AN INNOVATIVE ASSESSMENT OF THE BIOMECHANICAL PROPERTIES OF PLANTAR TISSUES AND DIABETIC FOOT ULCERS

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An innovative assessment of the biomechanical properties of plantar tissues and diabetic foot ulcers

by

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CERTIFICATE OF ORIGINALITY

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

_____ (Signed)

CHAO YUET LAN (Name of student)

Abstract of thesis entitled "An innovative assessment of the biomechanical properties of plantar tissues and diabetic foot ulcers" submitted by Clare Yuet-Lan Chao for the degree of Doctor of Philosophy at the Hong Kong Polytechnic University in January 2012.

ABSTRACT

Foot ulcers are a common complication of diabetes mellitus and the predisposing factors are multifactorial. Diabetic peripheral neuropathy and repetitive stress are the most well known causative factors for diabetic related foot injuries while microvascular dysfunction is throught to be an essential factor contributing to the pathogenesis of tissue breakdown in the diabetic foot. It may also play a role in the development of neuropathy and interact with the complex interchange of advanced glycosylation end products as induced by hyperglycaemia, causing potential pathological consequences of morphological change in skin and soft tissue properties. Yet the precise mechanism of this process remains unclear. Apart from the pathological changes that take place in plantar skin morphology, foot swelling and changes in the properties of plantar soft tissues may further increase the risk of foot ulceration in people with diabetes. In order to develop strategies to prevent or manage diabetic ulcers, it is vital to obtain a better understanding of the underlying pathophysiology of diabetic ulcers. Also, a precise and quantitative method for evaluating the healing of ulcers is essential for making appropriate treatment decisions and monitoring the efficacy of the treatments. Thus far, a precise quantitative method of assessing wound healing or the properties of ulcer tissues is lacking. The restoration of the mechanical properties of wound tissue is an important indicator of the quality of wound healing. Nonetheless, changes in the biomechanical properties of skin wound tissues across different phases of the wound healing process

have not been explored. In laboratory work, the evaluation of wound tissue properties can be achieved by testing an excised wound specimen using Material Testing Systems *in vitro*. An optical coherence tomography (OCT)-based air-jet indentation system is a novel non-contact method that has been recently developed for characterizing the biomechanical properties of soft tissues in a non-contact way. It can potentially be used for assessing the properties of wound tissues *in vivo*.

This project consists of four inter-related studies, with each study having specific objectives. They are: (I) The epidermal thickness and biomechanical properties of plantar tissues in the diabetic foot; (II) The association between skin blood flow and oedema on epidermal thickness in the diabetic foot; (III) A novel non-contact method to assess the biomechanical properties of wound tissue in humans; (IV) *In vivo* and *in vitro* approaches to studying the biomechanical properties of healing wounds in rat skin.

Study I: The objective of Study I was to examine the morphological changes in plantar epidermal thickness and in the properties of the soft tissues of the diabetic foot in humans.

Method and result of Study I: Seventy-two people with diabetes, namely 22 people with neuropathies, 16 with foot ulcerations, 34 with diabetics but without complications; and 40 healthy controls participated in the study. The thickness of the epidermal layer of the plantar skin was examined using high-frequency ultrasonography. Using the Tissue Ultrasound Palpation System, the thickness and stiffness of the total plantar soft tissue were measured at the big toe, the first, third, and fifth metatarsal heads, and the heel pad. As compared with the control group, the average epidermal thickness of plantar skin decreased by 15% in people with

diabetic foot ulcerations and 9% in people with neuropathy, but increased by 6% in those with diabetes without complications. An 8% increase in the total thickness of the plantar soft tissues was observed in all diabetic subjects at all testing sites (all $p<0.05$), with the exception of the first metatarsal head. The stiffness of the plantar soft tissues increased in all diabetic groups at all testing sites as compared with the control group (all $p<0.05$).

Study II: The aim of this study was to explore the association of skin blood flow and oedema on epidermal thickness in the feet of people with and without diabetes. Eighty-seven subjects, namely 19 people with diabetic neuropathy and foot ulcerations, 35 people with diabetes but without neuropathy, and 33 non-diabetic healthy controls participated in the study. High-frequency ultrasonography was used to measure the epidermal thickness and oedema in the papillary skin of the big toe as reflected by the thickness of the subepidermal low echogenic band (SLEB). The capillary nutritive blood flow was measured by the use of video capillaroscopy and skin blood flux was monitored by laser-Doppler flowmetry. We demonstrated that the thickness of the SLEB had increased in all diabetics, to a greater extent in people with neuropathy and ulceration than those without (64.7% vs 11.8%, $p<0.001$). Skin blood flux was shown to be higher in the groups with diabetes than in the controls (all $p<0.05$), but no significant difference was found in the resting nutritive capillary blood flow ($p>0.05$). A significant fair negative correlation ($p=0.002$, $r=-0.366$) was demonstrated between the SLEB and epidermal thickness at the pulp of the big toe, while no significant correlations were found among capillary blood flow, skin blood flux, and epidermal thickness (all $p>0.05$).

Study III: This study evaluated the stiffness of diabetic foot ulcer tissues and examined the test-retest reliability of the newly developed OCT-based air-jet indentation system for characterizing the biomechanical properties of wound tissues in humans. Eight subjects with diabetes (7 males, 1 female) participated in the study, and a total of 10 foot ulcers were assessed. Twenty measuring sites located either at the central wound bed (n=10) or in peri–ulcer areas (n=10) were identified and their biomechanical properties were assessed by the use of the air-jet indentation system. The test-retest reliability was examined at all measuring points. We found that the average stiffness of the peri-ulcer area (0.47±0.15 N/mm) was significantly higher than that of the central wound bed area (0.35±0.23 N/mm; p=0.042). Excellent test/retest reliability was demonstrated (ICC: 0.986; Pearson's correlation: r=0.972, p<0.001).

Study IV: This study examined the biomechanical properties of healing skin wounds *in vivo* using an air-jet indentation system and *in vitro* using a conventional material testing system in a rat model. Thirty male Sprague-Dawley rats, each with a 6 mm full-thickness circular punch biopsied wound at each posterior hind limb, were used. The stiffness at both the wound central and the margins was measured repeatedly in five rats at the same wound sites to monitor the longitudinal changes over various wound healing phases (i.e., before wounding, and on Days 0, 3, 7, 10, 14, and 21 after wounding) *in vivo* using an OCT-based air-jet indentation system. In addition, five rats were euthanized at each time point, and the biomechanical properties of the wound tissues were assessed *in vitro* using a material testing system. The size of the wound shrank significantly in the initial few days, closing almost completely by Day 10. At the central wound bed region, the stiffness at the baseline pre-wounding stage was 16.9±2.2 N/m, which increased significantly from Day 0

(19.8±5.3 N/m, 17.16%) and reached its peak on Day 7 (52.1±20.6 N/m, 208.28%), but then progressively decreased until Day 21 (23.7±3.2 N/m, 40.24%). In contrast, the biomechanical parameters of skin wound tissue measured by the material testing system showed a marked reduction upon wounding, and then gradually increased with time (all $p<0.05$). On Day 21, the ultimate tensile strength and stress of the skin wound tissue was about 50% of that of the unwounded skin; whereas the stiffness of the tissue recovered at a faster rate, reaching 97% of the pre-wounding status by Day 21.

