Leventhal, Furr, & Reiter, 2006; Oster, Kildal, & Ekselius, 2010).

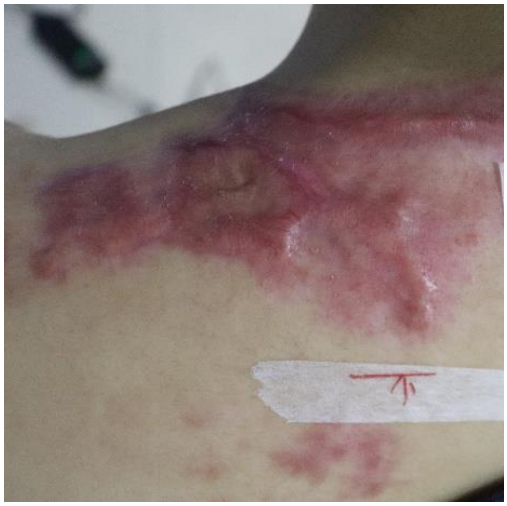
Except for functional problems, hypertrophic scars result in psychological issues. It has been shown that some patients felt depression and anxiety, and developed acute distress disorder and post-traumatic stress disorder (McGarry et al., 2014; J. C. Schneider, Holavanahalli, Helm, Goldstein, & Kowalske, 2006; Van Loey & Van Son, 2003). It was suggested that the depression history, gender of female, and facial disfigurement were risk factors of depression after burn injuries. A previous study also reported a significant correlation between scars and survivors' esteem (Lawrence, Mason, Schomer, & Klein, 2012). More importantly, scar severity had a direct relationship with their quality of life (Nitescu et al., 2012). A systematic review suggested that caregivers also experienced psychological problems. They might have the feeling of guilt, shame and blame (Kornhaber, Childs, & Cleary, 2018). Overall, hypertrophic scars commonly result in functional and psychological problems, which further have influence on survivors' daily life as well as returning to work and society.

Figure 1.1 The development process of a hypertrophic scar (a) at 2 months, (b) at 3 months and (c) at 4 months after burn injury

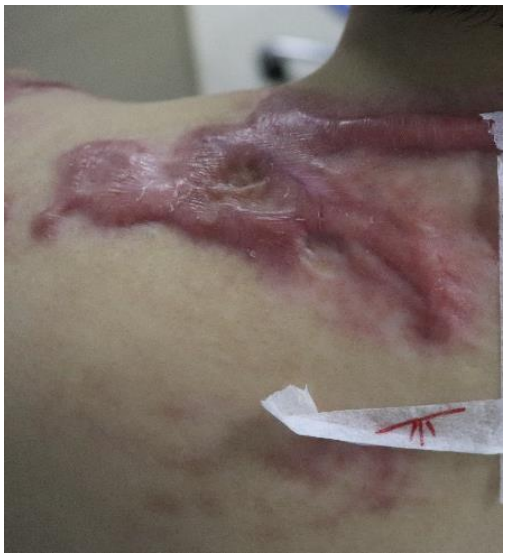
(a)



(b)



(c)



1.1.3 Scar assessment

Scar assessment presents scar properties and monitors scar changes over time that plays an important role in exploring effects of scar treatments and guiding treatment choices. There are multiple scar parameters, which are assessed in clinical work and research studies.

Scar color includes two components, scar erythema and scar pigmentation. Scar erythema is directly related to the amount of hemoglobin (Dawson et al., 1980; Takiwaki, 1998). Oxygenated hemoglobin reflects a high percentage of red light. As a result, a scar with a large amount of hemoglobin presents a deep color of redness. Therefore, measuring degree of redness is the major method to assess scar erythema. For subjective scales, scar erythema is rated as 'normal', 'pink', 'red' or 'purple' on the Vancouver Scar Scale (VSS) while it is rated from 1 to 10 with increase in redness on the Patient and Observer Scar Assessment Scale (POSAS) (Nguyen, Feldstein, Shumaker, & Krakowski, 2015). For objective tools, the color-measuring device is the most common used tool and is based on the principle of reflectance spectroscopy. It measures light absorption of hemoglobin to assess scar erythema. There are two sub-types of the color-measuring device including the tristimulus reflectance colorimeters and the narrow-band simple reflectance meters (Jones, 2012; Shriver & Parra, 2000). For devices based on the tristimulus reflectance colorimetry such as the LabScan XE and Spectrophotometer, the indices of a* is interpreted to measure the scar erythema. For devices based on the narrow-band simple reflectance meters such as the DermaSpectrometer and Mexameter, the erythema indices reflect the scar erythema.

As another main component of scar color, scar pigmentation is closely associated with

the amount of melanin. Scar pigmentation is different from healthy skin pigmentation (Figure 1.2) and usually changes over time (Figure 1.3). Pressure on skin is able to block hemoglobin from flowing into capillaries. Whereas it rarely affects scar pigmentation. Therefore, scar pigmentation is assessed in subjective scales by using a transparent glass to blanch out hemoglobin and then rating scar pigmentation as 'normal', 'hypopigmentation' or 'hyperpigmentation' on the VSS or rating from 1 to 10 with increase of melanin on the POSAS. For objective tools, the color-measuring device is based on quantifying light absorption of melanin to assess scar pigmentation. The L* value by the tristimulus reflectance colorimeters and the melanin value by the narrow-band simple reflectance meters are used to present scar pigmentation (L. J. Draaijers, Tempelman, et al., 2004). Given that light is absorbed by both hemoglobin and melanin, the measurement result of scar erythema by using the color-measuring device would be affected by melanin in scars and is related to its amount (Jones, 2012; Shriver & Parra, 2000).

Scar thickness is made up of the epidermal and dermal thickness and usually increases rapidly during scar active proliferation stage. The change in scar thickness is commonly used to reflect scar growth speed. On the VSS, scar thickness is rated as 'flat', '<2 mm', '2-5 mm', or '> 5mm' by a subjective judgement. This method potentially underestimates or overestimates the real scar thickness. Thus, increasing studies have adopted ultrasound to measure scar thickness (K. C. Lee, Dretzke, Grover, Logan, & Moiemen, 2016). The ultrasound device sends out acoustic pulse, returned signals by scar tissue are collected and an ultrasound image is produced. In the image, dark areas indicate areas with small changes in density while bright areas suggest areas with big changes in density (Szabo, 2004). Moreover, frequency of the ultrasound device

determines its penetration depth and resolution. An ultrasound device with a high frequency generates a high-resolution image with a shallow penetration depth, whereas an ultrasound device with a low frequency generates a low-resolution image with a deep penetration depth. A previous study explored the correlation between an ultrasound device and the VSS for measuring scar thickness (Lau, Li-Tsang, & Zheng, 2005). Their results showed that the scar thickness measurement by ultrasound had good inter-rater and test-retest reliability, and a moderate correlation with the scar thickness score on the VSS. Another study consistently reported that the ultrasound measurement had acceptable inter-rater and test-retest reliability, and a strong correlation with the scar thickness score of POSAS (Simons, Kee, Kimble, & Tyack, 2017).

Scar pliability presents scar contractile and elastic property, which is closely associated with the functional outcome. Patients with stiff scars are prone to limitation of movement especially for scars across joints (Stekelenburg et al., 2015). On the subjective scale of VSS, scar pliability is assessed as ‘normal’, ‘supple: flexible with minimal resistance’, ‘yielding: giving way to pressure’, ‘firm: inflexible, not easily moved, resistant to manual pressure’, ‘banding: rope-like tissue that blanches with extension of scar’, or ‘contracture: permanent shortening of scar producing deformity or distortion’. For objective assessment tools, there are different methods for measuring scar pliability and they mainly include the non-suction method and the suction method (Verhaegen, van der Wal, Middelkoop, & van Zuijlen, 2011). The non-suction method relies on stretching skin with a stable tension spring. However, this method is potentially affected by surrounding forces resulting from the deformation of surrounding tissues while stretching (Reihsner, Balogh, & Menzel, 1995). The

suction method is based on generating negative pressure. The scar deformation under negative pressure and the recovery process after releasing pressure is analyzed to quantify scar pliability. There are different types of devices adopting the suction method such as the elasticity probe of DermaLab and the Cutometer. A study reported that the Cutometer had acceptable reliability but varied correlations with a subjective pliability rating (L. J. Draaijers, Botman, et al., 2004).

Scar blood flow is related to red blood cells within scar capillaries. As the most commonly used tool for measuring blood flow, laser Doppler imaging (LDI) is a fast and non-contact assessment device and could scan a large injured area at one time. Laser light generated by LDI is reflected by moving red blood cells with Doppler frequency shifts, and then a color-coded image is produced. Thus, the measurement result by LDI depends on the concentration and speed of red blood cells in the scanned area. LDI has been applied to measurement of dermal inflammation and cutaneous ulceration (Murray, Herrick, & King, 2004). In the field of burn injury and hypertrophic scar, an early study reported that the newly healed wounds showed 18 times greater of blood flow than the healthy skin, and the wound, which developed into a hypertrophic scar, showed three times greater of blood perfusion than the healthy skin as well as four times greater of blood perfusion than the wound, which developed into a normal scar (Ehrlich & Kelley, 1992). Given its measurement mechanism, LDI is commonly used for burn depth assessment (Devgan, Bhat, Aylward, & Spence, 2006). A study compared the LDI measurement results with the clinical assessment results and wound healing time, and demonstrated that LDI was feasible to discriminate the superficial dermal burns from the deep dermal burns (Hoeksema et al., 2009).

Trans-epidermal water loss (TEWL) and scar hydration are two scar parameters to reflect the skin barrier function. TEWL shows the amount of water evaporating through a scar to environment while scar hydration presents the amount of water in a scar. Both TEWL and scar hydration could be measured by two different probes of DermaLab. It has been reported that the value of TEWL in scars was significantly different from the value in healthy skin and significantly correlated with the time after injury (Anthonissen et al., 2013). Decrease of hydration was detected in scars (Xu et al., 2015).

Assessment of scar microstructure morphology previously depends on histological tests and biopsy. However, their infeasibility of repeated and dynamic measurements as well as direct damage to skin facilitate the development of non-invasive devices to assess scar morphology. As one of the assessment devices, the optical coherence tomography (OCT) measures the echo delay time and magnitude of backscattered or back-reflected light to generate cross-sectional images of tissue microstructures. The measurement results by OCT reported that blood vessel density in the scar was higher than it in the healthy skin (Gong, Chin, et al., 2014; Gong, McLaughlin, et al., 2014; Liew, McLaughlin, Gong, Wood, & Sampson, 2013). The OCT is also applied to assessment of inflammatory skin conditions, blistering diseases and skin tumors (Gambichler et al., 2005). To improve the OCT image quality, growing studies have explored different methods such as the combination use with other devices to reduce noise (Choi, Reif, Yousefi, & Wang, 2014; Kwon et al., 2017).

Comparing with the OCT, the dermoscopy, with utilizing optical magnification and cross-polarized light, provides a more direct way to observe scar structures. Only the

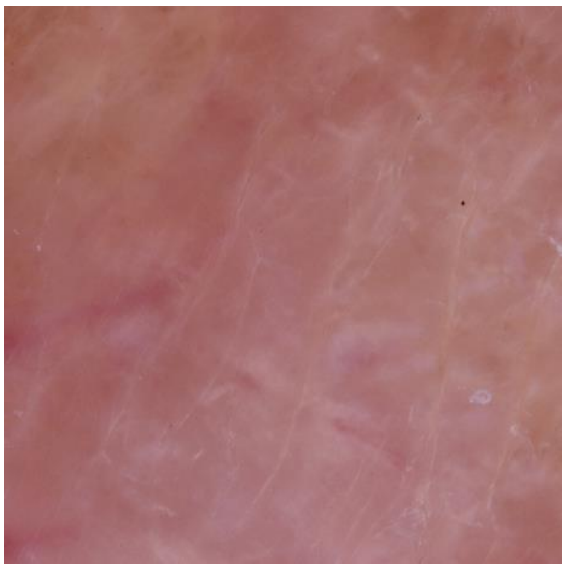
polarized light generated by dermoscopy, which changes its direction with sufficient scattering, could pass through the cross-polarizing filter and is captured. Thus, the dermoscopy with cross-polarized light allows to look clearer and deeper than naked eyes and allows visualization of the superficial dermis.

As a non-invasive and handheld device, the dermoscopy is widely used in the dermatology field such as assessment of psoriasis and skin tumor (Lallas et al., 2014). Growing evidence has supported the use of dermoscopy for assisting in the melanoma diagnosis (Bafounta, Beauchet, Aegerter, & Saiag, 2001; Kittler, Pehamberger, Wolff, & Binder, 2002; Vestergaard, Macaskill, Holt, & Menzies, 2008). In reference to its application in skin cancer, the methods of quantifying dermoscopy captured images mainly include color measurement and structure evaluation. ABCD rules, rule of seven points, rule of three points, Menzies' method and analysis of patterns are different methods of structure evaluation. A systematic review indicated insignificant differences in diagnosis abilities among different methods (Rajpara, Botello, Townend, & Ormerod, 2009). Another study reviewed the use of dermoscopy in non-pigmented skin tumors and summarized methods of quantifying vascular structures, which consist of morphological category of vascular pattern such as comma-like and linear-irregular vessel, architecture category of vessel such as string-like and branched vessel, as well as additional criteria such as hair and ulceration (Zalaudek et al., 2010). However, these methods greatly rely on assessors' subjective judgements. To get a more objective method of identifying vascular structures, methods with using different image processing algorithms have been developed (Celebi, Iyatomi, Schaefer, & Stoecker, 2009; Garnavi, Aldeen, Celebi, Varigos, & Finch, 2011; Gharabaghi, Daneshvar, & Sedaaghi, 2013; Moccia, De Momi, El Hadji, & Mattos, 2018). Despite the less use of

dermoscopy in scar assessment, its measurement results showed the increase and dilation of blood vessels during the scar development process that were consistent with the histological findings (Campanati et al., 2010).

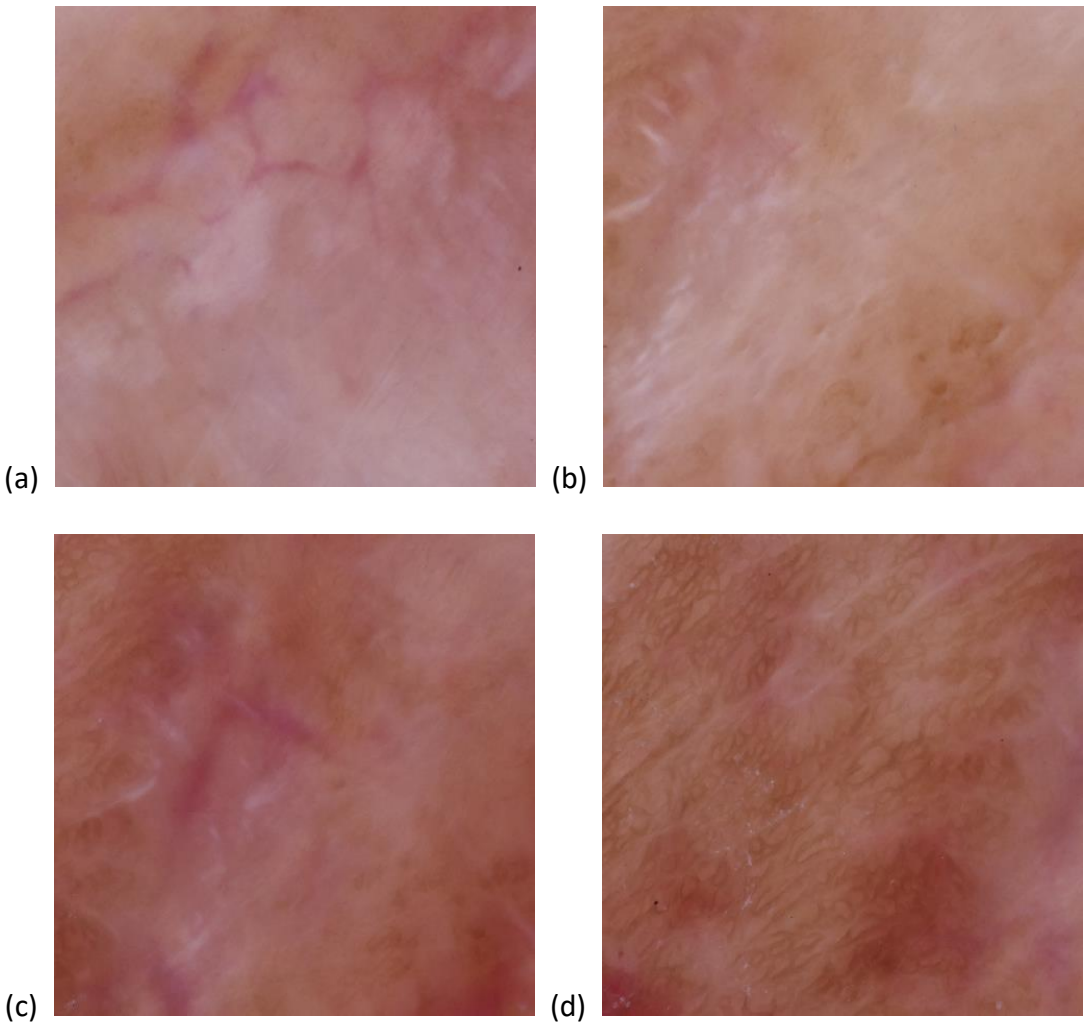
Figure 1.2 The pigmentation in a burn patient (a) in the scar and (b) in the healthy skin. The transparent glass in the front of dermoscopy was used to blanch out hemoglobin. When pressure could not further change the scar or skin color, the image was captured by using dermoscopy with cross-polarized light. This method was also used for taking the Figure 1.3.

(a)



(b)

Figure 1.3 The pigmentation change in a burn scar (a) at 5 months (b) at 6 months (c) at 7 months and (d) at 8 months after injury



1.1.4 Scar treatment

A wide range of treatments are currently in clinical use to manage hypertrophic scars (Friedstat & Hultman, 2014; Leventhal et al., 2006).

As one of the first-line and non-invasive scar management treatments, pressure therapy is widely utilized in clinical settings (Committee, Steering, & Advisory, 2016). It is indicated that pressure therapy limits scar formation by decreasing nutrition supply and collagen synthesis (Atiyeh, El Khatib, & Dibo, 2013). A systematic review reported that pressure therapy significantly reduced scar thickness, pigmentation and hardness (Ai et al., 2017). However, there was not a significant difference in scar vascularity. Pressure dosage is a key factor determining its effect on managing hypertrophic scars. Pressure dosage from 15 to 25 mmHg is recommended as safe and effective dosage to manage hypertrophic scars (Ai et al., 2017). Due to concave and convex shape of human bodies, there are different types of pressure products such as pressure garment, pressure padding and facemask to ensure sufficient pressure dosage on different scar sites. Given that it is difficult to apply pressure on an uneven face and patients who have facial hypertrophic scars are at high risk of developing psychological problems, providing optimal pressure dosage for facial hypertrophic scars is important and challenging. Pressure garment was firstly used to manage facial hypertrophic scars. However, holes for eyes, nose and mouth in a pressure garment make it difficult to reach a promising and even pressure dosage. The rigid facemask is increasingly used (Rivers, Strate, & Solem, 1979; Ward, 1991). Comparing with pressure garment, the facemask with fixed shape is more customized and shows better therapeutic effects on preventing facial disfigurement. The traditional method of fabricating a facemask is based on a face model, which duplicates the shape of

patient's face and is made up of plaster. The technician adjusts the face model to ensure optimal pressure dosages on different areas. Then, the high-temperature thermoplastic material is heated and molded on the face model to get the facemask. This process is labor intensive and usually costs around two weeks. With advances in technology, 3D scanners have been used in some burn centers that shortens the fabricating process and is more accurate than the traditional method (Y. Wei et al., 2018). Due to the fact that the facemask pressure is affected by its anchors and straps, the 4-point harness method is suggested as the cheapest and easiest method to fix the facemask (Parry et al., 2013).