Overall, the present thesis demonstrated that for people with diabetes, particularly for those with neuropathy or ulceration, the epidermal plantar skin became thinner and the plantar soft tissues stiffened. In addition, an increase in subepidermal oedema was demonstrated in people with diabetic neuropathy and ulceration, which may partly contribute to a reduction in epidermal thickness at the pulp of the big toe. All of these changes may subsequently lead to the breaking down of skin in the diabetic foot. This implies that diabetes-associated changes in the biomechanical properties of plantar skin, plantar soft tissues, and foot swelling are potential risk factors of foot ulceration in people with diabetes. Therefore, regular examinations of the sole of the foot of people with diabetes and the wearing of proper shoes should be reinforced in order to prevent foot complications. As for the stiffness of diabetic foot ulcer tissues, we demonstrated that the peri-ulcer area was stiffer than the ulcerated tissues in a diabetic foot with ulcers. This greater magnitude of hardness and inelasticity in the peri-ulcer region may scatter some of the contractile forces for wound contraction during the healing process. The newly developed OCT-based air-jet indentation system is a reliable tool for characterizing the stiffness of soft tissues around the wound in a non-contact way *in vivo*. As for the

changes in the biomechanical properties of skin wound tissues across different phases of the wound healing process, we found that stiffness recovered at a faster rate than tensile strength in rat skin wounds that were healing. Measurements made by the air-jet-indentation system and by the material testing systems involve different principles, but both systems can reflect the biomechanical properties of wound tissue.

PUBLICATIONS ARISING FROM THE THESIS

(A) Peer Review Journal

Chao CY, Ng GY, Zheng YP, Cheung KK, Wang LK, Cheing GL. *In vivo* and *ex vivo* approaches to studying the biomechanical properties of healing wounds in rat skin. *Wound Repair Regen* 2012 (conditional acceptance).

Chao CY, Zheng YP, Cheing GL. The association between skin blood flow and oedema on epidermal thickness in the diabetic foot. *Diabetes Technol Ther* 2012; 14(7): 602-9.

Chao CY, Zheng YP, Cheing GL. Epidermal thickness and biomechanical properties of plantar tissues in diabetic foot. *Ultrasound in Med Bio* 2011; 37(7): 1029-38.

Chao CY, Zheng YP, Cheing GL. A novel non-contact method to assess the biomechanical properties of wound tissue. *Wound Repair Regen* 2011; 19 (3): 324-9.

Chao CY, Zheng YP, Hung YP. Cheing GL. Biomechanical properties of the forefoot plantar soft tissue as measured by an optical coherence tomography (OCT)-based air-jet indentation system and tissue ultrasound palpation system. *Clin Biomech (Bristol, Avon)* 2010; 25 (6): 594-600.

Chao CY, Cheing GL. Microvascular dysfunction in diabetic foot disease and ulceration. *Diabetes Metab Res Rev* 2009; 25(7): 604-614.

(B) Conference Papers

Cheing GL, **Chao CY**, Zheng YP. Subepidermal oedema in diabetic foot. *The 3rd Asia-Oceanian Conference of Physical and Rehabilitation Medicine*; Bali Indonesia, 20-23 May 2012 (Poster presentation).

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Cheing GL, **Chao CY**, Zheng YP,. The association between skin blood flow and oedema on epidermal thickness in the diabetic foot. *Seventh International Symposium on Healthy Aging "Live Well Age Well"*, Hong Kong, 3-4 March 2012.

Chao CYL, Zheng YP, Cheing GLY. Epidermal thickness and biomechanical properties of plantar tissues in diabetic foot. *World Confederation for Physical Therapy (WCPT) congress 2011*; Amsterdam, Netherlands 20-23 June 2011 (Poster presentation).

Chao CYL, Zheng YP, Huang YP, Cheing, GLY. *In vivo*-monitoring of diabetic foot ulcer healing using OCT air-jet indentation. *Kowloon Central Cluster Convention 2010* p40 (Poster presentation).

Chao CYL, Zheng YP, Huang YP, Cheing, GLY. *In vivo*-monitoring of diabetic foot ulcer healing using OCT air-jet indentation. *The International Conference on the Ultrasonic Measurement and Imaging of Tissue Elasticity*, Zeeland, The Netherlands, September 14-17, 2009 (Oral presentation).

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CHAPTER 7 SUMMARY AND CONCLUSION

LIST OF ABBREVIATIONS

au	Arbitrary units
BMI	Body mass index
CBV	Capillary blood cell velocity
CI	Confidence interval
DM	Diabetes mellitus
DPN	Diabetic peripheral neuropathy
DU	Diabetic ulcer
FPG	Fasting plasma glucose
HbA1c	Glycated haemoglobin
ICGFA	Indocyanin green fuorescence angiography
MHz	Mega hertz
MTH	Metatarsal head
LDF	Laser Doppler flowmetry
LDI	Laser Doppler imaging
OCT	Optical coherence tomography
OGTT	Oral glucose tolerance test
OPS	Orthogonal polarization spectral
PPG	Photo-plethysmography
SBP	Skin blood flow
SD	Standard deviation
SLEB	Subepidermal low echogenic band
$TcpO_2$	Transcutaneous oxygen tension measurement
TUPS	Tissue ultrasound palpation system
VAR	Venoarteriolar reflex