Silicone gel sheet firstly showed its potential effect on managing burn scars in 1980s (Perkins, Davey, & Wallis, 1983). Studies have showed that silicone gel sheet improved scar thickness, scar color and symptoms of pain and pruritus (Li-Tsang, Zheng, & Lau, 2010; O'Brien & Jones, 2013). A study recruited 96 patients with high or low risk of excessive scar formation (Gold, Foster, Adair, Burlison, & Lewis, 2001). The patients were randomly allocated to treatment of the silicone gel sheet or the routine care. Their results showed an insignificant difference in low-risk patients but a significant difference in high-risk patients between use of the silicone gel sheet and the routine care. Therefore, silicone gel sheet is recommended as the first-line scar treatment and could be combinedly used with pressure garment. The mechanism of silicone gel sheet in scar management has not been completely understood, and current evidence suggests that its effect of occlusion and hydration benefits of scar maturation (Mustoe, 2008). In normal skin, the epithelium is to conserve water and protect from infection. Burn injury causes damage to the epithelium. Thus, the increase in water evaporation and decrease in hydration of the stratum corneum were detected in scars. The

occlusion effect provided by silicone gel sheet decreases water loss from the epidermis and improves the scar hydration (O'Shaughnessy, De La Garza, Roy, & Mustoe, 2009). These changes might further send signals to fibroblasts in the dermis and decrease the collagen production (Stavrou et al., 2010).

Intralesional corticosteroid injection has been reported to improve scar appearance and relieve symptoms such as itchiness and pain (Chowdri, Masarat, Mattoo, & Darzi, 1999; R. E. Fitzpatrick, 1999). It is suggested that intralesional corticosteroid injection suppresses inflammation, decreases oxygen and nutrition supply and inhibits activities of keratinocytes and fibroblasts to reduce collagen deposition (Atiyeh, 2007). A study compared effects of intralesional triamcinolone acetonide (TAC), intralesional 5-fluorouracil (5-FU), intralesional mixed TAC and 5-FU, and pulsed dye laser (PDL) with no treatment (Manuskiatti & Fitzpatrick, 2002). All treatment groups showed significant improvements over time and the scars with receiving intralesional corticosteroid showed the faster treatment responses than the scars with PDL treatments. Some studies also explored its combination use with laser therapy (Cavalie et al., 2015; Waibel, Wulkan, & Shumaker, 2013). It is suggested that ablative zones caused by laser therapy might increase drug delivery and strengthen the treatment effect of Intralesional corticosteroid injection. Meanwhile, some side effects following intralesional corticosteroid injection have been reported such as skin atrophy, hypopigmentation and rebound effects (Arno, Gauglitz, Barret, & Jeschke, 2014).

Cryotherapy leads to necrosis of hypertrophic scar tissues by contact or spray cryosurgery. However, cryotherapy is limited to managing small scars (Mustoe et al., 2002). To achieve a better treatment outcome, a study adjusted the cryotherapy

method by adding an intralesional needle. Their results showed an increase in freezing area of the deep scar tissue, which further increased the treatment efficiency of cryotherapy (Har-Shai, Amar, & Sabo, 2003). Side effects after cryotherapy were also reported including hypopigmentation, skin atrophy and pain (Rusciani, Rossi, & Bono, 1993).

With advances in technology over recent years, laser therapy is increasingly used to manage hypertrophic scars (Jin et al., 2013). There are different types of laser therapy mainly including ablative laser and non-ablative laser (Jin et al., 2013; Khatri, Mahoney, & McCartney, 2011). As the representatives of ablative laser, CO_2 laser and Er:YAG laser target at intracellular water and lead to tissue vaporization. Er:YAG laser has shown its effect on treating wrinkles and acne scars (Weinstein, 1999). CO_2 laser also demonstrated the effect on improving scar appearance and treating atrophic scars (Manuskiatti, Triwongwaranat, Varothai, Eimpunth, & Wanitphakdeedecha, 2010). The combination use of CO_2 laser and Er:YAG laser has been reported to cause cumulative damage to tissues. Moreover, CO_2 laser with a higher penetration depth led to more residual thermal damage than Er:YAG laser (de Noronha et al., 2001).

Use of ablative laser is commonly related to side effects as well as a prolonged recovery period. Thus, non-ablative laser such as Q-switched Nd:YAG laser, Nd:YAG laser and PDL were developed for less healing time and patient discomfort. Non-ablative laser is based on the theory of selective photothermolysis, which was firstly proposed in 1983 (Anderson & Parrish, 1983). The laser light with a specific wavelength causes damage to the selective structure without damaging the surrounding tissue. Setting of proper fluences and an optimal pulse duration is essential to ensure its positive therapeutic

effects. The optimal pulse duration is determined by size of the targeted chromophore. To reduce laser damage to the epidermis, the epidermis cooling is recommended by direct contact, cold air and cryogen spray after laser therapy. It is supported that non-ablative laser is effective in remodeling scars. The clinical improvements in skin were shown after Nd:YAG laser, and the histological results demonstrated new collagen formation after the laser treatment (Goldberg, 2000). In addition, Nd:YAG laser showed its effects on improving acne scars (Rostan, & Fitzpatrick, 2001).

As one of the first developed lasers, PDL targets at hemoglobin and causes damage to microvascular structures. PDL was primarily used for treating port wine stain (PWS). Some studies also reported the use of PDL for treating tuberous sclerosis (Michel et al., 2004), acne vulgaris, lupus erythematosus (Karsai, Roos, Hammes, & Raulin, 2007) and atrophy (Mansouri et al., 2015). Meanwhile, increasing studies have supported its use for managing hypertrophic scars (Brewin & Lister, 2014). A study performed PDL on patients with breast reduction surgeries and their results showed that PDL disrupted scar vascular patterns (Shakespeare, Tiernan, Dewar, Hambleton, & Alster, 2000). An animal study also reported that PDL caused damage to capillaries in the superficial dermis and resulted in decrease of the capillary number (M. L. Wei et al., 2016). These results are consistent with the theory that PDL is based on causing damage to microvascular structures to manage scars.

A 40.7% of scar height improvement and a 65.3% of scar erythema improvement in 15 Asian patients were shown after receiving two times of PDL irradiation (Kono, Ercocen, Nakazawa, & Nozaki, 2005). It was also reported that PDL improved persistent scar erythema in mature scars (Donelan, Parrett, & Sheridan, 2008). Early use of PDL, which

was conducted within two weeks after incision, showed its effect on preventing growth of hypertrophic scars (McCraw, McCraw, McMellin, & Bettencourt, 1999). Significant improvements in VSS scores were also reported after three PDL sessions with the combination use of pressure therapy (Bailey et al., 2012). A study compared the effect of PDL with silicone gel sheet on managing scars (Wittenberg et al., 1999). Significant changes in scar erythema were observed between baseline and 40 weeks. However, there was no difference between groups. A systematic review included eight randomized controlled studies and their results showed that PDL improved the overall scar scores despite inconsistent results in specific scar parameters (De Las Alas, Siripunvarapon, & Dofitas, 2012).

The parameter setting of PDL has been explored by previous studies. Due to the fact that melanin competes with hemoglobin for laser energy absorption, a higher fluence is recommended for people with more melanin present in skin. Manuskiatti et al. compared effects of different fluence of 3, 5 and 7 $J \cdot cm^{-2}$ on improving scar thickness, erythema and pliability (Manuskiatti, Fitzpatrick, & Goldman, 2001). Each group showed significant improvements after laser treatments. However, there was no difference in different groups. In another study, they compared different pulse width of 0.45 and 0.40 ms (Manuskiatti, Wanitphakdeedecha, & Fitzpatrick, 2007). Two segments of each median sternotomy scar randomly received 0.45 ms or 0.40 ms. Their results reported that pulse width of 0.45 ms was better than pulse width of 0.40 ms to reduce scar volume and thickness as well as improving scar pliability. However, there were no significant differences in scar lightening from baseline to 24 weeks and between groups. Moreover, there are some studies comparing PDL with other types of laser therapy. Lee et al. compared PDL with Nd:YAG laser for treating acne scars and

treatment effects were reported in both two types of laser therapy (D. H. Lee, Choi, Min, Yoon, & Suh, 2009). Another study compared PDL with intense pulsed light (IPL) for treating facial telangiectasias (Nymann, Hedelund, & Haedersdal, 2010). Their results indicated that patients with PDL showed better vessel clearance and felt less pain than patients with IPL.

There are other treatments utilized in clinical practice for managing scars such as needling therapy (Alam et al., 2014), radiotherapy (Scrimali, Lomeo, Tamburino, Catalani, & Perrotta, 2012), scar massage (Chen & Davidson, 2005; Nedelec et al., 2019), and exercise (Diego et al., 2013; Gittings et al., 2018).

1.2 STUDY RATIONALE

Hypertrophic scar after burn injury is a challenging clinical problem, which leads to functional loss, results in psychological and social issues, and has dramatic influence on patients' quality of life.

New blood vessel formation, which provides oxygen and nutrition for wound healing and scar formation, is an essential process in response to skin injury (Gurtner et al., 2008). An immature scar with robust growth of blood vessels tends to be hypertrophic (van der Veer et al., 2009). It has been reported that the capillary density in hypertrophic scar was significantly higher than it in non-hypertrophic scar (van der Veer et al., 2011). Furthermore, growth of blood vessels decreases when the scar progresses to mature (DiPietro, 2016). Therefore, scar vascularity is regarded as a key indicator of scar maturation. Measurement of scar vascularity is important to present degree of scar maturation and guide treatments for managing hypertrophic scars.

Currently, there are different tools used to measure scar vascularity. However, it has been shown that they were not equivalent for scar vascularity measurement (Jaspers et al., 2017). There is a need to summarize current evidence and have a better understanding of scar vascularity measurement.

Given that scar vascularity plays an important role in hypertrophic scar development, controlling scar vascularity at an early stage might be an effective way to limit scar growth and promote scar maturation. As one type of laser therapy, PDL directly causes damage to scar microvascular structures and has been preliminarily supported its effect on managing hypertrophic scars. However, the relationship between controlling scar vascularity by PDL and limiting scar growth is not fully understood. Exploring this relationship would contribute to a better clinical use of PDL for managing hypertrophic scars.

1.3 STUDY OBJECTIVES

There are two phases of this study. Phase one aims to systematically review clinical tools on scar vascularity measurement and validate the use of dermoscopy to measure scar vascularity. Phase two aims to explore the effect of controlling scar vascularity by PDL on managing hypertrophic scars. Overall, this study contributes to a better understanding of scar vascularity measurement and the relationship between controlling scar vascularity by PDL and managing hypertrophic scars.

1.4 THESIS OUTLINE

This thesis consists of five chapters. Chapter one presents study background, study rationale, study objectives and thesis outline. Background knowledge of burn injury,

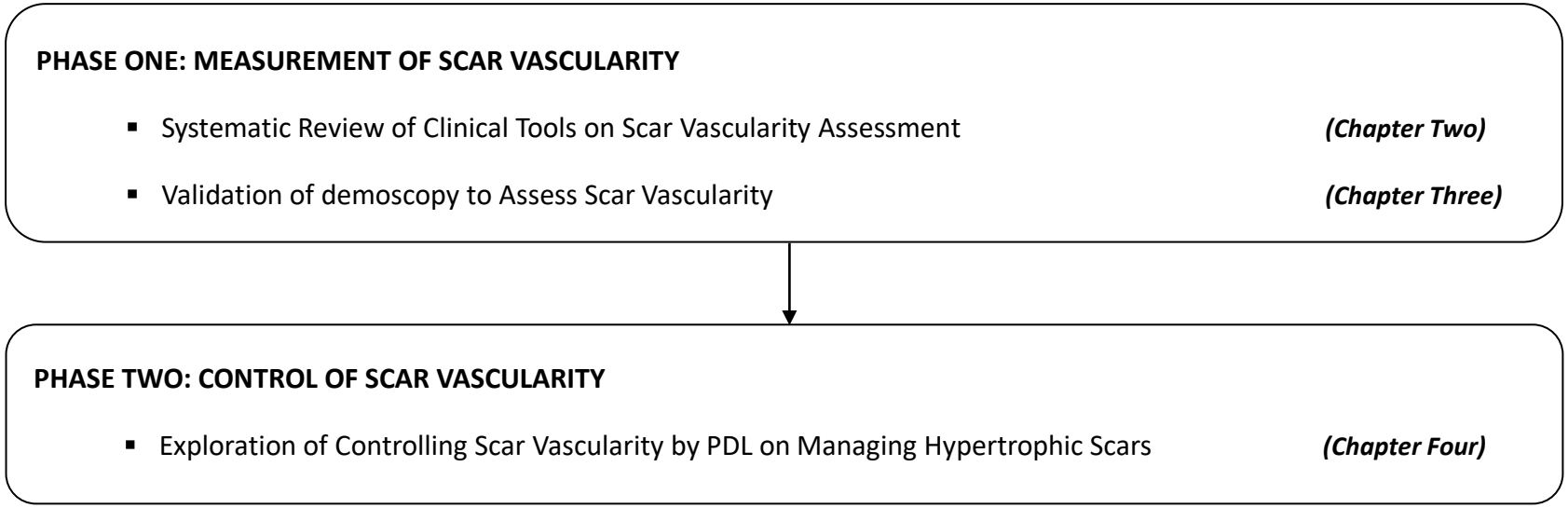
hypertrophic scar, different scar assessment parameters and scar treatments are introduced in this chapter.

Chapter two is a systematic review of the available clinical tools of measuring scar vascularity. They are divided into two types, subjective measurement scales including the POSAS, VSS and mVSS, and objective measurement tools including the color-measuring device, blood flow measuring device and morphological imaging device. Their measurement reliability and validity are presented in this chapter.

Based on the chapter two results, dermoscopy shows promising potential to measure scar vascularity. Chapter three validates the use of dermoscopy for measuring scar vascularity. It is a longitudinal exploratory study and patients with hypertrophic scars are enrolled. The construct validity of dermoscopy for measuring scar vascularity is examined by exploring its ability of discriminating hypertrophic scars from healthy skin, as well as its correlations with the measurement results by the POSAS and DermaLab Combo. Moreover, the predictive ability of scar thickness change is explored for different vascularity measurement tools.

Chapter four explores the effect of controlling scar vascularity by PDL on limiting scar thickness growth. It is a 3-month assessor-blinded experimental study. Patients with hypertrophic scars less than one year after injury are enrolled into the laser group or the control group. Changes in scar vascularity and thickness during the 3-month treatment period and differences between scar erythema and blood perfusion are explored in this chapter. Finally, chapter five suggests future research directions and summarizes this study.

Figure 1.4 The study framework



CHAPTER TWO

A SYSTEMATIC REVIEW OF SCAR VASCULARITY MEASUREMENT

2.0 CHAPTER ABSTRACT

Background: Vascularity is an important parameter closely associated with the scar maturation. Reliable and accurate measurement of vascularity helps to monitor the scar change and adopt targeted interventions to prevent excessive scarring and achieve promising outcomes. However, there is no consensus on the assessment tools for the scar vascularity measurement.

Objectives: This systematic review aimed to summarize and compare subjective and objective assessment tools available for the scar vascularity measurement and looked into their reliability and validity.

Methods: A systematic literature search was done using PubMed, CINAHL, Embase and Science Direct databases. Studies, which used non-invasive measurement tools and explored their clinimetric properties, were identified and included in this review.

Results: A total of 1458 articles were obtained, and 26 articles were finally included in this review. Subjective vascularity measurement scales include the POSAS, the VSS and the mVSS while objective vascularity measurement devices consist of the color-measuring device, the blood flow measuring device and the morphological imaging device.

Conclusion: Subjective scales are easy to use and have acceptable reliability to give a preliminary impression of scar vascularity. Three types of objective devices are not equivalent to measure scar vascularity.

2.1 INTRODUCTION

Scars can lead to different degrees of functional limitations and psychological difficulties, which further affect survivors' quality of life (Finnerty et al., 2016; J. C. Schneider et al., 2006). Various treatments for scar management are currently in clinical use such as pressure therapy, silicone gel sheeting and laser treatment (Friedstat & Hultman, 2014; Leventhal et al., 2006). Scar assessment is essential for evaluating and comparing the effectiveness of clinical treatments among different patient groups and is important for monitoring the progress of scar quality over time.

There are different parameters for scar assessment such as pliability, thickness and pigmentation (Durani, McGrouther, & Ferguson, 2009; Tyack, Simons, Spinks, & Wasiak, 2012). The measurement of scar vascularity is one of the most important scar parameters, which is closely associated with the scar maturation (Forbes-Duchart, Cooper, Nedelec, Ross, & Quanbury, 2009; Simons & Tyack, 2011; Stavrou, 2008). Therefore, reliable and accurate measurement of vascularity in the scar helps to identify the scar maturation and adopt targeted treatments in the early stage to prevent excessive scarring and achieve promising functional and cosmetic outcomes.

Different types of assessment tools are used to measure the scar vascularity and the majority of the assessment tools are based on evaluating the amount of redness to measure the scar vascularity (K. C. Lee et al., 2016; Tyack et al., 2012). For example, the VSS uses 'normal, pink, red, purple' to rate the scar vascularity. However, it is difficult for scar assessment scales to detect subtle changes of scar color and monitor scar progress resulting from the limitation of naked eyes. Therefore, increasing objective devices are of use in measuring the scar vascularity. As the most commonly

used measurement device in clinical settings and research work, the color-measuring device is also based on the scar redness to measure the vascularity. However, a poor correlation was reported between the color-measuring device and scar assessment scales (Jones, 2012; Verhaegen et al., 2011). It raises the concern about the mechanism of these assessment tools for vascularity measurement. Additionally, it is known that both the pigmentation and vascularity contribute to the skin color and interfere with each other. It is not clear about the accuracy of vascularity measurement by using these assessment tools.

Other assessment devices measure the scar vascularity based on different theories such as the LDI, which measures the blood flow. A study reported that the vascularity measurement by the LDI was not consistently associated with that by the color-measuring device during the scar maturation process (Mermans et al., 2013). A recent study also showed that there were no correlations for the vascularity measurement among the immunohistochemistry test, the LDI and the color-measuring device (Jaspers et al., 2017). These findings indicate that the assessment tools, which are developed to evaluate the scar vascularity, might measure different scar features. There is no consensus on which available measurement tool has better performance in measuring the scar vascularity.

This systematic review aimed to summarize and compare subjective and objective assessment tools available for the scar vascularity measurement and looked into their reliability and validity.

2.2 METHODS

2.2.1 Data source and search strategy

A systematic literature search was conducted using PubMed, CINAHL, Embase and Science Direct databases. The following searching terms were used: "(scar OR cicatrix OR fibrosis) AND (evaluation OR evaluate OR assessment OR assess OR measurement OR measure) AND (vascularity OR vascularization OR vascularisation)". The reference lists of potential articles were manually searched to identify additional relevant articles.

2.2.2 Inclusion and exclusion criteria

Articles were identified for this review according to the following inclusion criteria: 1) evaluating the reliability or validity of scales or devices, which measure the vascularity in the scar; 2) publishing in English from January 2007 to August 2017. Exclusion criteria included: 1) only using the vascularity measurement results to compare effects of different treatments; 2) only adopting invasive methods; 3) were not human studies; 4) were review papers, books, reports or lectures.

2.2.3 Data selection and extraction

Two reviewers independently assessed the titles and abstracts of first retrieved articles. Full-text of potentially relevant articles were further read to verify their eligibility based on the inclusion and exclusion criteria. The selection process was shown in Figure 2.1. Data extracted from the eligible articles consisted of the number of subjects and raters, the vascularity measurement tools used in the study, the reliability and validity results related to the vascularity measurement.

2.2.4 Quality assessment

The reliability and validity results were extracted and interpreted using the following

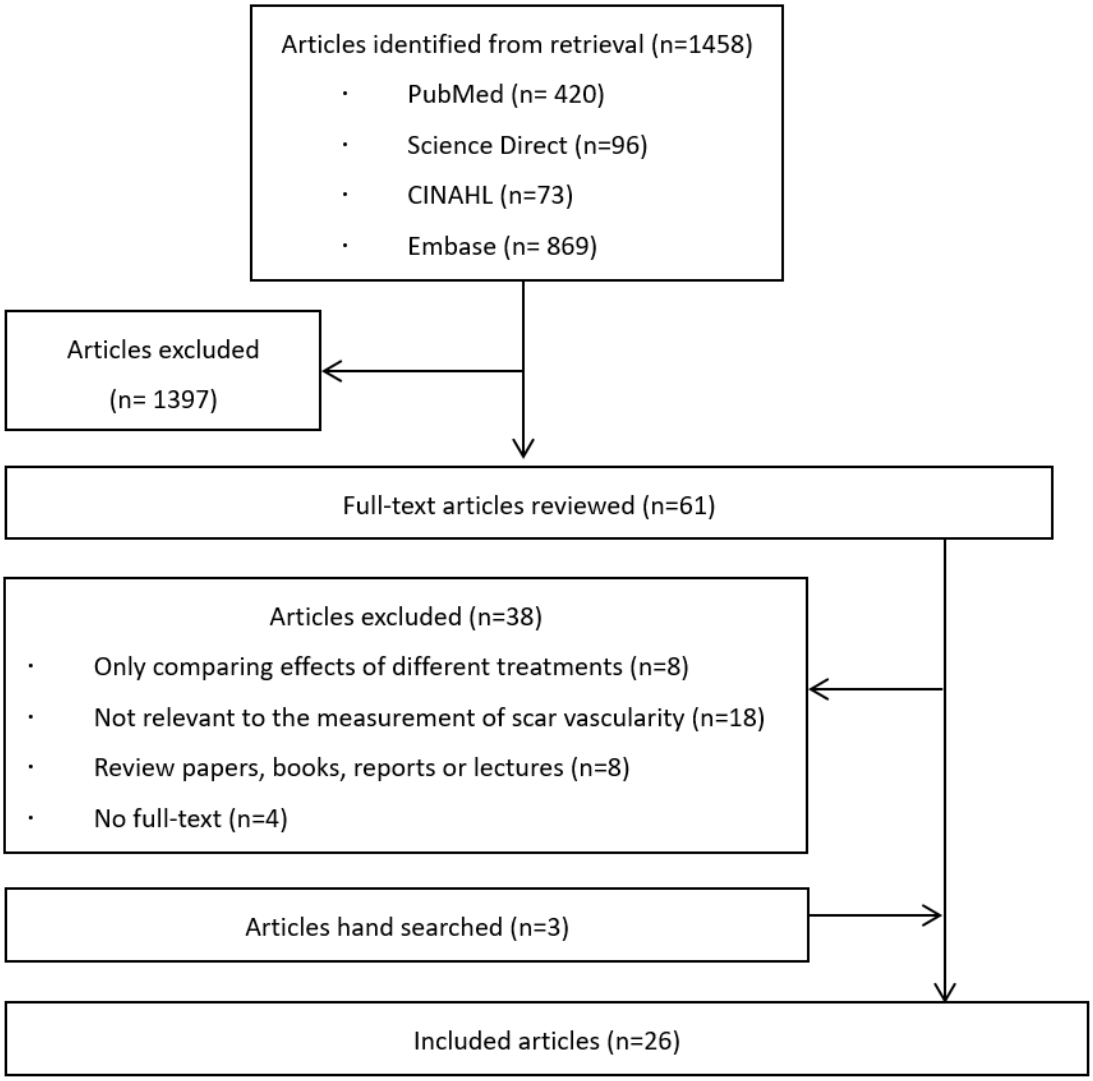
criteria.

The reliability is to evaluate the consistency of a measurement tool and there were five different types of reliability reported in this review: 1) the inter-rater reliability is to evaluate the agreement degree for the same subjects among different raters; 2) the intra-rater reliability is to evaluate the agreement degree for the same rater among different trials; 3) the test-retest reliability is to evaluate the consistency degree over time; 4) the internal consistency is to evaluate the agreement degree of different items in the same test; 5) the alternate forms reliability is to evaluate the consistency degree of different versions for the same test. In this review, the Intra-class Correlation Coefficient (ICC), the kappa coefficient, the Spearman's rank correlation coefficient, the Pearson correlation coefficient and the Cronbach's coefficient alpha are used to measure the reliability. An ICC below 0.4 is considered as 'poor agreement', between 0.4 and 0.75 as 'fair to good agreement', above 0.75 as 'excellent agreement' (Everitt, 1981). A kappa coefficient below 0.4 is interpreted as 'marginal agreement', between 0.4 and 0.6 as 'moderate agreement', between 0.6 and 0.8 as 'substantial agreement' and above 0.8 as 'perfect agreement' (McHugh, 2012). The Pearson correlation coefficient is used for the parametric data, while the Spearman's rank correlation coefficient is used for the nonparametric data (Mukaka, 2012). Both of them range from -1 to +1, and the larger absolute value indicates the stronger correlation. A Cronbach's alpha above 0.70 is considered as 'acceptable internal consistency' (Tavakol & Dennick, 2011).

Only the construct validity was explored in this review, which is to evaluate the degree of a tool measuring what it is purposed to measure. In this review, the construct

validity is examined through measuring its correlation with other tools which measure the same construct or providing evidence that the measurement tool could differentiate subjects with different characteristics. The Kendall rank correlation coefficient is used to present the correlation (Bottcher & Posthoff, 1975). The area under the curve (AUC) below 0.6 is considered as 'fail', between 0.6 and 0.7 as 'poor', between 0.7 to 0.8 as 'fair', between 0.8 and 0.9 as 'good', and above 0.9 as 'excellent' (Hand, 2009).

Figure 2.1 The selection process of articles included in this review



2.3 RESULTS

A total of 26 articles were included in this review. Based on the vascularity measurement tool that articles purposefully explored, they were classified into two types: 1) subjective vascularity measurement scales including the POSAS, the VSS and the mVSS; 2) objective vascularity measurement devices including the color-measuring device, the blood flow measuring device and the morphological imaging device. Table 2.1 summarized the frequency of different vascularity measurement tools used in all the included studies. Table 2.2 and table 2.3 summarized the reliability and validity results of different vascularity measurement tools.

2.3.1 Subjective vascularity measurement scales

Five studies explored the reliability and validity of the POSAS (Brölmann et al., 2013; Cai et al., 2016; Eskes et al., 2012; Goei et al., 2017; Mosterd, Arits, Nelemans, & Kelleners-Smeets, 2013), two studies of the VSS (Brandt et al., 2009; Thompson, Sood, Honari, Carrougher, & Gibran, 2015) and three studies of the mVSS (Forbes-Duchart, Marshall, Strock, & Cooper, 2007; Gankande et al., 2013; Simons, Ziviani, Thorley, McNee, & Tyack, 2013).

2.3.1.1 The Patient and Observer Scar Assessment Scale

The POSAS was first introduced in 2004 to assess burn scars, which combines the Patient Scar Assessment Scale (PSAS) rated by patients and the Observer Scar Assessment Scale (OSAS) rated by observers (L J 1 Draaijers et al., 2003; M. B. A. van der Wal et al., 2012). The vascularity is measured by observing the amount of redness after pressing and releasing the Plexiglas on the scar.

The vascularity measurement of the POSAS by using photographs or videos showed poor agreement with the on-site assessment (ICC = 0.27, 0.11) (Brölmann et al., 2013; Cai et al., 2016). Mosterd et al. reported fair to good agreement among three raters (ICC = 0.648). In addition, they found that the vascularity and pigmentation on the POSAS were the most predictive parameters of the overall scar quality (Mosterd et al., 2013). However, another two studies suggested the pigmentation and pliability (Eskes et al., 2012), and the pliability and relief (Goei et al., 2017) respectively.

2.3.1.2 The Vancouver Scar Scale

The VSS was the first scale used to quantify the pliability, vascularity, pigmentation and height in the scar. By observing the scar at rest and amount of blood refilling after blanching, the vascularity is rated as 'normal, pink, red or purple' (Sullivan, Smith, Kermode, McIver, & Courtemanche, 1990).

A study compared the method of Equal Appearing Interval (EAI) with the method of Direct Magnitude Estimation (DME) to assess four parameters on the VSS. Regression results showed that the curvilinear function was better than the linear function to present the scar vascularity, which indicated that the current rating method of VSS was not appropriate (Brandt et al., 2009). For the best parameter contributing to hypertrophic scar diagnosis, a survey suggested the measurement of scar height in the VSS (Thompson et al., 2015).

2.3.1.3 The modified Vancouver Scar Scale

For the mVSS, one study developed separate vascularity subtest for the Caucasian skin and the Aboriginal skin, and slight agreement was found among raters for the

vascularity measurement (k = 0.04, 0.12, 0.25) (Forbes-Duchart et al., 2007). Another modified version of VSS combined with the Total Body Surface Area (TBSA) showed the moderate to good agreement of the vascularity measurement (k = 0.44-0.76) (Gankande et al., 2013). Simons et al. reported similar results of good to excellent inter-rater reliability of the vascularity measurement on the mVSS (ICC = 0.78) as well as on the POSAS (ICC = 0.74, 0.75). In this study, correlation coefficients between the on-site assessment and the photographs assessment were 0.55 for the vascularity of POSAS and 0.45 for the vascularity of mVSS respectively (Simons et al., 2013).

2.3.2 Objective vascularity measurement devices

Three categories of vascularity measurement devices were included in this review, which consisted of the color-measuring device (Gankande et al., 2014b; Gankande, Duke, Wood, & Wallace, 2015; Jaspers et al., 2017; Kaartinen, Välisuo, Alander, & Kuokkanen, 2011; Nedelec, Correa, Rachelska, Armour, & LaSalle, 2008a, 2008b; Seo, Kang, Yoon, Lee, & Kim, 2017; M. van der Wal et al., 2013), the blood flow measuring device (Lobos, Wortsman, Valenzuela, & Alonso, 2017; Mermans et al., 2013) and the morphological imaging device (Gangemi, Carnino, & Stella, 2010; Gong, Chin, et al., 2014; Gong, McLaughlin, et al., 2014; Liew et al., 2013; Y. Wei et al., 2015; Yoo & Kim, 2014).

2.3.2.1 The color-measuring device

The color-measuring devices include devices based on the tristimulus reflectance colorimetry such as the LabScan XE and Spectrophotometer, and devices based on the narrow-band spectrophotometry such as the DermaSpectrometer and Mexameter.

As the most commonly used device for the measurement of scar vascularity, the color-measuring device was explored in eight studies. Three studies explored the reliability and validity of the Mexameter. Acceptable level of agreement was reached for all the measurements (ICC = 0.74-0.97) (Nedelec et al., 2008a, 2008b), and significant correlation was reported between the Mexameter and the vascularity subtest of mVSS (r = 0.52-0.65) (Nedelec et al., 2008a). However, Seo et al. showed its poor correlation with the vascularity subtest of VSS (r = 0.372) (Seo et al., 2017). As another type of the color-measuring devices, the color probe of DermaLab Combo showed good to excellent inter-rater reliability for the vascularity measurement (ICC = 0.66-0.84) and fair test-retest reliability for the worst scar sites (ICC = 0.42), whereas poor test-retest reliability was reported for the best scar sites (ICC = 0.29) (Gankande et al., 2014b).

A study compared the Mexameter, the Colorimeter which is based on the principle of tristimulus reflectance colorimetry, and the DSM II ColorMeter which combines the narrow-band spectrophotometry and the tristimulus reflectance colorimetry to measure the vascularity of burn scars. This study supported their reliable inter-rater reliability (ICC = 0.84-0.95) and good correlations with the POSAS (r = 0.52-0.69) (M. van der Wal et al., 2013). Kaartinen et al. reported a similar result by using a color-measuring device with a modified method (r = 0.63) (Kaartinen et al., 2011).

Of the 26 included articles, a study compared the performance of four different methods to measure the vascularity in the scar consisting of one color-measuring device (DSM II ColorMeter), one blood flow measuring device (LDI), one scar assessment scale (POSAS) and the immunohistochemistry test. Thirty-two patients with hypertrophic scars were recruited and assessed. Only significant correlation was

found between the DSM II ColorMeter and the vascularity score of POSAS (r = 0.403, *p* = 0.03) (Jaspers et al., 2017).

2.3.2.2 The blood flow measuring device

The blood flow measuring device adopts a non-invasive way to measure the blood flow in the scar. Lobos et al. suggested increased vessel thickness, transverse and longitudinal axis, and volume in active keloids comparing with inactive keloids. However, it failed to reach the significant *p* value (Lobos et al., 2017). Another study showed inconsistent correlations for the scar vascularity measurement between the Colorimeter and the LDI at interval of 6 weeks, 3 months, 6 months and 9 months (Mermans et al., 2013).

2.3.2.3 The morphological imaging device

The OCT, the Dermoscopy and the Videocapillaroscopy are three types of devices based on morphological imaging technique, and they are used to measure the scar vascularity in recent years.

Studies using the OCT showed significant differences in vascular density and vessel diameter (Liew et al., 2013), attenuation coefficient (Gong, McLaughlin, et al., 2014) and birefringence ratio (Gong, Chin, et al., 2014) among hypertrophic scars, mild scars and normal skin. The Dermoscopy study reported that frequency of arborizing vascular structure significantly differed between in keloid scars and in hypertrophic scars (Yoo & Kim, 2014). Additionally, excellent inter-rater (ICC = 0.93) and test-retest reliability (ICC = 0.98), and strong correlations with other vascularity measurement tools (r = 0.625-0.891) were reported for the Dermoscopy (Y. Wei et al., 2015). Only one study

in this review used the Videocapillaroscopy, which provides a direct way to observe microvascular structures. Comparing with normal skin, significant increase of capillary loop diameter and length, and specific vascular structures were identified in scars (Gangemi et al., 2010).

Table 2.1 The Frequency of vascularity measurement tools used in the included studies

Scar Vascularity Measurement Tools	Frequency
Total of included studies	26
Subjective Vascularity Measurement Scales	**21**
The Patient and Observer Scar Assessment Scale	9
The Vancouver Scar Scale	6
The modified Vancouver Scar Scale	6
Objective Vascularity Measurement Devices	**22**
The Color-measuring Device	13
The Blood Flow Measuring Device	3
The Morphological Imaging Device	6
‧ The Optical Coherence Tomography	3
‧ The Dermoscopy	2
‧ The Videocapillaroscopy	1

Table 2.2 Summary of the reliability and validity results for subjective vascularity measurement scales

Study	Number of Subjects	Number of Raters	Vascularity Measurement Tool	Reliability Result	Validity Result
The Patient and Observer Scar Assessment Scale					
Cai et al.	17 subjects	3 raters	POSAS*	<u>Interrater reliability</u> 1) Vascularity (by POSAS): ICC = 0.04 (single rater), 95%CI -0.07-0.18; p>0.05 ICC = 0.11 (average rater), 95%CI -0.27-0.40; p>0.05	N/A
Brolmann et al.	119 subjects	12 raters	OSAS*	<u>Alternate forms reliability</u> 1) Agreement between onsite and photographs assessment: ICC = 0.27 (vascularity of POSAS), 95%CI 0.09-0.43; p^N	N/A
Mosterd et al.	54 subjects	3 raters	POSAS*	<u>Interrater reliability</u> 1) Vascularity (by POSAS): ICC = 0.381 (single rater), 95%CI 0.215-0.547; p^N ICC = 0.648 (average rater), 95%CI 0.451-0.783; p^N	N/A
Goei et al.	130 subjects	2 raters	POSAS*; Dermaspectrometer	<u>Internal consistency</u> 1) OSAS: α^1 = 0.916 (T3) / 0.871 (T>18) 2) PSAS:	<u>Construct validity</u> 1) Correlation between Vascularity (by POSAS) and erythema (by Dermaspectrometer): r^2 = 0.403 (T3); p<0.001

				α^1 = 0.846 (T3) / 0.818 (T>18) <u>Interrater reliability</u> 1) POSAS: ICC = 0.950 (T3); 95%CI 0.921-0.969; *p*<0.001 ICC = 0.687 (T>18); 95%CI 0.558-0.779; *p*<0.001	r^2 = 0.319 (T>18); *p*<0.001 2) OSAS for long-term scar quality: AUC = 0.854 (T3); 95%CI 0.781-0.911; *p*<0.001 3) PSAS for long-term scar quality: AUC = 0.728 (T3); 95%CI 0.640-0.804; *p*<0.001
Eskes et al.	106 subjects	11 raters	POSAS*	<u>Internal consistency</u> 1) Vascularity (by POSAS) with overall opinion: r^2 = 0.32; p<0.10	N/A
The Vancouver Scar Scale					
Brandt et al.	30 scar photos	27 raters	VSS*	<u>Intrarater reliability</u> 1) VSS: r^3 = 0.822 (EAI); p^N r^3 = 0.809 (DME); p^N <u>Alternate forms reliability</u> 1) Regression analysis of Vascularity (by VSS): *p*<0.002 (r square = 0.716 (EAI) vs. 0.866 (DME))	N/A
Thompson et al.	5 scar photos	130 survey responses	VSS*	N/A	<u>Construct validity</u> 1) Vascularity (by VSS) for hypertrophic scar diagnosis:

				AUC = 0.78, 95%CI 0.73-0.83; p<0.001	
The modified Vancouver Scar Scale					
Forbes-Duchart et al.	14 subjects	3 raters	mVSS*	<u>Interrater reliability</u> 1) Vascularity (by mVSS): k^4 = 0.12 (R1 vs. R2), 0.04 (R2 vs. R3), 0.25 (R1 vs. R3); p>0.05	N/A
Gankande et al.	30 subjects	3 raters	mVSS*	<u>Interrater reliability</u> 1) Vascularity (by mVSS): k_w^5 ('worst' scar) = 0.76 (R1 vs. R2), 0.75 (R2 vs. R3), 0.64 (R1 vs. R3); p^N k_w^5 ('best' scar) = 0.44 (R1 vs. R2), 0.63 (R2 vs. R3), 0.71 (R1 vs. R3); p^N 2) mVSS: ICC = 0.85-0.88 ('worst' scar); p^N ICC = 0.65-0.73 ('best' scar); p^N	N/A
Simons et al.	12 subjects	5 raters	mVSS*; POSAS	<u>Interrater reliability</u> 1) Vascularity (by POSAS): ICC (mean) = 0.74 (onsite), 0.75 (photographs); p^N ICC(single rater) = 0.36 (onsite), 0.37 (photographs); p^N	N/A

2) Vascularity (by mVSS):

ICC (mean)= 0.78 (onsite), 0.78 (photographs); p^N

ICC (single rater)= 0.42 (onsite), 0.41 (photographs); p^N

Alternate forms reliability

2) Agreement between onsite and photographs assessment:

r^2 = 0.55 (vascularity of POSAS); p^N

r^2 = 0.45 (vascularity of mVSS); p^N

Note. AUC = Area under the curve; DME = Direct magnitude estimation; EAI = Equal appearing interval; ICC = Intra-class correlation coefficient; N/A = Not applicable; OSAS = Oberver scar assessmnet scale; R1 = Rater 1; R2 = Rater 2; R3 = Rater 3; T3 = 3 months post-burn; T>18 = At least 18 months post-burn.

[1]: The Cronbach's alpha; [2]: The Spearman's rank correlation coefficient; [3]: The Pearson's correlation coefficient; [4]: The kappa statistic; [5]: The weighted kappa statistic; *: The vascularity measurement tool this study purposefully explored.

p^N: *p*-value is not reported.