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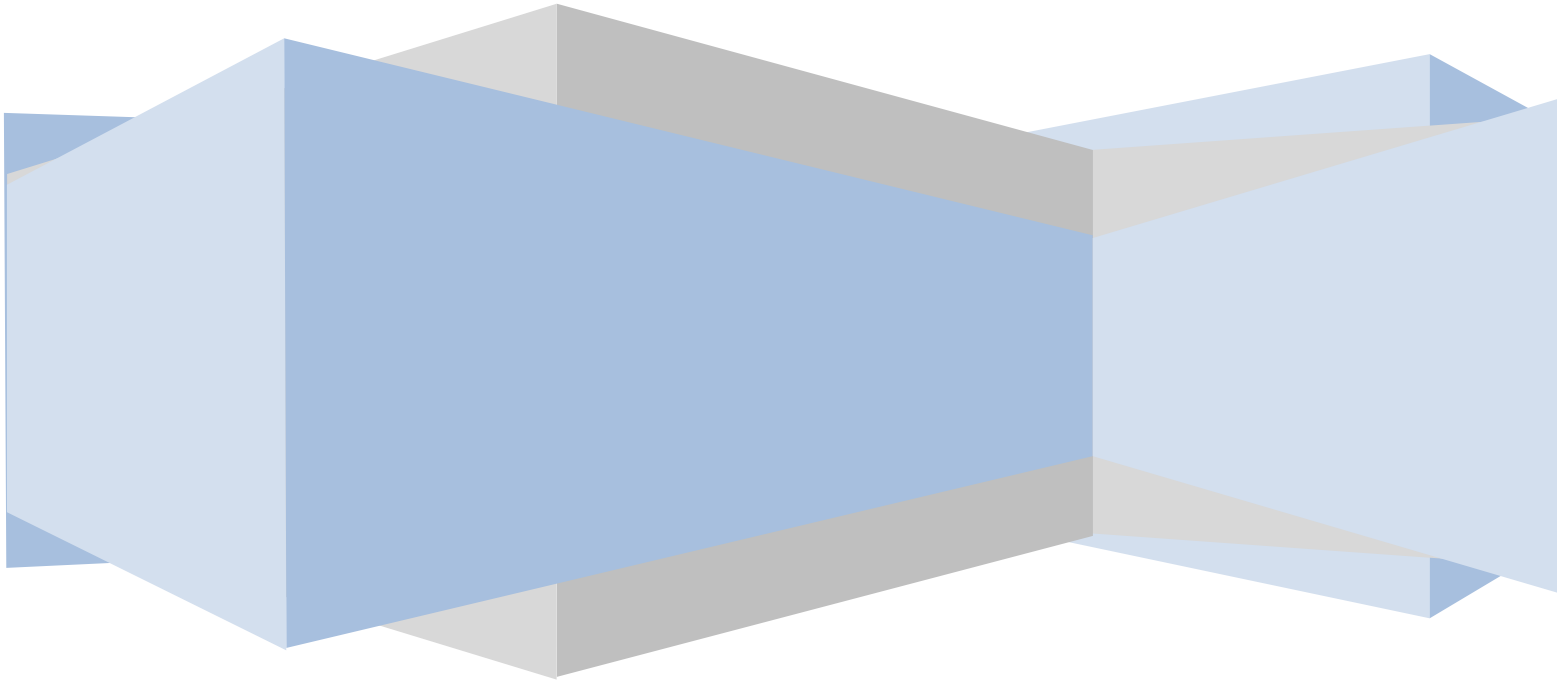
CHAPTER ONE

General Introduction

Clare YL Chao



1.1 Introduction

Diabetes mellitus is a global lifelong health problem with increasing prevalence worldwide. In tandem with the aging population, and the rising incidence of obesity due to lack of physical activity and rich in high-fat diet lifestyle, the number of diabetes patients is estimated to increase from 20.8 million in 2000 to 42.3 million in 2030 in China (Boutayeb and Boutayeb 2005). Worldwide, diabetes is estimated to affect 171 million people, with an increase projected to rise to 366 million by the year 2030 (Wild *et al.* 2004).

1.2 Diagnosis and Classification of Diabetes Mellitus

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from either impairment in insulin secretion or defects in insulin action, or both. The classical symptoms include polyuria, polydipsia and polyphagia (American Diabetes Association 2004). It is diagnosed by demonstrating any one of the following clinical presentations (American Diabetes Association 2004).

(i) Fasting plasma glucose (FPG) level ≥ 7.0 mmol/L (126 mg/dL)

(ii) Plasma glucose in oral glucose tolerance test (OGTT) (2 hours after a 75g oral glucose load) ≥ 11.1 mmol/L (200 mg/dL)

(iii) Symptoms of diabetes plus casual plasma glucose (any time of day without regard to time since last meal) ≥ 11.1 mmol/L (200 mg/dL)

People are classified to be in pre-diabetic state if they are considered to have impaired fasting plasma glucose level fall between 5.6 to 6.9 mmol/L (100 to 125 mg/dL) or impaired glucose tolerance with plasma glucose fall within

7.8mmol/L to 11.1mmol/L (140mg/dL to 200mg/dL) in OGTT (American Diabetes Association 2004).

There are three major subtypes of diabetes, namely type 1 diabetes, type 2 diabetes, and gestational diabetes (American Diabetes Association 2004). Type 1 diabetes is characterized by insulin deficiency that caused by loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas. Type 2 diabetes is characterized by insulin resistance that refers to the defective responsiveness of body tissues to insulin. Gestational diabetes refers to a non-diabetic pregnant women developed high blood glucose level during pregnancy.

Around 90% of all diabetes cases belong to Type 2 diabetes (Amos *et al.* 1997). Boulton (2000) reported that up to 50% of older Type 2 diabetic patients have risk factors for foot ulceration, and over 5% have a history of foot ulceration. Fifteen percent of diabetic people develop foot ulcers during their lifetime and 85% of all non-traumatic lower limb amputations are preceded by foot ulcers (Reiber *et al.* 1999). Diabetic foot pathology such as ulceration, infection and gangrene may lead to hospitalization and this significantly increases the burden on individuals and health care system.

1.3 Pathogenesis of Diabetic Foot Ulcers

A diabetic foot ulcer is defined as any skin breakdown with cutaneous erosions on the foot in a diabetic person. It is characterized by a loss of epithelium that extends into or through the dermis to deeper tissues. It develops when the mechanical loading exceeds the tolerance of skin tissue of a localized area for a

prolonged period of time, resulting in ischaemia followed by necrosis (Hagisawa and Shimada 2005). It mostly happens in the pressure sensitive site of the heel pad, metatarsal heads, medial and lateral border of the foot in diabetic persons (Reiber *et al.* 1998). Among all, the medial plantar forefoot soft tissues are the site that withstands the highest plantar pressure during weight bearing (Armstrong *et al.* 1998; Duckworth *et al.* 1985). Abnormal foot biomechanics caused by changes in the properties of tissue has been postulated as a key risk factor for the breakdown (Cevera *et al.* 1997; Whitney 2003). Foot lesion is commonly occur in the older population in particularly for people with diabetes (Whitney 2003). A high incidence of foot problems such as symptomatic metatarsalgia was reported.

1.3.1 Risk factors for developing diabetic foot ulcer

Majority of the diabetic foot ulcers are chronic wounds as it fail to heal in a timely and orderly manner to restore its anatomic and functional integrity. The non-healing foot ulcers significantly increase the risk for infection, and may end up with subsequently lower limb amputation. The most frequent risk factors for diabetic foot ulcers are neuropathy, vascular insufficiency, plantar callus, elevated plantar pressures, and a limited range of motion in the joints (Bennett *et al.* 1996; Fernando *et al.* 1991; Frykberg 2002; Mueller *et al.* 2005). Local changes in the skin morphology and biomechanical properties of plantar soft tissue may further increase the risk of developing foot ulcer.