Table 2.3 Summary of the reliability and validity results for objective vascularity measurement devices

Study	Number of Subjects	Number of Raters	Vascularity Measurement Tool	Reliability Result	Validity Result
The Color-measuring Device					
Nedelec et al.	32 subjects	3 raters	Mexameter*; mVSS	<u>Interrater reliability</u> 1) Erythema (by Mexameter): ICC = 0.85 (S1), 95%CI 0.75-0.92; p^N ICC = 0.82 (S2), 95%CI 0.71-0.90; p^N ICC = 0.97 (D), 95%CI 0.95-0.99; p^N 2) Vascularity (by mVSS): k^4 = 0.14 (S1); p^N k^4 = 0.25 (S2); p^N k^4 = 0.25 (D); p^N	<u>Construct validity</u> 1) Correlation between Erythema (by Mexameter) and vascularity (by mVSS): r^2 = 0.56 (S1); p<0.0001 r^2 = 0.52 (S2); p=0.003 r^2 = 0.65 (D); p<0.0001
Nedelec et al.	30 subjects	1 rater	Mexameter*; mVSS	<u>Intrarater Reliability</u> 1) Erythema (by Mexameter): ICC = 0.84 (S1), 95%CI 0.72-0.91; p^N ICC = 0.74 (S2), 95%CI 0.59-0.86; p^N ICC = 0.90 (D), 95%CI 0.83-0.95; p^N	<u>Construct validity</u> 1) Erythema (by Mexameter): p<0.05 (324.8±109.98 (scar) vs. 238.56±68.17 (normal skin))
Seo et al.	25 subjects	2 raters	Mexameter*;	<u>Interrater reliability</u>	<u>Construct validity</u>

			VSS	1) Vascularity (by VSS): k^4 = 0.624; p^N	1) Correlation between Erythema (by Mexameter) and vascularity (by VSS): r^3 = 0.372; p<0.001
Gankande et al.	30 subjects	3 raters	DermaLab Combo*	<u>Interrater reliability</u> 1) Erythema (by DermaLab Combo): ICC ('best' scar) = 0.74 (R1 vs. R2), 95%CI 0.60-0.83; p^N ICC ('best' scar) = 0.66 (R1 vs. R3), 95%CI 0.48-0.79; p^N ICC ('best' scar) = 0.78 (R2 vs. R3), 95%CI 0.66-0.85; p^N ICC ('worst' scar) = 0.84 (R1 vs. R2), 95%CI 0.76-0.89; p^N ICC ('worst' scar) = 0.67 (R1 vs. R3), 95%CI 0.50-0.78; p^N ICC ('worst' scar) = 0.73 (R2 vs. R3), 95%CI 0.59-0.82; p^N <u>Test–retest reliability</u> 1) Erythema (by DermaLab Combo): ICC = 0.42 ('worst' area), 95%CI 0.19-0.58; p^N ICC = 0.29 ('best' area), 95%CI 0.01-0.48; p^N	N/A
Gankande et al.	100 subjects	3 raters	DermaLab Combo*; mVSS	N/A	<u>Construct validity</u> 1) Correlation between EI% values (by DermaLab Combo) and vascularity (by mVSS): tb^6 = 0.4 (R1); p<0.001 tb^6 = 0.3 (R3); p<0.001

van der Wal et al.	50 subjects	2 raters	Mexameter*; Colorimeter; DSM II ColorMeter; POSAS	<u>Interrater reliability</u> 1) Erythema (by Mexameter): ICC = 0.90, 95%CI 0.83-0.94; p^N 2) LAB2 (by Colorimeter): ICC = 0.95, 95%CI 0.91-0.97; p^N 3) Erythema (by DSM II): ICC = 0.84, 95%CI 0.73-0.91; p^N 4) a* (by DSM II): ICC = 0.94, 95%CI 0.90-0.97; p^N 5) Vascularity (by POSAS): ICC = 0.71 (single), 95%CI 0.54-0.82; p^N ICC = 0.83 (average), 95%CI 0.70-0.90; p^N	<u>Construct validity</u> 1) Correlation between Erythema (by Mexameter) and vascularity (by POSAS): r^3 = 0.59, 95%CI 0.37-0.74; p^N 2) Correlation between LAB2 (by Colorimeter) and vascularity (by POSAS): r^3 = 0.69, 95%CI 0.51-0.81; p^N 3) Correlation between Erythema (by DSM II) and vascularity (by POSAS): r^3 = 0.66, 95%CI 0.47-0.80; p^N 4) Correlation between a* (by DSM II) and vascularity (by POSAS): r^3 = 0.52, 95%CI 0.28-0.70; p^N
Kaartinen et al.	14 subjects	3 raters	SDI and SpM*; POSAS; VSS	<u>Interrater reliability</u> 1) Vascularity (by POSAS): ICC = 0.51 (1^{st}), 0.56 (2^{nd}); p<0.05 2) Vascularity (by VSS): ICC = 0.40 (1^{st}), 0.32 (2^{nd}); p<0.05	<u>Construct validity</u> 1) Correlation between Hemoglobin concentration and vascularity (by POSAS): r^2 = 0.63; p<0.001 2) Correlation between Hemoglobin concentration and vascularity (by VSS): r^2 = 0.74; p<0.001

Jaspers et al.	32 subjects	3 raters	DSM II ColorMeter*; Laser Doppler imaging; POSAS; Immunohistochemistry test	N/A	<u>Construct validity</u> 1) Correlation between Micro-vessel density score (by immunohistochemistry) and blood flow (by LDI): r^3 = 0.139; *p*=0.450 2) Correlation between Erythema (by DSM II ColorMeter) and blood flow (by LDI): r^3 = -0.115; *p*=0.551 3) Correlation between Micro-vessel density score (by immunohistochemistry) and erythema (by DSM II ColorMeter): r^3 = -0.157; *p*=0.417 4) Correlation between Erythema difference score (by DSM II ColorMeter) and vascularization score (by POSAS): r^3 = 0.403; *p*=0.030
The Blood Flow Measuring Device					
Lobos et al.	35 subjects	2 raters	Color Doppler Ultrasound*	N/A	<u>Construct validity</u> 1) Thickness (by CDU): P^7=0.07 (6.5mm (in active keloids) vs. 3.5mm (in

					inactive keloids)) 2) Transverse (by CDU): P^{8}=0.36 (24.7mm (in active keloids) vs. 17mm (in inactive keloids)) 3) Longitudinal (by CDU): P^{7}=0.76 (25.4mm (in active keloids) vs. 23.1mm (in inactive keloids)) 4) Volume (by CDU): P^{8}=0.41 (3377.1mm^3 (in active keloids) vs. 1470mm^3 (in inactive keloids))
Mermans et al.	24 subjects	2 raters	Laser Doppler imager*; Colorimeter	N/A	<u>Construct validity</u> 1) Correlation between redness (by Colorimeter) and perfusion (by LDI): r^2 (after 6 weeks) = 0.233 (breast scar); p=0.368 r^2 (after 6 weeks) = 0.414 (abdominal scar); p=0.099 r^2 (after 12 weeks) = 0.622 (breast scar); p=0.002 r^2 (after 12 weeks) = 0.353 (abdominal scar); p=0.127 r^2 (after 24 weeks) = 0.343 (breast scar); p=0.151

					r^2 (after 24 weeks) = 0.244 (abdomen scar); *p*=0.313 r^2 (after 36 weeks) = 0.211 (breast scar); *p*=0.372 r^2 (after 36 weeks) = 0.501 (abdomen scar); *p*=0.029
The Morphological Imaging Device					
Liew et al.	8 scar areas	N/A	OCT*	N/A	<u>Construct validity</u> 1) Vascular density: 38±3.2% (in scar) vs. 22±1.4% (in normal skin) 2) Median vessel diameter: 34±3.2um (in scar) vs. 23±0.7um (in normal skin)
Gong et al.	6 scar areas	N/A	OCT*	N/A	<u>Construct validity</u> 1) Attenuation coefficient: p^8<0.001 (3.8±0.4 mm^{-1} (in hypertrophic scar) vs. 4.2±0.9 mm^{-1} (in normotrophic scar) vs. 6.3±0.5 mm^{-1} (in normal skin))
Gong et al.	13 subjects	N/A	OCT*	N/A	<u>Construct validity</u> 1) Birefringence: Ratio = 2.2 (hypertrophic scar vs. normal skin) Ratio = 1.1 (normotrophic scar vs. normal skin)

					2) Median birefringence ratio: p^{δ}<0.001 ((hypertrophic scar to normal skin) vs. (normotrophic scar to normal skin))
Yoo et al.	41 subjects	N/A	Dermocopy*	N/A	<u>Construct validity</u> 1) Vascular structures (arborizing): OR = 8.750 (keloid scars vs. hypertrophic scars); *p*=0.033 2) Vascular structures (linear irregular): OR = 4.286 (keloid scars vs. hypertrophic scars); *p*=0.238 3) Vascular structures (comma-shaped): OR = 1.538 (keloid scars vs. hypertrophic scars); *p*=1.000
Wei et al.	18 subjects	2 raters	Dermoscopy*; Spectrocolorimeter; VSS	<u>Interrater reliability</u> 1) Redness (by Dermoscope): ICC = 0.930, 95%CI 0.842–0.969; *p*<0.01 <u>Test-retest reliability</u> 1) Redness (by Dermoscope): ICC = 0.980, 95%CI 0.964-0.989; *p*<0.01	<u>Construct validity</u> 1) Correlation between redness (by Dermoscopy) and redness (by spectrocolorimete): r^3 = 0.890(mean), 0.891(R1), 0.881(R2); *p*<0.01 2) Correlation between redness (by Dermoscope) and vascularity (by VSS): r^2 = 0.625; *p*<0.01

Gangemi et al.	12 subjects	2 raters	Videocapillaroscopy*; VSS	N/A	<u>Construct validity</u> 1) Capillary diameters: p^8=0.04 (20.5um (AHS) vs. 16.2um (control)) p^8<0.01 (16.2um (RHS) vs. 12.4um (control)) p^8>0.05 (14.6um (NT) vs. 12.0um (control)) 2) Capillary length: p^8=0.03 (467.2um (AHS) vs. 241.0um (control)) p^8<0.01 (443.4um (RHS) vs. 287.4um (control)) p^8=0.01 (398.8um (NT) vs. 272.1um (control)) 3) Neoangiogenesis (‘bush-like’ / ‘deer horn-like’): p^9<0.01 (AHS: 3.04 vs. 0.15) p^9=0.01 (RHS: 1.02 vs. 0.09)

Note. AHS = Active hypertrophic scar; CDU = Color doppler ultrasound; D = Donor site; EI% = Erythema index%; ICC = Intra-class correlation coefficient; LDI = Laser doppler imager; mVSS = modified vancouver scar scale; N/A = Not applicable; NT = Normotrophic scar; OCT = Optical coherence tomography; OR = Odds ratio; POSAS = Patient and observer scar assessment scale; R1 = Rater 1; R2 = Rater 2; R3 = Rater 3; RHS = Remitted hypertrophic scar; S1 = The most severe scar site; S2 = The less severe scar site; SDI and SpM = Standardized digital imaging and spectral modelling; VSS = Vancouver scar scale.

[1]: The Cronbach’s alpha; [2]: The Spearman’s rank correlation coefficient; [3]: The Pearson’s correlation coefficient; [4]: The kappa statistic; [5]: The weighted kappa statistic; [6]: The Kendall tau-b rank correlation; [7]: The Wilcoxon-Mann-Whitney test; [8]: The student’s t-test; [9]: The x^2 test; *: The vascularity measurement tool this study purposefully explored.

p^N: *p*-value is not reported.

2.4 DISCUSSION

This is the first systematic review to summarize subjective and objective assessment tools for the measurement of scar vascularity which is a vital indicator of the scar maturation (Simons & Tyack, 2011).

All the three subjective assessment scales measure the scar vascularity through evaluating the amount of redness in the scar. The Plexiglas is used to blanch the scar for decreasing the influence of scar pigmentation on the vascularity judgment (Roques & Téot, 2007). Acceptable inter-rater reliability of the VSS (Brandt et al., 2009), the mVSS (Gankande et al., 2013; Simons et al., 2013) and the POSAS (Mosterd et al., 2013; Simons et al., 2013) were reported for the measurement of vascularity. Because of their easy to use, subjective assessment scales are widely implemented in clinical settings to preliminarily evaluate the scar. The VSS is the first used assessment scale. However, increasing evidence suggested its irrelevant and nonspecific scar component measurement, poor reliability and difficult use for identifying scar progress (Fearmonti, Bond, Erdmann, & Levinson, 2010; Thompson et al., 2015; Tyack et al., 2012). As a newly developed assessment scale, the POSAS rates the scar vascularity from 1 to 10, which is sensitive to identify scar changes (Franchignoni et al., 2019). In addition, patients' perception is involved into the assessment and provides reference for individualized scar management plans.

In this review, using photographs to evaluate the scar vascularity on the POSAS showed a poor agreement with on-site assessment results (Brölmann et al., 2013; Cai et al., 2016). The vascularity measurement is a dynamic process of observing the blood refilling after blanching the scar, however, photographs only present static images of

scar color and it is difficult for raters to distinguish the scar vascularity from the pigmentation especially for the scar with hyper-pigmentation and increased vascularity at the same time (Forbes-Duchart et al., 2007; Verhaegen et al., 2011). For future vascularity measurement by using photographs, taking a short video is recommended to show the process of scar redness change after using the Plexiglas, and attention should be paid to the environment lighting, setting up of camera and quality of internet, which potentially affect the quality of photographs or videos. Some scales are specifically developed for the scar photograph assessment such as the Manchester Scar Scale, the Yeong's Burn Scar Assessment and the Hamilton Scar Rating Scale. However, limited evidence has reported the reliability and validity to support their use in clinical and research work (Idriss & Maibach, 2009).

As the most commonly adopted assessment device, the color-measuring device could quantitatively measure the amount of skin redness to present the scar vascularity. Our review supports that the color-measuring device provides reliable measurement results among raters and provides continuous numerical data, which is more sensitive than scar assessment scales to detect subtle changes over time (Gankande et al., 2014b; Nedelec et al., 2008a, 2008b; M. van der Wal et al., 2013). Acceptable correlations were also reported between the color-measuring device and the mVSS (Gankande et al., 2015; Nedelec et al., 2008a), the VSS (Kaartinen et al., 2011) or the POSAS (Jaspers et al., 2017; Kaartinen et al., 2011; M. van der Wal et al., 2013) separately. However, two studies reported a weak correlation with the assessment scale (Seo et al., 2017) and a non-significant correlation with other vascularity measurement devices (Jaspers et al., 2017). As a commonly reported limitation, the color-measuring device with open chamber is easily affected by the environment

lighting and hair. Therefore, it is suggested to trim the hair before the color measurement and control the environment lighting during the assessment process. Given that pressure on skin will change the skin color, raters are required to lightly put the color-measuring device in contact with scars without causing additional pressure. To reduce the influence of pressure, some devices provide a spring in the probe head such as the Mexameter to ensure constant pressure on the skin. Another limitation of the color-measuring device is their limited measurement area at one time caused by the small size of probe, which increases the difficulty in comprehensively presenting the color of a large size scar and applying to patients with large burned area.

The blood flow measuring device, which has been proved useful in evaluating the burn depth and skin inflammation (Hoeksema et al., 2009; McGill, Sorensen, MacKay, Taggart, & Watson, 2007), depends on the velocity and concentration of red blood cells to quantify the scar vascularity. Studies suggested that more active scars were associated with increased blood perfusion (Lobos et al., 2017; Mermans et al., 2013). However, these two studies failed to give reference scores to show scars in different stages. It is worthwhile for future studies to explore the blood perfusion in different stage scars as well as normal skin. It will give a clear clue of the blood perfusion change during the scar maturation process. Because of the high sensitivity of skin perfusion to external stimulation and changes in body temperature and breathing movement (Briers, 2001), it is vital to follow a standardized protocol to measure the scar vascularity by using the blood flow measuring device such as guiding patients to remove pressure garment and sit on a chair for rest 20 minutes prior to the assessment, as well as keeping a consistent temperature and humidity of the assessment room.

With increasing concern about the safety of measurement tools, the morphological imaging technique is developed as an alternative to biopsy. Our results support its feasibility of measuring the vascularity to distinguish scars from normal skin (Gangemi et al., 2010; Gong, Chin, et al., 2014; Gong, McLaughlin, et al., 2014; Liew et al., 2013) or from keloid scars (Yoo & Kim, 2014). However, the reliability was only explored for the Dermoscopy and the result indicated its good inter-rater and test-retest reliability (Y. Wei et al., 2015).

The penetration depth and resolution decide on the performance of morphological imaging devices to measure the scar vascularity. The OCT was firstly utilized in ophthalmology for identifying eye diseases and has become increasingly used in dermatology to image the microvasculature of skin tissue. The penetration depth of the OCT is approximately 2mm with the resolution of 4-10um (Dalimier & Salomon, 2012). Both the Dermoscopy and the Videocapillaroscopy are based on the theory of magnification. Therefore, their penetration depth is limited to the superficial layer of skin. The Dermoscopy can provide the magnification of up to 70 fold while the Videocapillaroscopy provides greater magnification of 200 to 600 fold. The Dermoscopy lacks the micrometer-scale resolution to distinguish individual capillary vessels and is generally used to identify the presence of specific vascular pattern such as comma-shaped pattern (Bafounta et al., 2001), while the capillary length and diameter can be measured by using the Videocapillaroscopy. With technology development, some new versions of Dermoscopy adopt the cross-polarized light, which allows more light pass through the stratum corneum till the dermis (Campos-do-Carmo & Ramos-e-Silva, 2008).

Cost and portability are closely related to the utilization of devices in clinical settings. Comparing with low cost of the Dermoscopy (around USD 2000), the cost of the OCT (around USD 40,000) and the Videocapillaroscopy (around USD 37,000 to 70,000) are much more expensive (Bhakuni et al., 2012). High sensitivity to motion resulting in distortion of images and dependence on system further limit the portability of OCT. Comparing with the OCT, handheld Dermoscopy and Videocapillaroscopy appear to be more convenient for clinical and research use.

Evidence demonstrated that increased blood flow and angiogenesis took place during the wound healing and scar formation process (DiPietro, 2013). Angiogenesis refers to the process of growing new blood vessels (Carmeliet, 2005). For objective vascularity measurement tools, the color-measuring device measures the reflected light by the hemoglobin in vessels, the blood flow measuring device evaluates the blood flow, and the morphological imaging device assesses the angiogenesis to quantify the vascularity in scars. Therefore, the three types of devices are not equivalent for the measurement of scar vascularity. Comparing with the blood flow, which is easily affected by the environment and stimulations, the angiogenesis is more stable for measurement. In addition, our results support the feasibility of differentiating scars from normal skin or from keloid scars by using the morphological imaging device. Future studies are needed to explore its ability of predicting the scar maturation for further supporting the use of morphological imaging device.

Overall, this review presents available evidence on subjective and objective scar vascularity measurement tools. Because of acceptable reliability and easy to use, subjective vascularity measurement scales are widely used to give a preliminary

impression of the scar vascularity. The VSS and mVSS are easier to present an overall impression of scar vascularity while the POSAS is more sensitive to show changes in scar vascularity and includes patients' rating. Therefore, the VSS and mVSS are recommended for scars screening, while POSAS is recommended for routine scar assessments. For objective vascularity measurement devices, the color-measuring device, the blood flow measuring device and the morphological imaging device measure different dimensions of scar vascularity.

CHAPTER THREE

MEASURING SCAR VASCULARITY BY DERMOSCOPY

3.0 CHAPTER ABSTRACT

Background: Vascularity of hypertrophic scar is a key indicator of scar maturation and a vital parameter of evaluating effects of scar management interventions.

Objectives: This study aimed to explore the construct validity of dermoscopy for measuring vascularity of hypertrophic scar and its predictive ability of scar thickness change.

Methods: Patients with hypertrophic scars were recruited for scar assessments at baseline and at one-month follow-up, which consisted of the POSAS, DermaLab Combo, ultrasound and dermoscopy.

Results: Forty hypertrophic scars in the active proliferation stage were included in this study. The dermoscopic measurements based on color significantly discriminated the hypertrophic scars from the healthy skin ($p < 0.001$). In addition, they showed moderate to strong correlations with the vascularity component of the POSAS ($r = -0.438$, $p < 0.01$; $r = -0.461$, $p < 0.01$; and $r = -0.437$, $p < 0.01$) and the erythema value as measured by DermaLab Combo ($r = -0.474$, $p < 0.01$; $r = -0.603$, $p < 0.01$; and $r = -0.498$, $p < 0.01$). Weak to moderate correlations of the micro-vessel percentage were observed with the vascularity of POSAS ($r = 0.385$, $p < 0.01$) and the erythema of DermaLab Combo ($r = 0.444$, $p < 0.01$). For prediction of the scars with high risk of thickness change, the green value by dermoscopy was the strongest predictor (AUC = 0.738, $p = 0.034$, 95%CI = 0.570-0.906).

Conclusion: Dermoscopy, which measures scar color and provides a view of vascular structures, could be used as an objective assessment tool to evaluate scar vascularity.