Generally speaking, the etiologies are broadly classified as vascular (10%), neuropathic (40%) or neuro-ischaemic (40%) origin (Boulton; Grunfeld 1992). Vascular ulcer usually refers to the absence of foot pulses, mainly at the posterior

tibial and dorsalis pedis, and with an abnormal ankle brachial index of less than 0.9. Neuro-ischaemic ulcer is a further addition of impairment of foot sensation to vascular ulcer (Sandeman and Shearman 1999). Pure neuropathic ulcer is characterized by having no clinical evidence of macrovascular disease, and loss of protective sensation associated with diabetic sensory neuropathy (Brand 1990).

1.3.1.1 Diabetic peripheral neuropathy

Diabetic peripheral neuropathy (DPN) is the most frequent complications of diabetes with its prevalence rate approaches 70% (Dobretsov *et al.* 2009). It may present with or without signs and symptoms of peripheral nerve dysfunction such as numbness, prickling/tingling, burning, aching, lancinating pain, and allodynia. The pathophysiology of DPN involves (i) oxidative stress, (ii) advanced glycation end products, (iii) polyol pathway flux, and (iv) protein kinase C activation. All contribute to microvascular disease and nerve dysfunction (Duby *et al.* 2004).

In DPN persons, the myelin sheath and structure of the axon fibers of the nerves are damaged due to hyperglycemia. All the sensory, motor and autonomic systems may be affected. Sensory neuropathy results in loss of protective sensation to pain, pressure or temperature. It can be classified as distal symmetric polyneuropathy, focal neuropathy (e.g., diabetic mononeuropathy), and diabetic amyotrophy. Motor neuropathy causes muscle weakness, atrophy or paresis of the intrinsic muscles of the foot and leads to imbalance of the tendons, hyperextension of the toes, displacement of the fat pads, and splaying of the foot, which clinically results in hammer or claw toes. Automonic neuropathy presents as a loss of peripheral sympathetic vascular tone. This impairs peripheral circulation and

microvascular skin blood flow and promotes decreased production of the sweat and oil glands. This further makes foot dry, and stiff, resulting in fissure and crack that provides a portal for infection (Delmas 2006). It may be classified by the system that is involved (e.g., endocrine, gastrointestinal, genitourinary). Among all, sensorimotor neuropathy is the most common form of neuropathy in diabetes. It affects large and small afferent nerve fibers to varying degrees, resulting in mixed symptoms and sensory loss. Large afferent nerve fibers transmit proprioception, cold, and vibration sensation. Small afferent fibers are responsible for conducting nociceptive stimuli, touch, and warmth sensation. Both the sensory and motor neuropathy increases the chance of foot tissue damages over the pressure points. However, the neuropathic foot does not ulcerate spontaneously. Rather, it usually associates with a combination of sensory impairment, deformity and trauma.

For diagnostic purpose, people should include at least two of the five abnormality including symptoms, signs, electrodiagnostic test, quantitative sensory, and autonomic testing (Consensus statement 1988; Dyck 1988). Electrodiagnostic test refers to the nerve conduction test. People may demonstrate slowing of nerve conduction velocity owing to demyelination and loss of large myelinated fibers, and a decrease in nerve action potentials owing to loss of axons (Said 2007). Such alteration only happens after involvement of larger myelinated fibers. Those small myelinated and unmyelinated nerve fibers could be detected by skin biopsies in showing reduced density of intraepidermal nerve fibers (Walk 2009). Quantitative sensory testing is the most commonly used method to screen individuals who lose their protective sensation. One recommended way is with a 10-g (5.07) Semmes-Weinstein monofilament. This examination tests an individual’s pressure perception

by pressing filament against the skin of the distal plantar foot in either a 4-point (great toe and base of first, third, and fifth metatarsals) or 10-point (pulp of great toe, third and fifth toes; base of first, third, and fifth metatarsals; two at mid-plantar region, heel pad and dorsal web space between first and second toes) testing sites while with the eyes closed. Inability to perceive 1 to 4 sites is associated with clinically significant large-fiber neuropathy and loss of protective sensation (Singh *et al.* 2005). An alternative quantitative sensory testing method is the vibration perception threshold testing using the calibrated biothesiometer. People having vibration perception threshold values over *25* V are accepted to have neuropathy (Damci *et al.* 1999). For those who have impaired pressure sensation, they may also demonstrate reduced or absent ankle or knee reflexes (Ellenberg 1961). Autonomic dysfunction could be screened by using valsalva maneuver (Vinik *et al.* 2003).

1.3.1.2 Vascular Insufficiency and foot swelling

Impaired blood supply is another significant risk factor for the development of diabetic foot ulcers and subsequently prolongs its wound healing. Diabetes was a risk factor for macrovascular and microvascular complications, and both macro- and microvascular dysfunction may contribute to tissue ischaemia (Gregg *et al.* 2000; Maty *et al.* 2004). The diabetic associated hyperglycemia alters cellular function, damaging the endothelium of the vessel walls. This predisposes patients to plaque build-up and narrowing (American Diabetes Association 2003; Brownlee 2005). Macrovascular changes such as peripheral arterial disease, characterized by arterial stenosis and occlusions, can develop atherosclerotic plaque, which seriously impairs peripheral blood flow that requires early revascularization (Brem *et al.* 2006). It commonly affects the femoral, dorsalis pedis, popliteal, and posterior tibial arteries.

It is anticipated that reduced flow through large arteries leads to diminished flow through small blood vessels and to impaired microvascular regulatory mechanisms (Jacobs *et al.* 1992). It reduces blood perfusion and causes peripheral ischaemia, then subsequently reduces tissue viability. The foot is therefore less tolerable to the mechanical stresses during weight bearing, thereby predisposing to diabetic foot ulceration development (Jeffcoate and Harding 2003). The presence of peripheral vascular disease also leads to poor wound healing and increased risk for amputation.

Apart from the macrovascular dysfunction, it is suggested that microvascular abnormalities may also lead to diabetic foot ulceration. The development of foot ulceration in the presence of strong peripheral pulse in diabetes has led to the hypothesis that microvascular dysfunction plays a significant role in the breaking down of tissues in the diabetic foot. However, the precise mechanisms of this process remain unclear and poorly understood. It has been suggested that the earliest manifestation of microcirculatory disorder is oedema (Zimny *et al.* 2001). Yet, the precise relationship between skin blood flow, oedema and skin morphology in a diabetic foot remains unclear. Microvasculature in the skin comprises nutritive capillaries and thermoregulatory arteriovenous shunt flow. It is regulated through complex interaction of neurogenic and neurovascular control. The interplay among endothelial dysfunction, impaired nerve-axon reflex activities, and microvascular regulation in diabetic patient results in poor wound healing. In people with diabetes especially for those complicated with neuropathy, an increase in foot swelling is a commonly observed feature precede to noticable skin lesions and breakdown. Under normal circumstances, the development of distal interstitial oedema is prevented by the venoarteriolar reflex via limiting the rise in capillary hydrostatic pressure during

leg dependency (Flynn and Tooke 1995). Such a vasoconstriction reflex response is impaired in people with diabetes, especially for those complicated with neuropathy and ulceration (Belcaro and Nicolaides 1991; Belcaro *et al.* 1992; Iwase *et al.* 2007). It has been suggested that skin microvasculature undergoes both morphologic changes as well as functional deficits when parts of the body come under stress or injury. Two important theories that have been put forward to explain the observed abnormalities are the haemodynamic hypothesis and capillary steal syndrome. Details of the microvascular dysfunction in diabetic foot disease and ulceration will be discussed in later part of this chapter.