3.1 INTRODUCTION

Hypertrophic scars are a common problem following burn injury and other associated skin trauma, reportedly occurring in 67% of Caucasian people and 74% of Chinese people after severe burn injuries (Brown & Bayat, 2009; Li-Tsang et al., 2010; Ud-Din & Bayat, 2014). With the characteristics of red color, raised appearance and poor pliability, hypertrophic scars leave survivors with physical and psychological problems. To meet the metabolic demand for wound healing and scar formation after burn injury, increased capillary blood flow and newly formed micro-vessels arise in injured areas (DiPietro, 2016). These reactions lead to scar growth and gradually decrease with scar becoming mature (DiPietro, 2013; van der Veer et al., 2011). Therefore, vascularity of hypertrophic scar is regarded as a key indicator of scar maturation. An accurate and comprehensive measurement of scar vascularity has the benefit of identifying scars with active proliferation; thus, targeted treatments can be adopted at an early stage to prevent excessive scarring and achieve promising functional and cosmetic outcomes.

By quantifying capillary blood flow and micro-vessels, different types of assessment scales and devices are currently used to measure vascularity of hypertrophic scars (H. Deng & Li-Tsang, 2018; Tyack et al., 2012). Rating the degree of scar redness is the most common method used for vascularity assessment scales and gives a preliminary impression of scar vascularity, such as ‘normal, pink, red or purple’ on the VSS. However, their dependence on subjective judgement and clinical experience results in poor inter-rater reliability and difficulty in detecting changes. Accordingly, assessment devices have been developed. Similar to the assessment scales, the major type of device measures scar color to evaluate scar vascularity such as DermaLab Combo. The other types of devices image scars to quantify micro-vessels or measure blood

perfusion such as LDI.

Dermoscopy, a non-invasive optical imaging device, was firstly used in optometry and is increasingly utilized in dermatology. Growing evidence supports its contribution to the diagnosis of melanoma by assessing its color and structures (Bafounta et al., 2001; Kittler et al., 2002; Rajpara et al., 2009), which shows potential to evaluate scar vascularity by measuring scar color and identifying micro-vessels. Two studies to date have applied dermoscopy to measuring vascularity of hypertrophic scars. One study measured the vascular pattern of hypertrophic scars (Yoo & Kim, 2014). However, the classification method of vascular pattern was subjective. The other study evaluated scar vascularity by measuring the red value of captured scar images by dermoscopy (Y. Wei et al., 2015). There is not a study measuring both scar color and micro-vessels. Moreover, the cross-sectional nature of these two studies limits their ability to explore the value of dermoscopically measured scar vascularity as a predictor of scar thickness change and an indicator of scar maturation.

In this study, we proposed the dermoscopic measurement of scar vascularity by measuring scar color and micro-vessel percentage. By exploring the ability to discriminate hypertrophic scars from healthy skin and its relationship with accepted vascularity measurement tools, this study aimed to explore the construct validity of dermoscopy for measuring vascularity of hypertrophic scars, as well as its predictive ability of scar thickness change.

3.2 METHODS

3.2.1 Study design

The present work was a longitudinal exploratory study and was approved by the Ethical Committee (reference number: HSEARS20180119003). All patients were assessed at baseline and were followed up for one month. The principles outlined in the Declaration of Helsinki were followed.

3.2.2 Patients

Patients were recruited from the Guangdong Provincial Work Injury Rehabilitation Hospital. Eligible patients were enrolled in this study based on the following criteria. The inclusion criteria included: a) age over 18 years; b) scars caused by burn injuries; c) length of wound healing post-burn injuries over 3 weeks; d) days post-injury less than 12 months; and e) ability and willingness to adhere to all the assessment procedures. The exclusion criteria included: a) open wounds or active infection; b) conditions that affect wound healing, such as diabetes mellitus; c) graft surgery for the chosen scar sites; and d) history of steroid injection or laser treatment.

The sample size was determined using the G*Power 3.0 (Franz Faul, Universitat Kiel, Germany) based on a 2-tailed alpha level of 0.05 and power of 0.8. A previous study reported strong correlations between the dermoscopic measurements and the VSS, with Spearman's rank correlation coefficient (r) equal to 0.625, and between the dermoscopic and the spectrocolorimetric measurements, with an r-value of 0.890 (Y. Wei et al., 2015). Assuming a correlation coefficient of 0.625, a minimum sample size of 15 was required. Given the assumed 20% attrition rate, this study aimed to recruit at least 18 patients.

3.2.3 Study process

The written informed consent was obtained from the eligible patient prior to assessment procedure. Background information was collected, including age, gender, skin type, cause of injury and days post-injury. Skin type was assessed on the Fitzpatrick Scale, a numerical scale that ranges from 1 to 6 with increasing melanin present in the skin (T. B. Fitzpatrick, 1988). The causes of injury comprised flame, scald, chemical burns and electric burns. The number of days post-injury represented the duration from injury to enrolment in this study.

For each patient, two 2×2 cm scar sites that had the highest vascularity score on the POSAS, as well as one 2×2 cm healthy skin site that located anatomically opposite or adjacent to the scar site, were selected. Hair was shaved from the chosen scar sites and healthy skin sites, and photographs were taken for record.

A total of two scar assessments were conducted for each patient: the first at baseline and the second at one-month follow-up. The scar sites were assessed by the POSAS, DermaLab Combo, dermoscopy and ultrasound, while the healthy skin sites were assessed by the DermaLab Combo and dermoscopy. There was a total of two assessors who had no less than two years of burns working experience. One assessor, who was blind to measurements of the POSAS, DermaLab Combo and ultrasound, did demoscopic measurements. During the scar assessment, the POSAS, which required blanching the scar with a piece of Plexiglas, was applied first. Then, the DermaLab Combo, ultrasound and dermoscopy were applied with a rest interval of 10 minutes between measurements. To reduce environmental effects, all the assessments were performed in the same room with consistent lighting and temperature.

3.2.4 Measurements

3.2.4.1 The Patient and Observer Scar Assessment Scale

The POSAS was first introduced in 2004 and consists of two separate numeric rating scales for observers and patients. This scale has been used extensively in research on scars, and evidence supports its good reliability and validity (Nguyen et al., 2015; Tyack et al., 2012). This study used the observer scale consisting of parameters of vascularity, pigmentation, thickness, relief, pliability and surface area. A lower score suggests better scar performance on the parameter.

3.2.4.2 DermaLab Combo

The color probe of DermaLab Combo (Cortex Technology, Hadsund, Denmark) uses light-emitting diode (LED) light to measure scar color. The reliability and validity of the instrument are supported by previous studies (Gankande et al., 2014a; Gankande et al., 2015). Each site was measured four times, and the average result was recorded for the following five parameters: erythema, melanin, L*, a* and b*. The parameters of erythema and a* are regarded as the measurement of scar vascularity, and higher values of these two parameters suggest a more vascularized scar.

3.2.4.3 Ultrasound

Previous work has validated the use of ultrasound for scar thickness measurement (J. Q. Li, Li-Tsang, Huang, Chen, & Zheng, 2013). In this study, the Mindray M5 ultrasound (Mindray M5, Mindray, China) was used to measure scar thickness. The center of the probe was placed on the marked scar area. Scar was identified from the ultrasound image and scar thickness was measured.

3.2.4.4 Dermoscopy

The dermoscopy system used in this study consisted of the DermLite Foto II Pro (3GEN Inc., San Juan Capistrano, CA, USA) and a connected digital camera (Canon EOS 750D; Canon, Tokyo, Japan). The camera was set to an ISO speed of 400 and a focal length of 22. The images captured by dermoscopy with cross-polarized light were processed in ImageJ program to measure the color and micro-vessel percentage (Figure 3.1) (C. A. Schneider, Rasband, & Eliceiri, 2012). The color measurement was based on the red-green-blue (RGB) color space. The green, red and blue values, which range from 0 to 255, present the intensity on each color axis (Figure 3.2). After subtracting background with the rolling-ball algorithm, filtering noises and enhancing contrast (J. Li et al., 2012), the vessel edges were identified in the captured image. The micro-vessel percentage presented the percentage of blood vessel regions in the image.

3.2.5 Data analysis

Data analysis was performed in SPSS Statistics version 23.0 (SPSS Inc., Chicago, IL, USA), and the level of significance was set to $p < 0.05$. Descriptive data, including gender, skin type and cause of injury, were described in terms of frequency and percentage, while age and days post injury were described in terms of mean and standard deviation.

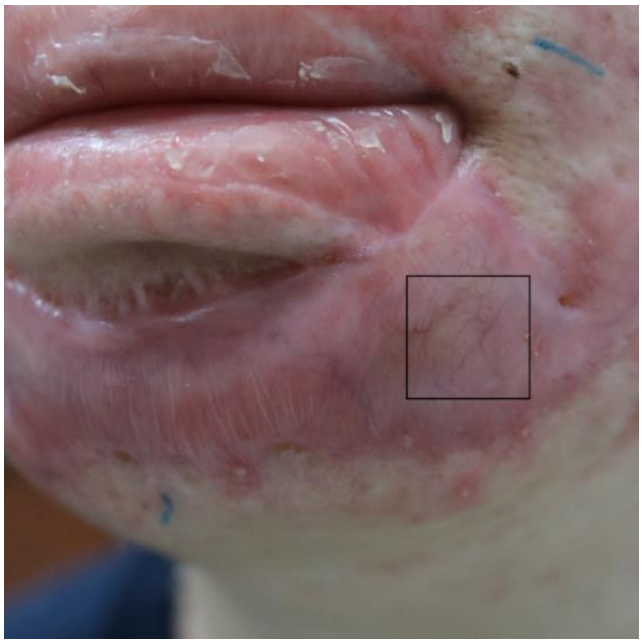
To explore the construct validity of dermoscopy, we used the independent t test for parametric data and the Mann-Whitney U test for non-parametric data to examine differences in dermoscopic measurement results between hypertrophic scars and healthy skin. In addition, the Pearson correlation coefficient for parametric data and the Spearman's rank correlation coefficient for non-parametric data were used to examine the correlations of dermoscopic measurements with the POSAS scores and

DermaLab Combo measurements. The correlation coefficient ranges from -1 to +1 (0.0 to 0.2 / 0.0 to -0.2: very weak or no correlation; 0.2 to 0.4 / -0.2 to -0.4: weak correlation; 0.4 to 0.6 / -0.4 to -0.6: moderate correlation; 0.6 to 0.8 / -0.6 to -0.8: strong correlation; 0.8 to 1.0 / -0.8 to -1.0: very strong correlation) (Porteney & Watkins, 2008). Due to the fact that the red, green and blue values measured by dermoscopy change in the opposite direction of the vascularity component of the POSAS and the erythema and a* value from DermaLab Combo, negative correlations between them were hypothesized. The ability of dermoscopy to discriminate hypertrophic scars from healthy skin, as well as moderate or strong correlations with the POSAS and DermaLab Combo, was regarded as support for the construct validity of dermoscopy (Downing, 2003).

To explore the predictive ability of scar thickness change, we used the Receiver Operating Characteristic (ROC) Curve. The percentage of scar thickness change, defined as ((Scar Thickness $_{\text{1-month follow-up}}$ - Scar Thickness $_{\text{baseline}}$) / Scar Thickness $_{\text{baseline}}$) × 100%, was calculated to represent the change of scar thickness in one month. The included scars were divided into a high-risk group and a low-risk group by using the median percentage of scar thickness change (46.67%) as the cut-off point. The AUC was calculated to explore the overall performance of different baseline vascularity measurement results in identifying the scars with high risk of thickness change. An AUC greater than 0.7 was considered acceptable (Mandrekar, 2010; Zweig & Campbell, 1993).

Figure 3.1 The images of a hypertrophic scar (a) by camera and (b) by dermoscopy

(a)



(b)

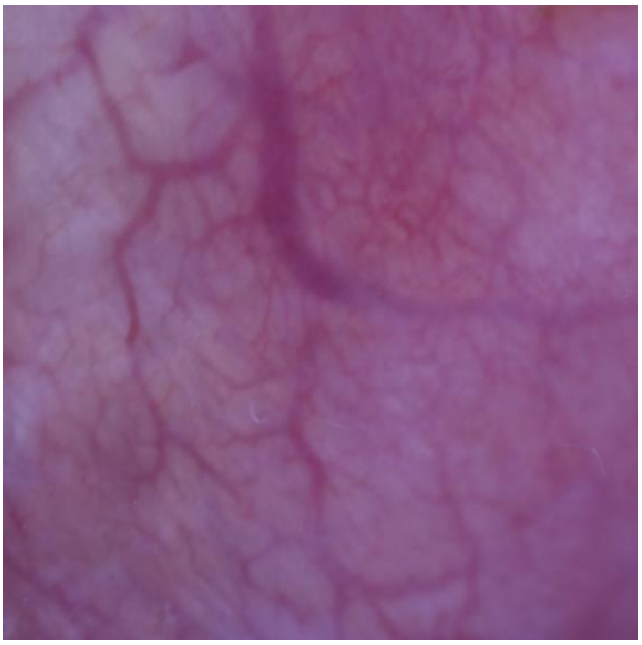
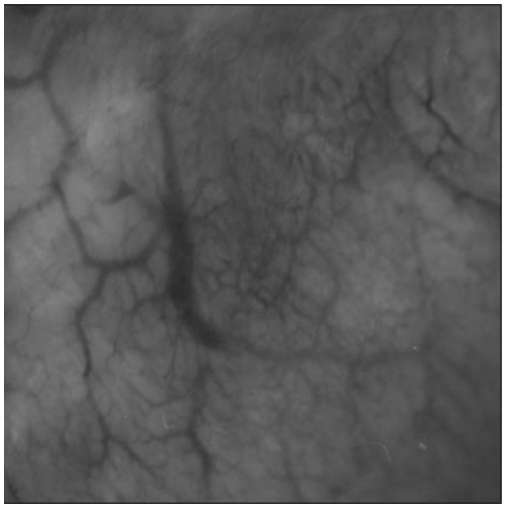
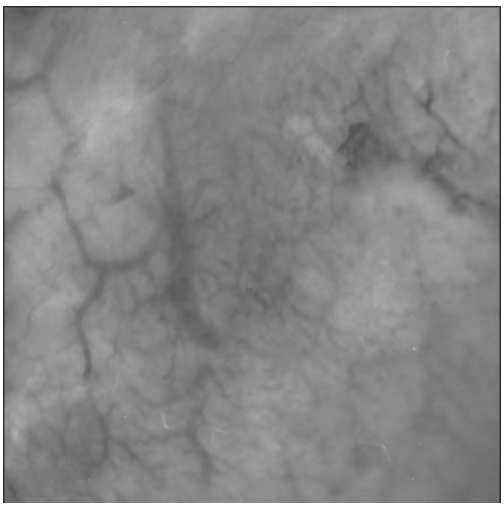


Figure 3.2 (a) Green, (b) red and (c) blue channel of a hypertrophic scar image by dermoscopy

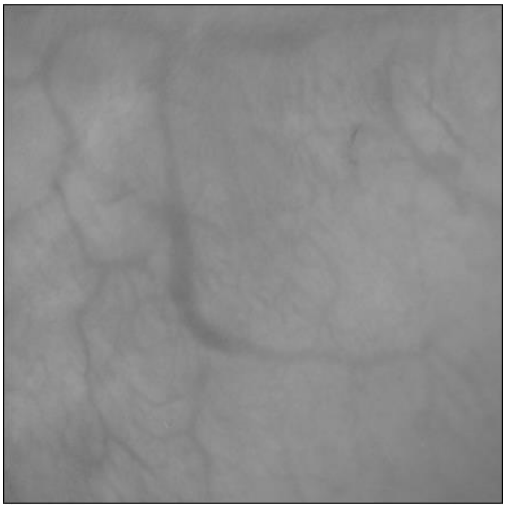
(a)



(b)



(c)



3.3 RESULTS

3.3.1 Demographics and injury characteristics

Twenty patients with a total of 40 hypertrophic scars were recruited into this study between January 2018 and December 2018. One hypertrophic scar was excluded from the follow-up assessment because of graft surgery for the chosen scar site. As shown in Table 3.1, the average age of the included patients was 37.25 years (SD 12.06 years), and the mean number of days from injury to enrolment in this study was 151.20 days (SD 89.81 days).

3.3.2 Scar assessment results

Except for the surface area, all the POSAS parameter scores increased from baseline assessment to one-month follow-up assessment. As shown in Table 3.2, erythema and a* as measured by DermaLab Combo slightly decreased after one month, while scar thickness measured by the ultrasound increased from 0.27cm (SD 0.19cm) to 0.32cm (SD 0.21cm). In Table 3.3, the red, green and blue values by dermoscopy increased, while the micro-vessel percentage decreased from 14.49% (SD 6.94%) to 11.72% (SD 8.23%).

3.3.3 Construct validity of dermoscopy

Table 3.3 shows that all the dermoscopic measurement results based on color for hypertrophic scars were significantly different from that for healthy skin ($p < 0.001$). Regarding the vascularity measurement of hypertrophic scars, Table 3.4 presents the correlations of the dermoscopy results with the POSAS scores and DermaLab Combo measurements. Overall, the red, green and blue values by dermoscopy showed moderate correlations with the vascularity component of the POSAS (r = -0.438, $p <$

0.01; r = -0.461, $p < 0.01$; and r = -0.437, $p < 0.01$, respectively), and moderate to strong correlations with erythema as measured by DermaLab Combo (r = -0.474, $p < 0.01$; r = -0.603, $p < 0.01$; and r = -0.498, $p < 0.01$, respectively). The micro-vessel percentage weakly correlated with the vascularity component of the POSAS (r = 0.385, $p < 0.01$) and moderately correlated with the erythema as measured by DermaLab Combo (r = 0.444, $p < 0.01$). Both the color and micro-vessel percentage showed weak or non-significant correlations with the a* by DermaLab Combo.

3.3.4 Predictive ability of dermoscopy

In Table 3.5, only the green value measured by dermoscopy significantly predicted the scars with high risk of thickness change (AUC = 0.738, $p = 0.034$, 95%CI = 0.570-0.906).

Table 3.1 Demographic characteristics and injury information of the study patients with hypertrophic scars

	(n=20)
Age (mean ± SD, years)	37.25 ± 12.06
Gender, n (%)	
- Male	15 (75%)
- Female	5 (25%)
Fitzpatrick skin type, n (%)	
- Type I	0 (0%)
- Type II	5 (25%)
- Type III	11 (55%)
- Type IV	4 (20%)
- Type V	0 (0%)
- Type VI	0 (0%)
Days post injury (mean ± SD, days)	151.20 ± 89.81
Cause of injury, n (%)	
- Flame	14 (70%)
- Scald	5 (25%)
- Chemical	1 (5%)
	(n=40)
Scar location, n (%)	
- Upper limbs	15 (37.5%)
- Lower limbs	1 (2.5%)
- Trunk	16 (40%)
- Head and neck	8 (20%)

Note. SD = Standard deviation.

Table 3.2 Measurement results of the hypertrophic scars at baseline and at one-month follow-up

(mean ± SD)	**Baseline** (n=40)	**1-month follow-up** (n=39)
POSAS		
- Vascularity	3.59 ± 1.41	4.31 ± 1.42
- Pigmentation	3.10 ± 0.97	3.82 ± 0.91
- Thickness	3.23 ± 1.20	4.03 ± 1.11
- Relief	3.28 ± 0.92	3.59 ± 0.97
- Pliability	3.18 ± 1.23	3.97 ± 1.25
- Surface area	1.95 ± 0.46	1.95 ± 0.22
DermaLab Combo		
- Melanin	52.14 ± 10.70	51.14 ± 9.40
- Erythema	18.09 ± 3.89	17.18 ± 3.85
- L*	22.23 ± 7.07	23.06 ± 6.29
- a*	17.23 ± 3.50	16.66 ± 3.43
- b*	5.29 ± 2.60	6.24 ± 2.77
Ultrasound (cm)	0.27 ± 0.19	0.32 ± 0.21

Note. POSAS = Patient and observer scar assessment scale; SD = Standard deviation.