1.3.1.3 Plantar callus, elevated plantar pressure and limited joint range of motion

Plantar callus and limited joint mobility in the foot have been shown to be associated with high local plantar pressures (Payne *et al.* 2002; Duffin *et al.* 2003). High plantar pressures usually occur at sites with bony prominence. Moreover, the presence of plantar callus has been suggested to be highly predictive of subsequent ulceration (Murray *et al*. 1996). So, all diabetic foot should be screened regularly and any detection of plantar callus should be removed accordingly.

1.3.1.4 Pathological change in plantar skin morphology

The morphological structure of the skin is composed of two layers including a thinner outer epidermis and inner dermis. Epidermis contains keratin and has no blood supply, its nutrition is provided via the papillary layer of the dermis. The dermis consists of papillary and reticular layers of collagen and elastic fibres, blood vessels, sweat glands, hair follicles and nerves. A basement membrane is present between the epidermal-dermal interfaces (Hagisawa and Shimada 2005). It is wavy

in shape with finger-like projections into the dermis, and its structure place a great impact on tissue integrity. In the foot, nutritional capillaries supply blood to the skin and are organized into functional units, with each dermal papilla supplied by one to three capillary loops (Fontaine *et al.* 2006). Capillary endothelium lies on the basement membrane. Blood is supplied to the capillaries through side branching of the smallest arterioles (Tooke *et al.* 1996). The exchange of nutrients and metabolites between blood and tissues occurs at the capillary level. As the skin capillaries are situated just under the reticulin sheet of the papillary layer of the dermis, thus if the basement membrane is thickened, it would compromise the vital exchange of nutrients and collection of waste products (Hagisawa and Shimada 2005; Tooke 1999). Skin thickening has been observed in people with diabetes and has been postulated to be due to the excessive accumulation of advanced glycosylation end products in the collagen of the dermis (Seirafi *et al.* 2009). However, such skin thickening seems to appear only at selected body parts, including the fingers, hands, and upper back region (Paron and Lambert 2000). The morphological changes that take place in the plantar skin of people with diabetes remain unclear. Moreover, the role of the structural changes of the plantar skin tissues in the pathogenesis of foot ulceration, and its interrelationship to the microcirculation and the biomechanical properties of plantar tissue in a diabetic foot are still not well established yet.

1.3.1.5 Pathological change in biomechanical properties of plantar soft tissues

Evidence suggests that diabetic foot injuries may initially develop not entirely on the surface of the skin, but also in deeper layers of plantar soft tissue (Gefen 2003; Thomas *et al.* 2003). The whole plantar soft tissue layers comprises of a complex framework of skin, fat cells, fascia layers, and muscles (Bojsen-Moller

and Flagstad 1976). Each layer of this multilayered composite of tissues has various mechanical properties and the extent to which it deforms in response to external stress may vary. However, the total thickness of the plantar soft tissue composed of various layers can work together and serves as a cushion for optimizing load-bearing during ambulation.

Tissue breakdown of the foot frequently occurs at the plantar aspect of the metatarsal head region (Reiber *et al.* 1998). This area is subject to micro-tears due to the compound stresses of tension, compression, and shearing force during repetitive gait cycles, which make it more susceptible to the breakdown of tissue (Reiber *et al.* 1998). Abnormal foot biomechanics caused by changes in the properties of tissue has been postulated as a key risk factor for the breakdown (Cevera *et al.* 1997; Whitney 2003). It is apparent that foot lesions occur mostly in the older population (Whitney 2003) or in pathological conditions such as diabetes. A substantially stiffer tissue may cause a reduction in the cushioning effect of plantar tissue, hence less shock-absorbing ability during the loading periods of ambulation. It may lead to foot complications such as metatarsalgia or development of ulcers. Nevertheless, as human tissue has the capability to adapt to mechanical loading by changing its structure and composition, an understanding of the quantitative changes in the biomechanical properties of tissue is of crucial importance for many biomedical applications. Understanding these physiological changes may assist in both the prevention and treatment of the diabetes associated foot problems such as ulceration.

In the last decade, methodologies had primarily focus on accessing the plantar pressure in diabetic subjects. It was generally accepted that the plantar

pressure was significantly higher in diabetic persons than non-diabetic persons during walking (Caselli *et al.* 2002; Frykberg *et al.* 1998; Ledoux and Blevins 2007; Pataky *et al.* 2005). In recent years, techniques had been developed to access the mechanical properties of tissue thickness or stiffness. Zheng and Mak (1996) have developed a system that uses ultrasound to measure displacement in tandem with a load cell for force measurement. By using such technique, Zheng *et al.* (2000) found that the plantar soft tissues of the elderly diabetic neuropathy patients were significantly stiffer and thinner than that of the young healthy subjects on the plantar pressure points. Abouaesha *et al.* (2001) used the Plantsan (Toshiba Medical Systems Europe, Zoetermeer, Netherlandds) ultrasound technique to determine tissue depth while standing, and they demonstrated a significant inverse relationship between plantar tissue thickness and peak plantar pressure at all metatarsal heads in diabetic neuropathic patients. By using a computational numerical model of the foot structure, Gefen (2003) also found that the planar pad was significantly stiffer than in the forefoot region in diabetic persons in standing position. Klaesner *et al.* (2002) have developed an indentor device which consisted of a load cell mounted on a 3-dimensional measurement device to record the position and force data. They found that the plantar tissue of diabetic neuropathy subjects over the metatarsal head was significantly stiffer than age-matched control subjects. Previous studies have primarily focused on examining the change in plantar tissue stiffness precede ulcer development. There is a dearth of studies examining the changes in plantar skin morphology and soft tissue properties in subjects with diabetes. In particular, people who have developed complications in the foot are at a higher risk of developing skin problems and foot ulcers.

1.4 Microvascular Dysfunction in Diabetic Foot Disease and Ulceration

Microvascular dysfunction is an integral component of the pathological complications that occur in a diabetic foot. However, the aetiology of microvascular abnormalities in the pathogenesis of diabetic foot ulceration has not yet been established. The development of foot ulceration in the presence of strong peripheral pulse in diabetes has led to the hypothesis that microvascular dysfunction plays a significant role in the breaking down of tissues in the diabetic foot. Lower limb amputation then becomes a possibility which, should it occur, would cause great distress to a patient and imposes a significant burden on health care resources. Therefore, a significant increase amount of research on diabetic foot problems or ulceration is called for.

1.4.1 Types of Diabetic Foot Ulceration and its Causal Pathway

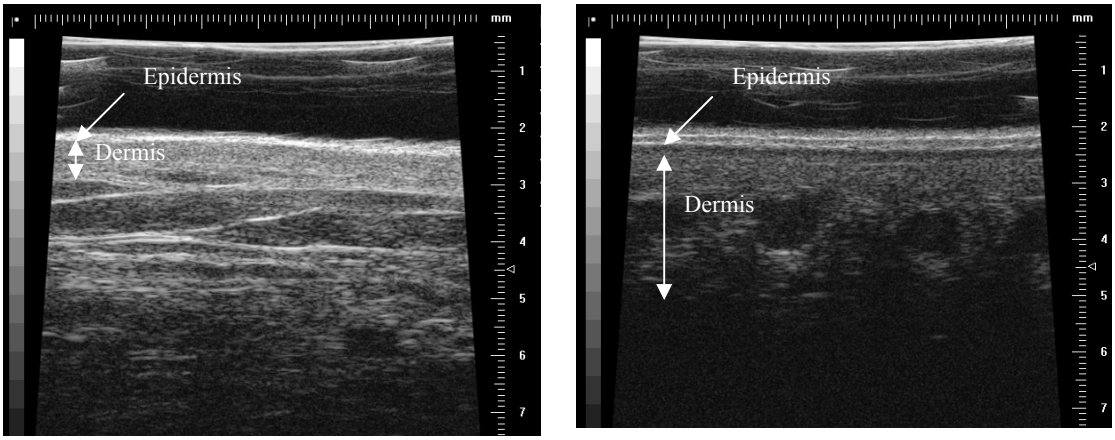
Diabetic foot ulceration can be classified as vascular (10%), neuropathic (40%) or neuro-ischaemic (40%) origin (Boulton; Grunfeld 1992). Vascular ulcer usually refers to the absence of foot pulses, mainly at the posterior tibial and dorsalis pedis, and with an abnormal ankle brachial index of less than 0.9. Neuro-ischaemic ulcer is a further addition of impairment of foot sensation to vascular ulcer (Sandeman and Shearman 1999). Pure neuropathic ulcer is characterized by having no clinical evidence of macrovascular disease, and loss of protective sensation associated with diabetic sensory neuropathy (Brand 1990). The most common causal pathway to diabetic foot ulceration can be identified as the combination of loss of sensation, abnormal foot structure and stress, unperceived trauma and poor management of associated foot injury (Brem *et al.* 2006). Large vessel diseases has been thought as the major cause of ischaemic foot ulceration and early revascularization in diabetes

with peripheral arterial disease are found to reduce risk of associated ulceration and subsequent amputation (Lioupis 2005). While occlusive small vessel diseases has been refuted to exist in diabetic foot (Goldenberg *et al.* 1959), alteration of diabetic foot microcirculation has been postulated to be an important factor in poor wound healing associated with chronic diabetic foot ulcerations (Dinh and Veves 2005). A thorough understanding of the specific microvascular impairment in diabetes is therefore helpful to consolidate concepts of pathogenesis of diabetic foot disease.

1.4.2 Microvascular Abnormalities in Diabetes

Microvasculature abnormalities specific to diabetics include micro-angiopathic complications of the retina, kidneys, skin, and peripheral nervous systems. What these tissues have in common is insulin-dependent intracellular glucose accumulation. Hyperglycemia is the unique central causative factor in vascular abnormalities induced by diabetes, including impaired vascular permeability, vascular tone, and the auto-regulation of blood flow (Dinh and Veves 2005). These changes are accomplished indirectly by the alteration of multiple metabolic pathways. Chronic hyperglycemia leads to structural and functional changes in the nerve microvasculature in people with diabetic peripheral neuropathy, in turn causing reduced endoneurial perfusion and hypoxia (Giannini and Dyck 1995; Malik *et al.* 1989; Malik *et al.* 1992; Yasuda and Dyck 1987). A reduction in the supply of oxygen to nerves and tissues causes a disturbance in the metabolism of cells, which significantly impedes viability of tissues and wound healing process.

1.4.3 Anatomy of Skin Morphology and Microcirculation



(A) (B)

Figure 1.1. Ultrasonic images of human skin showing different skin thickness at different region of the foot. Images are taken by high frequency ultrasound with central frequency at 55MHz (Visualsonic Inc, Vevo 708, Toronto, Canada). (A) Dorsum of the foot (non-glabrous skin). (B) Plantar skin at metatarsal head (glabrous skin).

The morphological structure of the skin is composed of two layers: an outer epidermis and an inner dermis (Figure 1.1). Epidermis contains keratin and has no blood supply, with nutrition provided by the papillary layer of the dermis. The dermis consists of papillary and reticular layers of collagen and elastic fibers. It consists of a microvascular network that provides tissue with nutrients and eliminates waste products (Hagisawa and Shimada, 2005). Skin microcirculation is composed of nutritive capillary blood flow and the thermoregulatory arteriovenous shunt flow. It is organized into two horizontal plexuses: an upper subpapillary plexus and a lower cutaneous plexus (Figure 1.2). The whole vascular network of the skin varies considerably from one area to another. In a normal foot, it has been estimated that 80-90% of total skin blood flow passes through the arteriovenous shunts circulation while the remaining 10-20% passes through the more distal nutritive

capillary bed (Flynn *et al.* 1988). These nutritional capillaries are organized into functional units, with each dermal papilla supplied by one to three capillary loops (Fontaine *et al.* 2006). As the exchange of nutrients and metabolites between blood and tissues occurs at the capillary level, the integrity of the capillary circulation has an impact on the health of the entire skin.