Table 3.3 Measurement results by dermoscopy at baseline and at one-month follow-up

	Baseline (n=40)			**1-month follow-up** (n=39)		
(mean ± SD)	Scar	Healthy skin	*p*	Scar	Healthy skin	*p*
Color						
- Red value	132.12 ± 27.38	169.49 ± 22.01	<0.001†	135.76 ± 30.82	173.50 ± 20.81	<0.001‡
- Green value	75.59 ± 24.42	137.45 ± 24.28	<0.001†	80.90 ± 27.52	140.71 ± 24.94	<0.001†
- Blue value	119.13 ± 26.28	153.53 ± 23.76	<0.001†	123.29 ± 29.28	154.70 ± 26.90	<0.001†
Micro-vessel percentage (%)	14.49 ± 6.94	/	/	11.72 ± 8.23	/	/

Note. The *p* presents the difference of measurement results between hypertrophic scars and healthy skin. SD = Standard deviation; / = Not applicable.

†: Independent t-test; ‡: Mann-Whitney U test.

Table 3.4 Correlations between dermoscopy and other tools for measuring the vascularity of hypertrophic scars

	POSAS	**DermaLab Combo**	
Dermoscopy	- Vascularity	- Erythema	- a*
Baseline (n=40)			
Color			
- Red value	-0.448 ‡**	-0.559 †**	0.305 †
- Green value	-0.490 ‡**	-0.640 †**	0.160 †
- Blue value	-0.477 ‡**	-0.573 †**	0.259 †
Micro-vessel percentage	0.477 ‡**	0.498 †**	-0.034 †
1-month follow-up (n=39)			
Color			
- Red value	-0.504 ‡**	-0.394 †*	0.309 †
- Green value	-0.534 ‡**	-0.564 †**	0.146 †
- Blue value	-0.488 ‡**	-0.424 †**	0.279 †
Micro-vessel percentage	0.426 ‡**	0.381 †*	-0.101 †
Overall (n=79)			
Color			
- Red value	-0.438 ‡**	-0.474 †**	0.299 †**
- Green value	-0.461 ‡**	-0.603 †**	0.142 †
- Blue value	-0.437 ‡**	-0.498 †**	0.261 †*
Micro-vessel percentage	0.385 ‡**	0.444 †**	-0.053 †

Note. POSAS = Patient and observer scar assessment scale.

†: Pearson correlation coefficient; ‡: Spearman's rank correlation coefficient.

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

Table 3.5 Prediction of the scars with high risk of thickness change by using different vascularity measurement tools

	Area	95% CI	*p* value
POSAS			
- Vascularity	0.365	0.139 - 0.591	0.229
DermaLab Combo			
- Erythema	0.391	0.144 - 0.638	0.330
- a*	0.601	0.385 - 0.818	0.367
Dermoscopy			
- Color			
Red value	0.710	0.538 - 0.883	0.061
Green value	0.738	0.570 - 0.906	0.034*
Blue value	0.702	0.529 - 0.876	0.071
- Micro-vessel percentage	0.421	0.173 - 0.668	0.479

Note. CI = Confidence interval; POSAS = Patient and observer scar assessment scale.

*: $p < 0.05$.

3.4 DISCUSSION

As a key indicator of scar maturation, vascularity of hypertrophic scar was measured in our study. The green value of scar color as measured by dermoscopy significantly differentiated hypertrophic scars from healthy skin. It also had moderate to strong correlations with the POSAS and DermaLab Combo, which have established validity. Furthermore, the green value was the strongest parameter to predict the scars with high risk of thickness change. Our study supports the use of dermoscopy as an assessment tool to evaluate vascularity of hypertrophic scars and identify scars with active proliferation.

Measurement of scar color is the most common method used to evaluate scar vascularity. Hemoglobin and melanin, the main visible chromophores in human skin, are major determinants of the visual color of skin. Within the visible light region, hemoglobin mainly absorbs light in the green region, whereas melanin has prominent absorption in the red region (Diffey, Oliver, & Farr, 1984; Stamatas, Zmudzka, Kollias, & Beer, 2004). Due to their absorption spectrum characteristics, the green value of the color is regarded as the measure of vascularity, and the red value serves as the measure of pigmentation. A scar with greater amount of hemoglobin has higher absorption of green light and presents a lower green value. Comparing with the red and blue channels, the green channel of images is supported to be clearer to identify blood vessels (Gharabaghi et al., 2013; Ricci & Perfetti, 2007; Soares, Leandro, Cesar, Jelinek, & Cree, 2006; Staal, Abramoff, Niemeijer, Viergever, & van Ginneken, 2004). Our results are consistent with previous research. Among the red, green and blue values measured by dermoscopy, the green value showed the highest correlations with the vascularity component of the POSAS and the erythema value from DermaLab

Combo. Moreover, the green value was the best predictor of a scar at a high risk of thickness change. Our findings support the idea that the green value of scar color is preferable for evaluating the vascularity of hypertrophic scar.

In our study, the dermoscopic measurement results showed moderate to strong correlations with the erythema value by DermaLab Combo. However, they showed weak and non-significant correlations with the a* value, which is based on the LAB color space and presents the green-red component of color. A higher positive value of a* indicates more red while a lower negative value of a* suggests more green. However, the erythema value, defined as $100 \times {}_{\log}$(intensity of the reflected red light / intensity of the reflected green light), measures light absorption by the hemoglobin and subtracts light absorption by the melanin (L. J. Draaijers, Tempelman, et al., 2004). It is common that burn injuries cause changes in the amount of melanin in injured areas, and the changes vary among individuals. Therefore, the agreement between a* and erythema for the measurement of scar vascularity becomes weak in a cohort of people with widely varied amounts of melanin (Shriver & Parra, 2000). This might be the reason of inconsistent correlations of the dermoscopic measurement results with the erythema and a* value by DermaLab Combo. In future studies of using color-measuring devices, the erythema value is recommended to measure scar vascularity instead of the a* value.

Growth of new blood vessels is a critical process for wound healing and scar formation (Tonnesen, Feng, & Clark, 2000). Histologic analysis is the gold standard of quantifying blood vessels in scar tissues. There have been a growing number of studies, which report that the vessel density of hypertrophic scars is significantly higher than that of

healthy skin (Amadeu et al., 2003; van der Veer et al., 2009; van der Veer et al., 2011). However, histologic analysis is limited to biopsy, which is invasive and potentially causes secondary damage to scar tissues. Dermoscopy, as a non-invasive optical imaging technique, could collect light reflected from dermis with utilization of cross-polarized light (Jacques, Ramella-Roman, & Lee, 2002). Therefore, blood vessels are identified in images captured by dermoscopy and the micro-vessel percentage was calculated in our study. However, the micro-vessel percentage was weak to predict the scar thickness change. One possible reason is that newly formed blood vessels have different degrees of occlusion (Kischer, Thies, & Chvapil, 1982); thus, they could not fulfill the role of delivering oxygen and nutrition to support scar growth. In a 52-week follow-up study, an increasing trend of micro-vessels in hypertrophic scars is reported by comparing with that in non-hypertrophic scars (van der Veer et al., 2011). Similar findings were observed in our study that scars with high risk of thickness change tended to increase micro-vessel percentage. These results indicate that the micro-vessel percentage presents amount of physical micro-vessels and is a different dimension from the scar color which is closely related to amount of hemoglobin. To have a comprehensive evaluation of the scar vascularity, the micro-vessel percentage is suggested to be interpreted with the scar color.

Previous evidence suggested that a more proliferative scar had a more vascularized activity which was supported by increased blood flow and formation of new blood vessels (Huang, Murphy, Akaishi, & Ogawa, 2013; Kose & Waseem, 2008). Therefore, measurement of scar vascularity potentially helps prediction of scar thickness change. In our study, the erythema and a* values by DermaLab Combo could not identify scars at a high risk of thickness change. Same as the majority of vascularity assessment

devices based on measuring scar color, DermaLab Combo uses LED light. The main light collected by this tool is the light reflected off stratum corneum due to its roughness (Anderson & Parrish, 1981). Thus, the measurement of scar vascularity by DermaLab Combo is limited to the superficial epidermis. However, dermoscopy with cross-polarized light is able to measure the vascularity deep to the dermis, and color measurement results by dermoscopy showed the ability of predicting scar thickness change. It appears that the scar vascularity in the dermis is more related to the change of scar thickness than that in the superficial epidermis. Comparing with traditional device of measuring scar vascularity, dermoscopy performs better to predict scar thickness change and provides a non-invasive way to observe blood vessels in scar tissues.

A previous study has shown that experience and expertise had significant influence on interpreting dermoscopy images (Reiter et al., 2019). In addition, blur resulting from movement while taking images and dust on the lens potentially affect the dermoscopy image quality and increase difficulty in identifying the vascular structures by image-processing programs. Therefore, training is necessary for dermoscopy users.

There are some limitations for this study. Light in the whole visible spectrum is absorbed by hemoglobin as well as melanin. Therefore, vascularity measurements based on color made by dermoscopy should be interpreted with due consideration of the influence of melanin. In addition, longer follow-up time would be beneficial for exploring the relationship between scar vascularity and thickness change. Notwithstanding the limitations, this study indicates that the green value of scar color measured by dermoscopy could be used to evaluate the scar vascularity and predict

the scars with high risk of thickness change. In addition, the micro-vessel percentage by dermoscopy contributes to a comprehensive evaluation of the scar vascularity. In future studies, dermoscopy could be used as an objective assessment tool to explore different treatments' effects on controlling scar vascularity.

CHAPTER FOUR

CONTROLLING SCAR VASCULARITY BY PDL FOR MANAGING HYPERTROPHIC SCARS

4.0 CHAPTER ABSTRACT

Background: Growth of capillaries is an essential process after a dermal injury. An immature scar with robust growth of capillaries tends to be hypertrophic. PDL causes damage to microvascular structures and is increasingly used for early erythematous scars to limit scar growth.

Objectives: To have a better understanding of the impact of PDL on scar vascularity and to optimize the clinical use of PDL for managing hypertrophic scars, this study aimed to explore changes in vascularity and thickness of immature hypertrophic scars in Asian patients who received PDL treatments at an early stage and compare changes between scar erythema and blood perfusion.

Methods: It was a 3-month, assessor-blinded, clinical study. There were two groups of patients, PDL group and control group, who had hypertrophic scars less than one year post-injury. Patients in the PDL group received three PDL sessions at 4-week intervals. A total of three assessments were performed, at baseline, 1 month and 3 months, consisting of the POSAS and objective measurements of scar erythema, blood perfusion and scar thickness.

Results: A total of 45 patients were enrolled, 22 in the PDL group and 23 in the control group. After the 3-month treatment, parameters of scar vascularity (p = 0.003), pigmentation (p = 0.026), color (p < 0.001), thickness (p < 0.05) and overall scores (p < 0.01) on the POSAS significantly decreased in the PDL group. Moreover, objective measurements of scar erythema and blood perfusion showed significant improvements in the PDL group (p = 0.009 and p = 0.022, respectively) but not in the control group (p = 0.296 and p = 0.115, respectively). In addition, patients in the PDL group maintained a stable scar thickness compared to the control group which significantly increased from baseline, 1 month to 3 months (p < 0.01).

Conclusion: Use of PDL at an early stage controls vascularity of immature hypertrophic scar by improving its poor perfusion that further limits scar thickness growth and promotes scar maturation.

4.1 INTRODUCTION

Hypertrophic scar commonly develops after a deep dermal burn or traumatic injury, and is characterized by a rapid growth phase, then gradually becomes mature over a period of a few years (Mahdavian Delavary, van der Veer, Ferreira, & Niessen, 2012). Hypertrophic scars can leave survivors with functional and cosmetic problems, which further affect their independence of daily living and lead to psychological and social problems (Finnerty et al., 2016). Therefore, scar management is vital to optimize survivors' quality of life and facilitate their reintegration into society.

Early during the scar growth process, an increase in the number of capillaries and dilation of the residual capillaries have been observed in injured areas (van der Veer et al., 2009). An immature scar with robust growth of capillaries tends to be hypertrophic (DiPietro, 2016). Vascularity of hypertrophic scar significantly increases during early stage of scar development and changes across time as the scar matures. It has been suggested that scar erythema and blood perfusion are two different dimensions of scar vascularity (Huan Deng & Li-Tsang, 2019). Scar erythema presents the overall visual impression of scar vascularity and is associated with the amount of hemoglobin in hypertrophic scar. Meanwhile, blood perfusion reflects the dynamic process of scar vascularity and is related to moving red blood cells within scar capillaries.

Laser therapy has been demonstrated as a safe modality and shows encouraging treatment effects on preventing excessive scar formation and managing hypertrophic scars (Jin et al., 2013). One form of laser therapy is the PDL, which was first applied for the treatment of port wine stain, and then was widely used for the treatment of

telangiectasia, psoriasis and other dermatologic disorders (Liu, Moy, Ross, Hamzavi, & Ozog, 2012). It is one of the most effective laser types and was the first laser accepted for treating scars (Gauglitz, Korting, Pavicic, Ruzicka, & Jeschke, 2011; Vrijman et al., 2012). Light energy of PDL is selectively absorbed by hemoglobin and leads to damage of microvascular structures in the scars (Reiken et al., 1997). Thus, PDL is primarily recommended for early erythematous scars to limit scar growth (Anderson et al., 2014). A review summarized previous studies, which mainly recruited Caucasian patients and used subjective assessment scales, and reported improvements in scar erythema as well as scar thickness following the use of PDL (Parrett & Donelan, 2010). It is worthwhile to objectively explore the effect of PDL on scar blood perfusion that contributes to a better understanding of the impact of PDL on scar vascularity and optimizes the clinical use of PDL for managing hypertrophic scars.

This study aimed to explore changes in vascularity and thickness of immature hypertrophic scars in Asian patients who received PDL treatments at an early stage and compare changes between scar erythema and blood perfusion using objective scar evaluation tools.

4.2 METHODS

4.2.1 Study design

This study was a 3-month, assessor-blinded, clinical study. This study was approved by Ethical Committee of Hong Kong Polytechnic University (reference number: HSEARS20190402002) and registered at ClinicalTrials.gov (reference number: NCT03986346). Eligible patients with immature hypertrophic scars were enrolled and data was collected between May 2019 and February 2020. All study patients signed an

informed consent and the principles outlined in the Declaration of Helsinki were followed.

4.2.2 Patients

Patients were recruited from the Rehabilitation Clinics of the Hong Kong Polytechnic University in Hong Kong and the Burn Unit of Southwest Hospital in Chongqing which is one of the largest burn units in China. The inclusion criteria included: a) age between 16 and 70 years; b) scar caused by a burn injury or trauma-related injury; c) time required for wound healing greater than three weeks; d) time post injury less than one year; e) ability and willingness to adhere to all treatment and assessment procedures. The exclusion criteria included: a) history of steroid injection or graft surgery; b) history of keloid scarring; c) open wound or active infection; d) conditions that affect wound healing such as diabetes mellitus.

4.2.3 Study procedures

There were two groups, PDL group and control group, in this study. Potential patients were screened for eligibility. Those who were eligible and agreed to participate were assigned to either the control or PDL group on a weekly, alternating basis and group for the first week was randomly selected. For example, all eligible participants in week one were assigned to the control group, in week two were assigned to the PDL group, in week three the control group, etc. All patients were treated for three months. Patients in the PDL group received three PDL sessions at 4-week intervals and the first PDL session was performed immediately after the first scar assessment. The 585-nm PDL of a 7-mm spot size without overlapping was set with fluence ranging from 8 to 12 $J \cdot cm^{-2}$ and pulse duration ranging from 0.5 to 2.0 ms (Cynergy, Cynosure Inc,

Westford, MA). To protect the epidermis from injury, a cooling device was utilized during the PDL session and an ice pack was applied after the laser session. All patients in the PDL and control group received the standardized scar care including pressure therapy, daily scar cleaning and scar massage. Different clinical professionals performed the PDL and standardized scar care separately for the two groups.

A total of three assessments were performed at baseline, 1 month and 3 months. The first two assessments were performed immediately prior to the PDL treatment and the third assessment was performed one month after the final PDL treatment. For each patient, one treated scar, and one healthy skin site located on an anatomically contralateral site or adjacent to the treated scar, were assessed. To exactly measure same locations at different time points, photographs were taken for the record and relocation strategy was used (Nedelec et al., 2008b). The assessment consisted of the POSAS, erythema, blood perfusion and thickness for the treated scars, as well as measurement of erythema and blood perfusion for the healthy skin. Evaluation of the POSAS and objective measurements were separately performed by two treatment-blinded assessors.

4.2.4 Outcome measures

4.2.4.1 The Patient and Observer Scar Assessment Scale

The POSAS is reliable and valid to assess scars which consists of two numeric rating scales (M. B. A. van der Wal et al., 2012). Parameters of scar vascularity, pigmentation, thickness, relief, pliability and surface area were evaluated by the treatment-blinded assessor, while parameters of scar pain, itchiness, color, stiffness, thickness and irregularity were rated by the patients. The score for each parameter ranges from 1 to

10 and a higher score indicates a worse performance on any given parameter.

4.2.4.2 Scar erythema

Scar erythema presents the degree of scar redness. It was quantified by the green value of the scar image which was captured by the Dermlite Foto II Pro (3GEN Inc., San Juan Capistrano, CA, USA) with cross-polarized light and a connected digital camera (Canon EOS 800D; Canon, Tokyo, Japan) (H. Deng, Li-Tsang, & Li, 2020). The camera was set with ISO speed at 400 and shutter speed at 1/50. A higher green value indicates a lower degree of scar erythema.

4.2.4.3 Scar blood perfusion and thickness

Scar blood perfusion and thickness were measured by Doppler ultrasound with a frequency transducer ranging from 8 to 12 MHz (Mindray M5, Mindray, China). All patients were required to sit and rest for 20 minutes prior to the measurement. Three scan planes for each measured site were marked, and angles between each of two planes were 60 degrees. The transducer was held vertically with minimal pressure applied for each scanned plane.

The power mode of ultrasound is effective in detecting microvascular perfusion while the color mode is suitable for measuring blood flow in large vessels (Albrecht, Muller-Ladner, & Strunk, 2007). Previous studies have supported the feasibility and sensitivity of applying the power mode to evaluate blood perfusion at the microvascular level (Nam et al., 2016; Naredo et al., 2005; Walther et al., 2001). In our study, the power mode of the ultrasound was used to detect scar blood perfusion. The wall filter was set at the lowest value and gain was adjusted until random noise artefacts were

optimally reduced. For each patient, three 10-s videos for the scar and three 10-s videos for the healthy skin were recorded and processed in ImageJ program (C. A. Schneider et al., 2012). The value of the average color pixel was calculated for each video (Rawool, Goldberg, Forsberg, Winder, & Hume, 2003). An increased value of the average color pixel indicates increased blood perfusion. To reduce the influence of blood perfusion variations in different individuals and in different body parts, scar blood perfusion was quantified as: scar blood perfusion = average color pixel $_{scar}$ - average color pixel $_{healthy\ skin}$.

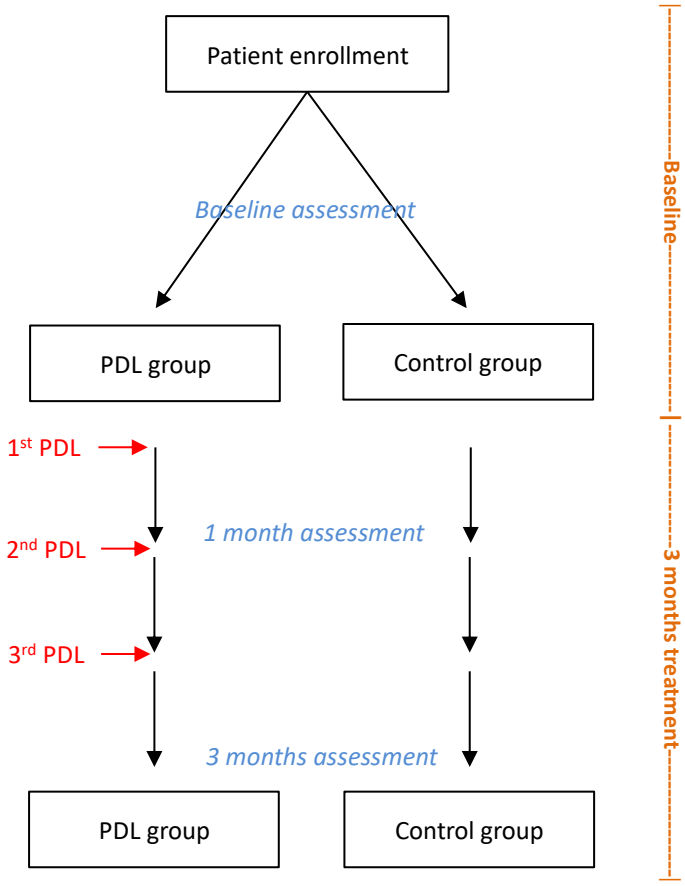
The B mode of the ultrasound was chosen to measure scar thickness (Ud-Din et al., 2019). The probe was placed on marked scan plane. Epidermal and dermal thickness were measured and the average of three measurements was used for the analysis of scar thickness.