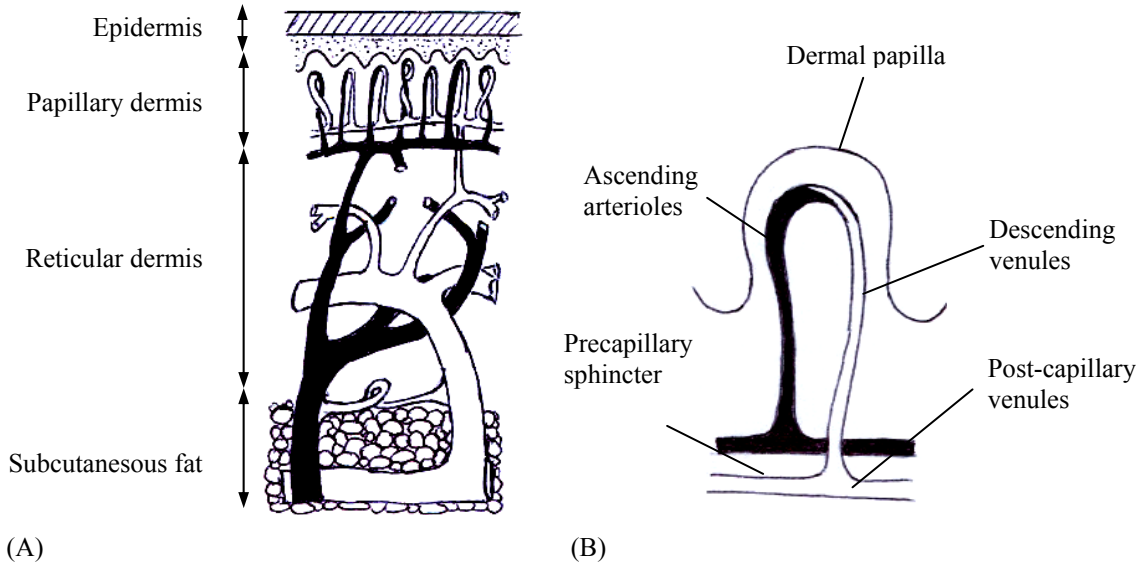


Figure 1.2. Schematic representation of the microvasculature in human skin. (A) An upper nutritive capillaries loops and a lower thermoregulatory arteriovenous shunt circulation in the dermal layer of the skin (B) A single capillary loop inside a dermal papilla.

1.4.4 Neurogenic and Neurovascular Regulation of Skin Blood Flow

Skin blood flow (SBF) is under the regulation of several humoral and neural factors including: (1) central neural reflex control from the long, descending autonomic fibers, (2) short reflex arcs though the spinal cord, and (3) local reflexes within the skin (Coffman and Cohen 1988; Henriksen 1991) and the integrity of endothelium.

1.4.4.1 *Central neural reflex control via sympathetic nerve fibers*

Human cutaneous microcirculation is controlled by both sympathetic adrenergic vasoconstrictor nerves and sympathetic vasodilator nerves (Charkoudian 2003; Kellogg DL 2006). SBF is controlled by the opening and closing of arteriovenous anatomoses and precapillary arterioles. Arteriovenous anatomoses are thick-walled and low in resistance, allowing blood to flow at high rate directly from arterioles to venules. In glabrous (hairless) skin, there are numerous arteriovenous anatomoses that are richly innervated by sympathetic vasoconstrictor nerves, while in non-glabrous (hairy) skin there are very few or even no arteriovenous anatomoses, which are innervated by both sympathetic vasodilator and vasoconstrictor nerves (Table 1.1) (Johnson *et al.* 1986). Only the vasoconstrictor system is tonically active in a thermoneutral environment. Due to the sympathetic tone on the vasculature, the arteriovenous shunts are maintained in a constricted state under normothermic conditions. The loss of this tone due to sympathetic neuropathy may result in the opening of the shunt and cause deviation of blood flow from the skin (Vinik *et al.* 2001).

Table 1.1. The sympathetic vasodilatory and vasoconstrictor systems in the regulation of skin blood flow.

Thermoregulatory systems	Neurotransmitters	Function	Skin innervation
Vasodilatory system			
Sympathetic cholinergic nerves	Nitric Oxide Acetylcholine Vasoactive Intestinal peptide	Dissipate body heat	Non-glabrous skin
Vasoconstrictor system			
Sympathetic adrenergic nerves	Norepinephrine Noradrenaline Neuropeptide Y	Conserve body heat	Glabrous skin & non-glabrous skin

In diabetes, the role of the sympathetic nervous system in the development of diabetes-associated microcirculatory alternations remains unclear. A decrease in sympathetic tone (Hoffman *et al.* 1993; Wiernsperger 2001), an increase in capillary flow and pressure (Flynn *et al.* 1988; Netten *et al.* 1996; Sandeman *et al.* 1992), and an increase in capillary permeability (Jaap *et al.* 1993; Lefrandt *et al.* 2003) has been reported in people with diabetes in different stages. Cacciatori *et al.* (1997) found an almost complete abolition of peripheral sympathetic activity in type 2 diabetic patients with foot ulceration.

1.4.4.2 *Nerve axon reflex mediated vasodilation*

Nerve axon reflex mediated vasodilation is another important mechanism for the regulation of microcirculation. This mechanism is thought to be neurally mediated by nociceptive C-fiber (Cable 2006) (Figure 1.3). C-nociceptive nerve fiber can be stimulated by heat or other noxious stimulus to initiate orthodromic conduction to the spinal cord and antidromic conduction to other axon branches. This in turn leads to the release of local vasodilatory substances such as neuropeptides substance P, bradykinin, adenosine analog ATP, and calcitonin gene-related peptide (CGRP) from the terminals in the skin and tissues that they innervate. Such neuropeptides may either act directly on vascular smooth muscle or indirectly through secondary pathways that include the mast cell release of histamine and sweat gland secretion of bradykinin and vasoactive intestinal polypeptide, to cause vasodilation to the immediate area of the receptive field of particular sensory neurons (Kiernan 1972; Vinik *et al.* 2001). This axon reflex is also known as Lewis triple flare response when induced by antidromic stimulation of sensory nerves. This accounts for 75 to 90% of the dilatory capacity in mostly non-glabrous skin. In

healthy subjects, it was found that the nerve axon reflex-related vasodilation accounts for one-third of the total endothelium-dependent vasodilation at both the forearm and foot level (Hamdy *et al.* 2001). However, the presence of diabetic neuropathy results in a reduction in the percentage contribution of this neurovascular vasodilation to the total response (Hamdy *et al.* 2001). A reduction in sensory neurons for substance P and CGRP has been shown in diabetic patients (Levy *et al.* 1989; Lindberger *et al.* 1989). The nerve axon-reflex related vasodilation has also been reported to be impaired in diabetic peripheral neuropathy patients (Caselli *et al.* 2003; Uccioli *et al.* 1994) or even to be virtually absent in severe cases (Arora *et al.* 2002; Veves *et al.* 1998).