4.2.5 Data analysis

Data analyses were performed with SPSS statistics version 23.0 (SPSS Inc., Chicago, IL, USA) and the level of significance was set to $p < 0.05$. For continuous variables, parametric data was presented as mean (SD) while non-parametric data was presented as median (IQR). For categorical variables, data was presented as n (%). Independent t test for parametric data, Mann-Whitney U test for non-parametric data, and Chi-square test for categorical data, were used to compare age, gender, skin type, days post-injury, cause of injury, scar location and scar thickness between two groups. Paired t-test for parametric data, while Wilcoxon signed rank test for non-parametric data, were used to compare the assessment results of the POSAS at baseline with the assessment results at 3 months and to compare the scar thickness at two different

time points in the same group. Additionally, one-way repeated measures ANOVA for parametric data, while Friedman test for non-parametric data, were used to explore the changes in scar erythema and blood perfusion among baseline, 1 month and 3 months. Due to the imbalance of scar vascularity at baseline between two groups, one-way ANCOVA was used to compare the assessment results of scar erythema and blood perfusion at 3 months between the PDL and control group with controlling for their assessment results at baseline.

Figure 4.1 The study flowchart. PDL: Pulsed dye laser.



4.3 RESULTS

4.3.1 Demographics and injury characteristics

Forty-five patients with immature hypertrophic scars were enrolled in this study. As shown in Table 4.1, the majority of participants were Fitzpatrick skin type III and the cause of injury was mostly caused by flame. The median age of the PDL group was 35.0 years (IQR 29.0 to 47.0 years) while the median age of the control group was 42.0 years (IQR 31.0 to 54.0 years). The median post-injury days in the PDL group was 89.5 days (IQR 57.0 to 148.8 days) while it in the control group was 77.0 days (IQR 49.0 to 166.0 days). There were no significant differences between the PDL and control group.

4.3.2 Assessment results of POSAS

Comparison of the POSAS assessment results between baseline and 3 months showed that the scar vascularity, pigmentation and color significantly decreased in the PDL group ($p = 0.003$, $p = 0.026$ and $p < 0.001$, respectively), while the scar vascularity and color significantly reduced in the control group ($p = 0.010$ and $p = 0.025$, respectively). The assessor's evaluation of scar pliability on the POSAS significantly increased in the control group ($p = 0.005$) but not in the PDL group ($p = 0.095$). However, the scar thickness on the POSAS, which was evaluated by the assessor and patients, significantly decreased in the PDL group ($p = 0.029$ and $p = 0.028$, respectively) but not in the control group ($p = 0.116$ and $p = 0.397$, respectively). Meanwhile, the overall POSAS scores of the assessor and patients significantly reduced in the PDL group only ($p = 0.002$ and $p = 0.004$, respectively). All other measures did not significantly change in the PDL or the control group.

4.3.3 Objective measurement results of scar erythema by dermoscopy, blood

perfusion and scar thickness by Doppler ultrasound

Patients in the PDL group had significant improvements in scar erythema (p = 0.009) and blood perfusion (p = 0.022) during the 3-month laser treatment. Whereas there were no significant changes in the control group (p = 0.296 and p = 0.115, respectively). For comparison of scar vascularity at 3 months between two groups, there was a significant difference in blood perfusion (p = 0.002) but not in scar erythema (p = 0.684). As shown in Figure 4.2, the scar thickness in the control group significantly increased from baseline to 1 month (0.22 cm vs. 0.26 cm, $p < 0.01$), and from 1 month to 3 months (0.26 cm vs. 0.32 cm, $p < 0.01$). Moreover, the scar thickness at 3 months in the control group was significantly higher than the PDL group (0.32 cm vs. 0.22 cm, $p < 0.05$).

Table 4.1 Demographic characteristics and injury information for the PDL and control group

	PDL group (n=22)	**Control group** (n=23)	***p***
Age (years), median (IQR)	35.0 (29.0-47.0)	42.0 (31.0-54.0)	0.369†
Gender, n (%)			0.912‡
- Female	8 (36.4%)	8 (34.8%)	
- Male	14 (63.6%)	15 (65.2%)	
Fitzpatrick Scale, n (%)			0.589‡
- Type I	1 (4.5%)	0 (0%)	
- Type II	7 (31.8%)	8 (34.8%)	
- Type III	8 (36.4%)	11 (47.8%)	
- Type IV	6 (27.3%)	4 (17.4%)	
- Type V	0 (0%)	0 (0%)	
- Type VI	0 (0%)	0 (0%)	
Days post injury (days), median (IQR)	89.5 (57.0-148.8)	77.0 (49.0-166.0)	0.750†
Cause of injury, n (%)			0.339‡
- Flame	11 (50.0%)	13 (56.5%)	
- Scald	6 (27.3%)	3 (13.0%)	
- Chemical	2 (9.1%)	3 (13.0%)	
- Electric	0 (0%)	3 (13.0%)	
- Trauma-related	3 (13.6%)	1 (4.3%)	
Scar location, n (%)			0.820‡
- Head and neck	4 (18.2%)	2 (8.7%)	
- Trunk	11 (50.0%)	13 (56.5%)	
- Upper limbs	3 (13.6%)	3 (13.0%)	
- Lower limbs	4 (18.2%)	5 (21.7%)	

Note. The *p* presents the comparison between the PDL and control group. IQR = Interquartile range; PDL = Pulsed dye laser.

†: Mann-Whitney U test; ‡: Chi-square test.

Table 4.2 Assessment results of POSAS in the PDL and control group

	PDL group (n=22)			Control group (n=23)		
Variable	0 month	3 months	*p*	0 month	3 months	*p*
(Rated by assessor)						
Vascularity, median (IQR)	7.0 (6.0-8.0)	5.0 (3.0-6.3)	0.003‡**	7.0 (6.0-8.0)	6.0 (4.0-7.0)	0.010‡*
Pigmentation, mean (SD) / median (IQR)	5.8 (2.0)	5.1 (1.9)	0.026†*	5.0 (4.0-6.0)	5.0 (4.0-6.0)	0.722‡
Thickness, mean (SD) / median (IQR)	5.4 (1.4)	4.9 (1.7)	0.029†*	5.0 (4.0-7.0)	6.0 (4.0-7.0)	0.116‡
Relief, median (IQR)	4.5 (3.0-5.3)	4.0 (3.0-4.3)	0.093‡	3.0 (3.0-5.0)	4.0 (4.0-5.0)	0.095‡
Pliability, mean (SD) / median (IQR)	4.7 (1.7)	4.3 (1.7)	0.095†	4.0 (3.0-5.0)	5.0 (3.0-7.0)	0.005‡**
Surface area, median (IQR)	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.000‡	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.000‡
Overall, median (IQR)	6.0 (6.0-7.0)	5.0 (4.0-6.3)	0.002‡**	6.0 (5.0-7.0)	6.0 (5.0-7.0)	0.369‡
(Rated by patient)						
Pain, median (IQR)	1.0 (1.0-3.0)	1.0 (1.0-1.0)	0.673‡	1.0 (1.0-3.0)	1.0 (1.0-1.0)	1.000‡
Itchiness, median (IQR)	3.0 (1.0-6.5)	1.0 (1.0-5.0)	0.233‡	2.0 (1.0-5.0)	3.0 (1.0-5.0)	0.625‡
Color, median (IQR) / mean (SD)	8.0 (6.0-10.0)	5.0 (4.0-6.0)	0.000‡**	6.6 (2.3)	5.6 (2.2)	0.025†*
Stiffness, mean (SD) / median (IQR)	5.5 (2.6)	4.6 (2.2)	0.087†	5.0 (4.0-8.0)	5.0 (3.0-7.0)	0.385‡
Thickness, mean (SD)	5.8 (2.9)	4.6 (2.2)	0.028†*	5.0 (2.5)	5.3 (2.3)	0.397†

Irregularity, mean (SD) / median (IQR)	4.7 (2.8)	4.3 (2.5)	0.466†	5.0 (3.0-7.0)	4.0 (3.0-6.0)	0.070‡
Overall, median (IQR)	7.0 (4.0-9.0)	5.5 (4.0-7.0)	0.004‡**	6.0 (5.0-8.0)	5.0 (3.0-8.0)	0.079‡

Note. The *p* presents the comparison of the POSAS assessment results between 0 month and 3 months. IQR = Interquartile range; PDL = Pulsed dye laser; SD = Standard deviation.

†: Paired t-test; ‡: Wilcoxon signed rank test.

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

Table 4.3 Objective measurement results of scar erythema and blood perfusion in the PDL and control group

	PDL group (n=22)				Control group (n=23)			
Variable	0 month	1 month	3 months	*p*	0 month	1 month	3 months	*p*
Scar vascularity								
- Scar erythema, median (IQR)	52.70 (44.10-72.05)	58.51 (47.25-69.74)	67.68 (55.42-83.56)	0.009‡**	57.76 (46.75-72.25)	62.68 (52.92-81.84)	66.18 (54.57-80.05)	0.296‡
- Blood perfusion, mean (SD)	-15.89 (17.05)	-11.00 (13.88)	-6.26 (15.88)	0.022†*	-9.13 (16.86)	-11.53 (15.19)	-16.40 (21.26)	0.115†

Note. The *p* presents the comparison of the measurement results among 0 month, 1 month and 3 months. IQR = Interquartile range; PDL = Pulsed dye laser; SD = Standard deviation.

†: One-way repeated measures ANOVA; ‡: Friedman test.

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

Figure 4.2 Between group comparison of scar vascularity measurement results. The *p* presents the comparison of the measurement results at 3 months between the PDL and control group (one-way ANCOVA). PDL: Pulsed dye laser.

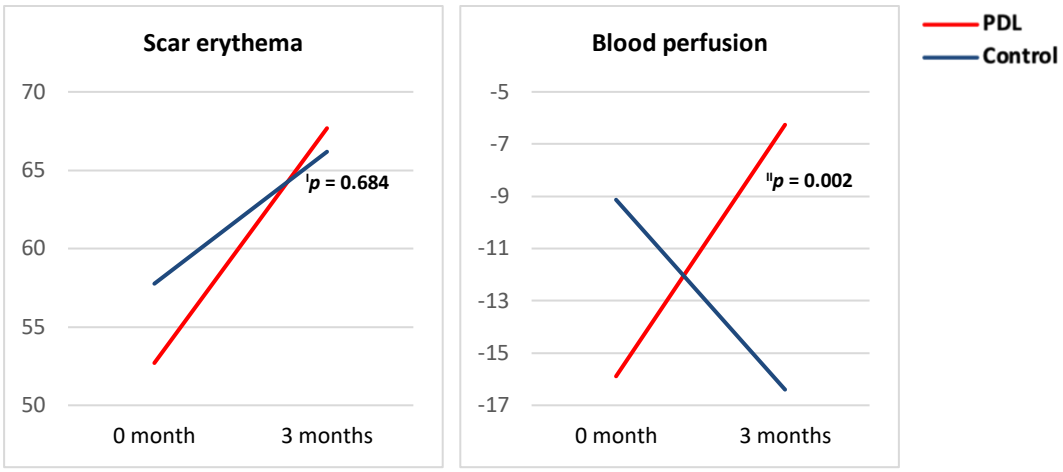


Figure 4.3 Objective measurement results of scar thickness in the PDL and control group. ^{I}p: Comparison of the scar thickness between 0 month and 1 month in the control group (Paired t-test); ^{II}p: Comparison of the scar thickness between 1 month and 3 months in the control group (Paired t-test); ^{III}p: Comparison of the scar thickness at 3 months between the PDL and control group (Mann-Whitney U test). PDL: Pulsed dye laser.

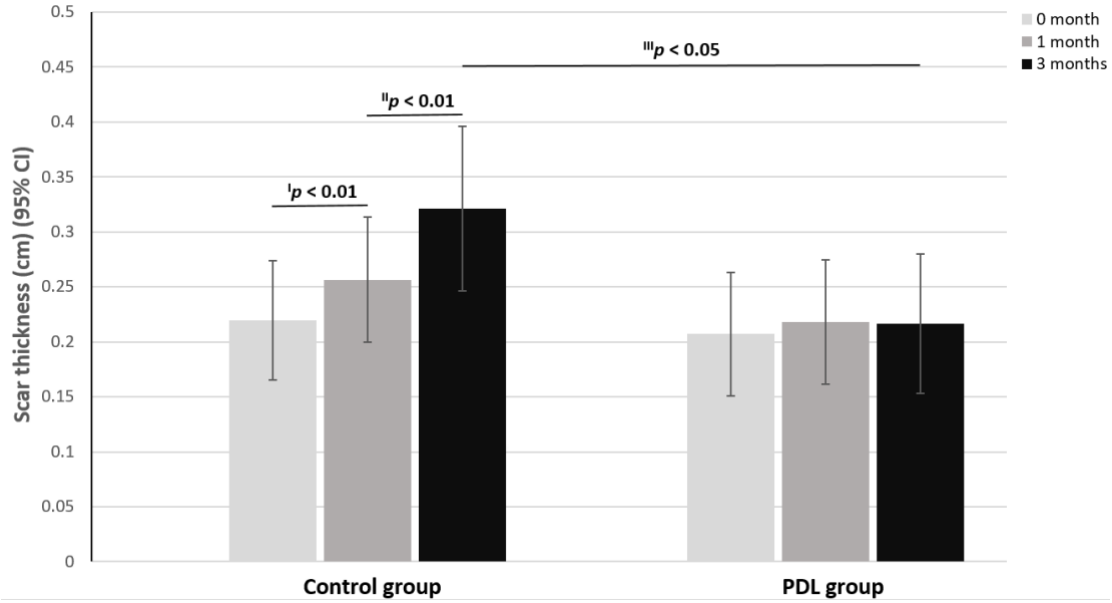
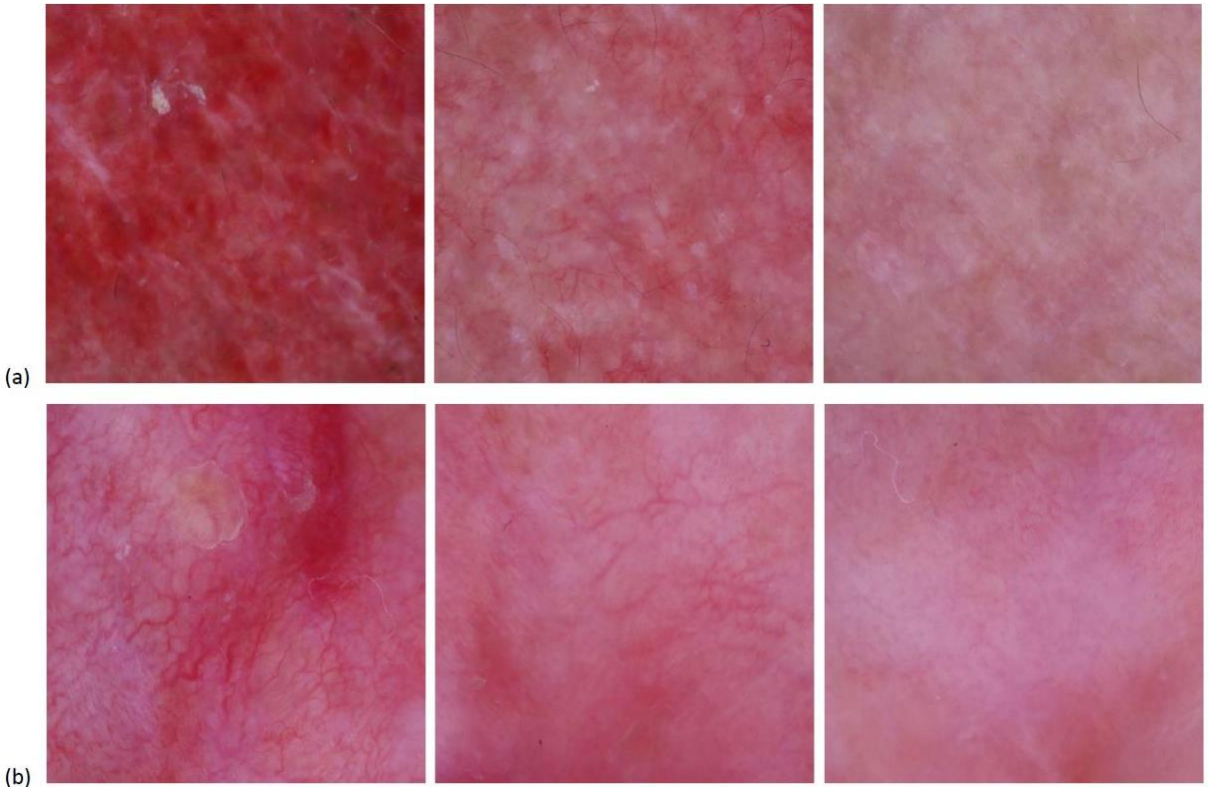


Figure 4.4 Dermoscopy images of treated immature hypertrophic scars in the PDL group (a) a 54-year-old male patient with an immature hypertrophic scar on the upper limb (b) a 48-year-old female patient with an immature hypertrophic scar on the trunk. Three images from left to right were taken at baseline (prior to PDL), 1 month (1 month after the 1st PDL treatment) and 3 months (1 month after the 3rd PDL treatment). PDL: Pulsed dye laser.



4.4 DISCUSSION

Management of hypertrophic scar is vital to reduce the scar's influence on survivors' daily living and social participation. Our results demonstrated that the use of PDL at an early stage improved scar erythema and blood perfusion, and limited thickness growth of immature hypertrophic scar.

In our study, scar erythema, as measured by dermoscopy, showed an insignificant difference between two groups but a significant change from baseline to 3 months in the PDL group only, which was consistent with the changes in scar vascularity rated by assessors and scar color rated by patients on the POSAS. Our results suggest that PDL, which caused damage to scar microvascular structures, contributed to decrease of scar erythema. A previous study, which recruited 15 Asian patients with hypertrophic scars and measured scar erythema using a Derma-spectrometer, reported a 65.3% of improvement in scar erythema following two PDL treatment sessions (Kono et al., 2005). Additionally, significant decreases of scar erythema were reported after PDL treatments of facial acne scars, keloid and hypertrophic sternotomy scars, and surgical scars (Alster & McMeekin, 1996; Alster & Williams, 1995; Kuo et al., 2004; Nouri et al., 2010).

Based on the quantification method, negative values of scar blood perfusion indicated that the scar blood perfusion was lower than the healthy skin blood perfusion. However, previous studies showed that the blood perfusion measured by LDI was higher in hypertrophic scar than healthy skin, and immature hypertrophic scar maintained the increased blood perfusion during the active proliferation period (Ehrlich & Kelley, 1992; Oliveira et al., 2005). LDI generates laser light, which penetrates scar tissue, and moving red blood cells in scar capillaries cause shifts of

Doppler frequency. Therefore, the LDI measurement results reflect the combined effect of the concentration and speed of the red blood cells (Stewart et al., 2005). Our study used the power mode of ultrasound to measure the blood perfusion of immature hypertrophic scar. Its measurement result presents concentration of moving red blood cells without providing information of their moving direction and speed (Torp-Pedersen & Terslev, 2008). Therefore, the blood perfusion measured by the power mode of ultrasound is not equivalent to the blood perfusion measured by LDI. Our blood perfusion measurement results demonstrated a smaller number of moving red blood cells in immature hypertrophic scar compared to healthy skin. Considering the fact that there is an increased total number of red blood cells in immature hypertrophic scar, which results from the robust growth of capillaries and is reflected by the increased scar erythema, a larger number of red blood cells in immature hypertrophic scar were in a relative static status compared with healthy skin. It suggests the poor efficiency of blood perfusion in immature hypertrophic scar.

Growth of capillaries is an essential process for wound healing and scar formation after a dermal injury (Gurtner et al., 2008). It was reported that partial newly formed capillaries were unstable and leaky (Yancopoulos et al., 2000). Thus, they were unable to serve their functional roles as compared to normal and mature capillaries in healthy skin and resulted in ineffective blood perfusion and tissue hypoxia. This is consistent with the low tissue O^2 tension, which has been reported in immature hypertrophic scar (Sloan, Brown, Wells, & Hilton, 1978; Zheng, Song, Lu, & Wang, 2014). As previously described, our blood perfusion results support the idea that the capillary network in immature hypertrophic scar is dense but poorly perfused.

In our study, the better blood perfusion was detected after the PDL treatment. Meanwhile, the control group, without PDL, developed the worse blood perfusion accompanied by a significant increase of scar thickness. Previous studies have supported that blood perfusion plays an important role in scar growth. A treatment of establishing a more functional perfusion network led to a better wound healing outcome and was less likely to form a hypertrophic scar (Bluff, O'Ceallaigh, O'Kane, Ferguson, & Ireland, 2006; Erba et al., 2011; Korntner et al., 2019). However, poor perfusion potentially resulted in tissue hypoxia which stimulated excessive production of collagen and scar growth (Lokmic, Musyoka, Hewitson, & Darby, 2012). Likewise, in our study, patients in the PDL group maintained a stable scar thickness during the 3-month laser treatment and patients in the control group showed a significant increase of scar thickness. The ultrasound-based scar thickness changes in the two groups were also consistent with the thickness evaluation results of both the assessors and patients using the POSAS. It appears that PDL establishes a more functional perfusion network in immature hypertrophic scar, which in turn limits scar growth and promotes scar maturation.

Scar thickness is determined by the amount and orientation of collagen deposition. PDL was first shown to improve scar texture in 1994 as the result of collagen remodelling (Alster, 1994). Increasingly clinical studies have reported the effect of PDL on inhibiting scar thickness growth (Brewin & Lister, 2014). Furthermore, histological studies showed that PDL decreased fibroblast proliferation and collagen deposition (Kuo et al., 2004; Kuo et al., 2005). However, the mechanism, by which PDL reduces scar thickness when its therapeutic target is the destruction of microvascular structures, is not fully understood. PDL causes damage to scar microvascular

structures. Leaky and unstable newly formed capillaries in immature hypertrophic scar might be more vulnerable and damaged by PDL. Thus, stable and functional capillaries are preserved that likely contributes to the better blood perfusion following the PDL treatment.

The overall scar scores rated by the assessors and patients significantly reduced in the PDL group after the 3-month treatment. Some studies also reported improvements in scar pliability, as well as related symptoms of pain and pruritus, following PDL treatments (Manuskiatti et al., 2001). However, there were no significant changes in our study. Compared with previous studies, which usually adopted fluence between 6 to 8 $J \cdot cm^{-2}$ and mainly included Caucasian patients, a slightly higher of PDL fluence was utilized in our study (Brewin & Lister, 2014). Theoretically, the penetration depth of PDL is around 0.12 cm, and melanin competes with hemoglobin for laser energy absorption (Parrett & Donelan, 2010). Thus, it is recommended to increase the PDL fluence for a patient with a thick scar and dark skin. Despite the fact that a higher fluence of PDL potentially increases the risk of complications, blisters were not observed in our study. Purpura disappeared within one week.

The major limitation of this study is the short treatment time. The 3-month treatment period is shorter than the scar maturation process which takes approximately 12 months. Future studies are required to follow participants for longer time to explore the long-term effects of PDL on scar vascularity and thickness and to determine the optimal number of treatments. Another limitation of this study is the imbalance of scar vascularity at baseline between two groups. Although two treatment-blinded assessors separately evaluated the POSAS and objective measures, and different

clinical professionals performed the PDL and standardized scar care. Patients in the PDL group showed worse scar erythema and blood perfusion at baseline compared to patients in the control group. It might be caused by the patient allocation method, which assigned eligible patients to either the control or PDL group on a weekly, alternating basis. Nevertheless, our results strengthen the link between controlling scar vascularity and limiting scar thickness growth through PDL treatment. More importantly, our study indicates that PDL inhibits scar growth probably through improving poor perfusion and establishing a functional perfusion network.

Given that PDL's efficacy is based on its selective photothermolysis of hemoglobin, it has a limited role in treating scars which show less erythema such as scars in a maturation stage. In addition, increasing the PDL fluence to maximize treatment effects on scars with a large amount of melanin would increase the risk of complications. Therefore, PDL is less recommended for scars with a large amount of melanin. In our study, scars with less erythema, much melanin or at a late stage were not recruited. It is necessary and important to apply PDL with considering scar properties while interpreting our results. To achieve a promising treatment outcome, PDL is recommended at an early stage for scars with high risk of developing to hypertrophic scars, a small amount of melanin and a significant increase in scar erythema.

CHAPTER FIVE

FUTURE RESEARCH SUGGESTIONS AND CONCLUSION

5.1 FUTURE RESEARCH SUGGESTIONS

5.1.1 Quantification of vascular patterns in dermoscopy captured images

Dermoscopy is a new assessment tool for measuring scar vascularity. There is no consensus on the method of quantifying dermoscopy captured images. Individual capillary could be measured in histological examinations (Woods, & Stirling, 2018)(Kurokawa, Ueda, & Tsuji, 2010). Due to the fact that the image captured by dermoscopy lacks micrometer-scale resolution, it is challenging to accurately identify individual capillaries in the image. Therefore, the method of quantifying vascular structures by dermoscopy relies more on vascular patterns. It has been reported that vascular patterns, which were identified in dermoscopy captured images, benefited of diagnosis of non-pigmented skin tumors (Zalaudek et al., 2010). A study preliminarily explored the vascular patterns in hypertrophic scars and keloids (Yoo & Kim, 2014). They subjectively classified capillaries into comma-shaped, arborizing and linear irregular patterns, and reported a significant difference in vascular patterns between hypertrophic scars and keloids.

It has been demonstrated that there are two different types of new blood vessel formation, the sprouting angiogenesis and the intussusceptive angiogenesis (Hillen & Griffioen, 2007). Furthermore, it is indicated that the intussusceptive angiogenesis generates more rapidly and has less leaky capillaries compared to the sprouting angiogenesis (Djonov, Baum, & Burri, 2003). Our results in chapter four suggested that formation of leaky capillaries might contribute to poor blood perfusion and stimulate scar thickness growth. Therefore, different predominant angiogenesis types in hypertrophic scars might lead to different outcomes. However, the relationship between angiogenesis type and hypertrophic scar development is unclear. Due to the

limitation of small sample size and short follow-up time in chapter three, our study is unable to answer this question.

Given that the sprouting angiogenesis is presented as the branch pattern while the intussusceptive angiogenesis is presented as the network pattern, developing a more accurate and sensitive method with highlight of angiogenesis type is required to quantify vascular patterns in hypertrophic scars. In addition, studies with big sample size and long follow-up time are needed to explore the relationship between vascular patterns and hypertrophic scars development. It will contribute to exploring characteristics of vascular patterns in hypertrophic scars at different stages.

5.1.2 Mechanism of scar capillary formation

Our measurement results in chapter four showed that the microvascular network in hypertrophic scar was poorly perfused, which was consistent with low tissue O_2 tension detected in hypertrophic scars (Z. Li, Liu, Wang, & Luan, 2017; Sloan et al., 1978). Furthermore, our results suggested that the poor blood perfusion in hypertrophic scars might be related to newly formed immature and leaky capillaries. However, the mechanism of forming immature and leaky capillaries has not been fully understood.

As one of the most recognized key factors, vascular endothelial growth factor (VEGF) plays an important role in regulating the blood vessel formation process in immature hypertrophic scars (van der Veer et al., 2009). It was reported that a scar with robust capillary growth, which was directly related to the high level of VEGF, tended to be hypertrophic (Wilgus, Ferreira, Oberyszyn, Bergdall, & Dipietro, 2008). A previous

study also showed that the amount of VEGF significantly decreased after PDL treatments (M. L. Wei et al., 2016). A review suggests that VEGF facilitates formation of immature and leaky blood vessels but Ang1 stimulates the opposite (Yancopoulos et al., 2000). It indicates that the vessel formation is a complex process and simultaneously affected by different factors such as VEGF, Ang and Ephrin. It is important for future studies to explore the mechanism of forming immature and leaky capillaries in hypertrophic scars, which potentially helps to understand its relationship with blood perfusion, establish a functional microvascular network at an early stage and prevent hypertrophic scar formation.

5.1.3 Optimal treatment time of PDL

A previous study compared early use of PDL on the day of suture removal with late use of PDL at nine weeks post suture removal (Davari et al., 2012). All patients with surgical scars received six PDL sessions at 3-week intervals. Their results showed that there were no differences in color and elasticity between early use and late use of PDL. In our study, PDL, which was used at an early stage of scar formation, decreased scar erythema and significantly improved poor perfusion after 3-month treatment. Another study explored the sequential use of laser therapy (Xie et al., 2018). PDL was firstly implemented for managing scars and was replaced by CO_2 fractional laser when scar vascularity decreased. Their results demonstrated significant treatment effects on scar vascularity, thickness and pliability. The sequential use of laser therapy has been recommended in clinical guidelines (Anderson et al., 2014; Hibbard et al., 2018; Lv & Xia, 2018).

Due to the fact that PDL targets at hemoglobin, its therapeutic effects reduce for scars with less erythema. Hypertrophic scars usually become mature over 12 months after injury and scar erythema changes during this period (Bond et al., 2008; Mahdavian Delavary et al., 2012). There is not a clear criterion of guiding the start and stop of PDL treatment. It is worthwhile to explore optimal treatment times and long-term effect of PDL on managing hypertrophic scars. It will contribute to maximizing the treatment efficiency and achieving promising outcomes.

It is also noticed that some PDL studies adopted the split-face design. One scar is divided into different segments with receiving different treatments. Given that laser energy might diffuse to the surrounding tissue, the control area is possibly affected by the laser therapy. This might be one of reasons that some PDL studies with adopting the split-face design reported insignificant differences between groups (Manuskiatti et al., 2001; Wittenberg et al., 1999). To reduce the influence, it is recommended for future laser studies to assign different treatments to different isolated scars.

5.1.4 Pain management with receiving PDL treatments

A previous study followed up 95 patients who received different types of laser therapy, in which patients with PDL made up of 71% of total patients (Clayton, Edkins, Cairns, & Hultman, 2013). Their reported complications after laser therapy included 37% of pain, 27% of blister, 12% of hypopigmentation, 10% of fever, 7% rash, 2% infection and 2% hyper-pigmentation. In our study, pain was also commonly complained during the PDL treatment. In addition, it was observed that pain significantly affected patients' compliance to the laser therapy in daily clinical practice.

To reduce pain, a study explored the use of opioids in pediatric patients (Wong et al., 2017). One group of patients received long-acting opioids, one group of patients received short-acting opioids while another group of patients received the combination treatment. They reported an insignificant difference in experiencing pain among different groups. In some hospitals, a topical anesthetic cream is applied to treated scar sites prior to laser treatments. It was observed that some types of topical anesthetic cream led to the contraction of scar capillaries, which affected PDL energy absorption by the hemoglobin. Thus, it became difficult for PDL to cause damage to the microvascular structures. Selection of suitable topical anesthetic cream is important to ensure PDL's treatment effects and manage patients' pain at the same time. The general anesthesia is used in some hospitals for patients who have large scar areas for PDL treatment. However, it is not proper as the routine care for all patients who are treated with PDL. There is a need for future studies to explore an effective way to reduce pain with receiving PDL treatments.

5.2 CONCLUSION

Scar vascularity is a key indicator of scar maturation. Available clinical tools could be divided into subjective and objective scar vascularity measurement tools. Due to acceptable reliability and easy to use, subjective vascularity measurement scales are widely used to give a preliminary impression of scar vascularity. It is recommended that the VSS and mVSS are used for screening scars and the POSAS with involvement of patients' rating is used in routine scar assessments. For objective tools, the color-measuring device, blood flow measuring device and morphological imaging device present different dimensions of scar vascularity and are not equivalent to measure scar vascularity. As one of assessment tools, dermoscopy shows better performance

in measuring scar vascularity than the color-measuring device and POSAS. Furthermore, dermoscopy shows its potential to predict scar thickness change and indicate scar maturation. The handheld and non-invasive dermoscopy, which measures scar color and provides a view of scar vascular structures, is recommended to assess scar vascularity. Additionally, PDL, which controls scar vascularity by significantly improving poor blood perfusion, appears to be an effective way to limit scar thickness growth and promote scar maturation. To achieve a promising treatment outcome, PDL is recommended at an early stage for erythematous and immature hypertrophic scars.

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APPENDICES

APPENDIX I: ETHICAL APPROVAL FOR CHAPTER THREE

THE HONG KONG POLYTECHNIC UNIVERSITY
香港理工大學

To	Tsang Wai Ping Cecilia (Department of Rehabilitation Sciences)		
From	Man Wai Kwong, Chair, Departmental Research Committee		
Email	david.man@	Date	22-Jan-2018

Application for Ethical Review for Teaching/Research Involving Human Subjects

I write to inform you that approval has been given to your application for human subjects ethics review of the following project for a period from 01-Jan-2018 to 31-Dec-2018:

Project Title:	Validation study of the Dermoscopy for the measurement of vascularity in the hypertrophic scar
Department:	Department of Rehabilitation Sciences
Principal Investigator:	Tsang Wai Ping Cecilia
Project Start Date:	01-Jan-2018
Reference Number:	HSEARS20180119003

You will be held responsible for the ethical approval granted for the project and the ethical conduct of the personnel involved in the project. In the case of the Co-PI, if any, has also obtained ethical approval for the project, the Co-PI will also assume the responsibility in respect of the ethical approval (in relation to the areas of expertise of respective Co-PI in accordance with the stipulations given by the approving authority).

You are responsible for informing the Human Subjects Ethics Sub-committee in advance of any changes in the proposal or procedures which may affect the validity of this ethical approval.

Man Wai Kwong

Chair

Departmental Research Committee

APPENDIX II: ETHICAL APPROVAL FOR CHAPTER FOUR

THE HONG KONG POLYTECHNIC UNIVERSITY 香港理工大學

To	Tsang Wai Ping Cecilia (Department of Rehabilitation Sciences)		
From	Man Wai Kwong, Chair, Departmental Research Committee		
Email	david.man@	Date	02-Apr-2019

Application for Ethical Review for Teaching/Research Involving Human Subjects

I write to inform you that approval has been given to your application for human subjects ethics review of the following project for a period from 01-Apr-2019 to 24-Apr-2020:

Project Title:	The vascular changes of scars with laser therapy: A prospective exploratory study
Department:	Department of Rehabilitation Sciences
Principal Investigator:	Tsang Wai Ping Cecilia
Project Start Date:	01-Apr-2019
Reference Number:	HSEARS20190402002

You will be held responsible for the ethical approval granted for the project and the ethical conduct of the personnel involved in the project. In case the Co-PI, if any, has also obtained ethical approval for the project, the Co-PI will also assume the responsibility in respect of the ethical approval (in relation to the areas of expertise of respective Co-PI in accordance with the stipulations given by the approving authority).

You are responsible for informing the Human Subjects Ethics Sub-committee in advance of any changes in the proposal or procedures which may affect the validity of this ethical approval.

Man Wai Kwong

Chair

Departmental Research Committee

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To: DENG, Huan RS [Student] <huan.rs.deng@ >
Subject: Request for permission (DOI:10.1111/srt.12812) Your case 05547905 [ref:_00Dd0eeku._5000W1bAiPM:ref]

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"Measuring vascularity of hypertrophic scars by dermoscopy: Construct validity and predictive ability of scar thickness change"
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APPENDIX V: THE PATIENT SCAR ASSESSMENT SCALE

POSAS Patient scale

The Patient and Observer Scar Assessment Scale v2.0 / EN

	1 = no, not at all								yes, very much = 10
	1	2	3	4	5	6	7	8	9	10
HAS THE SCAR BEEN PAINFUL THE PAST FEW WEEKS?	○	○	○	○	○	○	○	○	○	○
HAS THE SCAR BEEN ITCHING THE PAST FEW WEEKS?	○	○	○	○	○	○	○	○	○	○

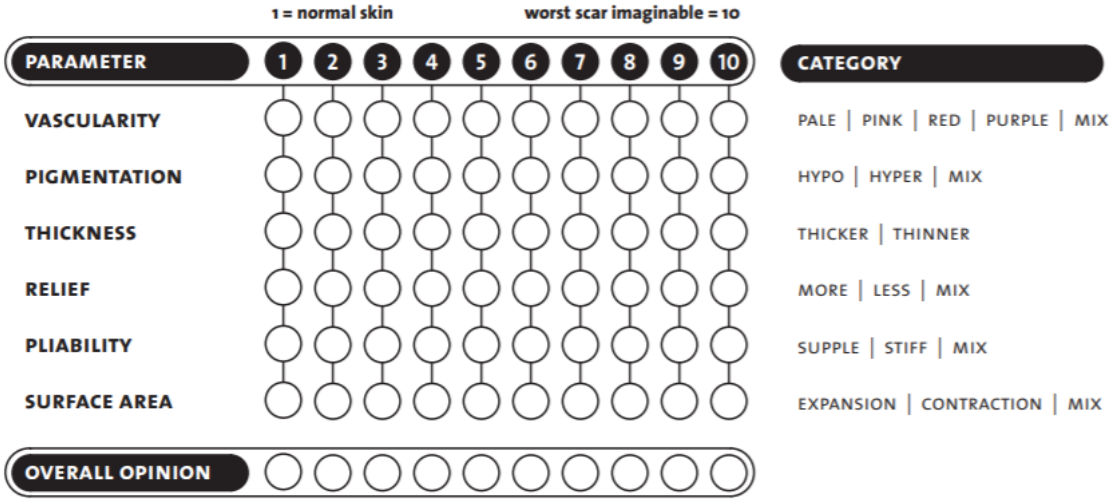
	1 = no, as normal skin								yes, very different = 10
	1	2	3	4	5	6	7	8	9	10
IS THE SCAR COLOR DIFFERENT FROM THE COLOR OF YOUR NORMAL SKIN AT PRESENT?	○	○	○	○	○	○	○	○	○	○
IS THE STIFFNESS OF THE SCAR DIFFERENT FROM YOUR NORMAL SKIN AT PRESENT?	○	○	○	○	○	○	○	○	○	○
IS THE THICKNESS OF THE SCAR DIFFERENT FROM YOUR NORMAL SKIN AT PRESENT?	○	○	○	○	○	○	○	○	○	○
IS THE SCAR MORE IRREGULAR THAN YOUR NORMAL SKIN AT PRESENT?	○	○	○	○	○	○	○	○	○	○

	1 = as normal skin								very different = 10
	1	2	3	4	5	6	7	8	9	10
WHAT IS YOUR OVERALL OPINION OF THE SCAR COMPARED TO NORMAL SKIN?	○	○	○	○	○	○	○	○	○	○

APPENDIX VI: THE OBSERVER SCAR ASSESSMENT SCALE

POSAS Observer scale

The Patient and Observer Scar Assessment Scale v2.0 / EN



APPENDIX VII: THE VANCOUVER SCAR SCALE

Parameter	Score
Vascularity	
Normal	0
Pink	1
Red	2
Purple	3
Pigmentation	
Normal	0
Hypopigmentation	1
Hyperpigmentation	2
Pliability	
Normal	0
Supple	1
Yielding	2
Firm	3
Ropes	4
Contracture	5
Height (mm)	
Flat	0
＜2	1
2～5	2
＞5	3
Total score	13

APPENDIX VIII: THE FITZPATRICK SKIN TYPE SCALE

Type I	Type II	Type III	Type IV	Type V	Type VI
Always burns, never tans	Usually burns, tans with difficulty	Sometimes mild burn, gradually tans to olive	Rarely burns, tans with ease to a moderate brown	Very rarely burns, tans very easily	Never burns, tans very easily, deeply pigmented

APPENDIX IX: THE DERMOSCOPY USED IN THIS STUDY