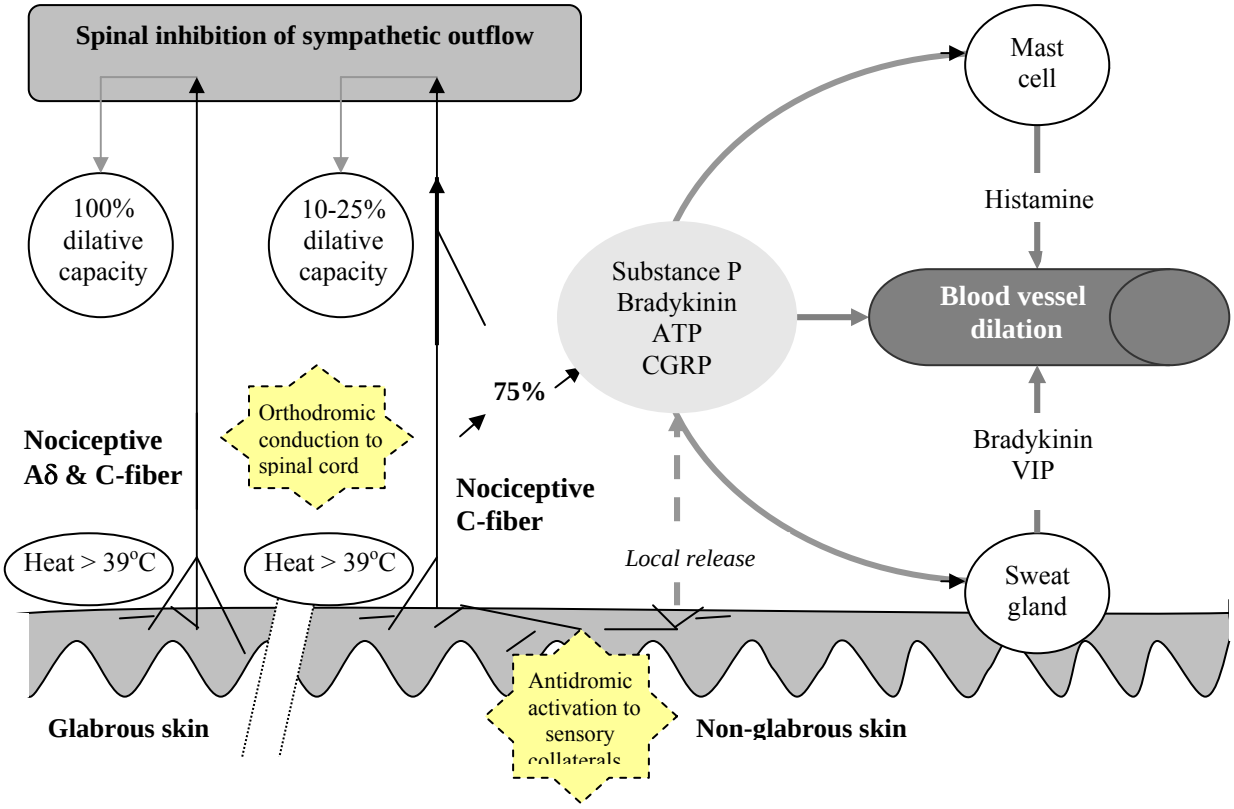


Figure 1.3. The nerve axon reflex mediated vasodilation in glabrous and non-glabrous skin.

1.4.4.3 *Local sympathetic veno-arteriolar axon reflex*

The sympathetically mediated vasoconstrictor reflex is also activated under gravitational stress (Figure 1.4). On standing, venous and arteriolar pressures increase due to the height of the column of blood between the heart and foot, resulting in an increase in capillary pressure in the foot. This could rapidly result in interstitial oedema. The increase in venous pressure also increases precapillary resistance and activates receptors in the small vein due to the force of the stretching on blood vessel walls (Rayman *et al.* 1986a). Oedema is prevented by a local vasoconstrictor reflex mediated by a local sympathetic axon reflex and a myogenic response (Belcaro and Nicolaides 1991; Henriksen and Sejrsen 1976; Rayman *et al.* 1986a). The nerve impulse in the vein is transmitted antidromically along the postganglionic sympathetic sudomotor axon to the branch point, and then orthodromically to the arteriolar, causing vasoconstriction in the precapillary sphincters. The degree of vasoconstriction is directly proportional to the change in the height of the column of blood between the heart and the limb.

Several investigators have shown that this veno-arteriolar reflex (VAR) is reduced in subjects with diabetic autonomic neuropathy, causing oedema and orthostatic hypotension (Belcaro and Nicolaides 1991; Belcaro *et al.* 1992; Golster *et al.* 2005; Iwase *et al.* 2007; Khodabandehlou *et al.* 1997; Yosipovitch *et al.* 1996). Thus, loss of postural regulation of blood flow and raised capillary pressure may have important consequences in the diabetic foot, due to an increase in fluid filtration and oedema formation. These abnormalities may initiate microvascular damage and contribute to the development of foot complications. The impaired VAR

is found in both type 1 (Yosipovitch *et al.* 1996) and type 2 diabetes (Cacciatori *et al.* 1997; Iwase *et al.* 2007; Nabuurs-Franssen *et al.* 2002; Zimny *et al.* 2001b).

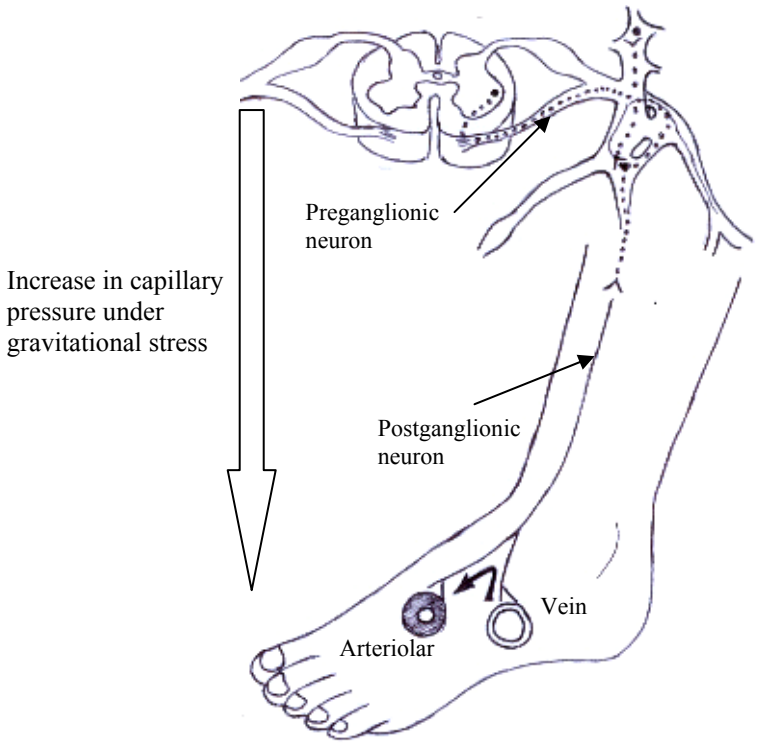


Figure 1.4. The sympathetic venoarteriolar axon reflex: pressure receptor in the vein is activated during leg dependency, and the nerve impulse is transmitted antidromically along the postganglionic sympathetic axon to the branch point, and then orthodromically to the arteriolar, causing vasoconstriction.

1.4.4.4 *Role of endothelium in the regulation of skin blood flow*

Endothelium is a flat monolayer of cells that covers the vascular lumen throughout the body. It is well known that vascular endothelium plays an important role in controlling microvascular tone by synthesizing and releasing several vasodilator substances such as nitric oxide (NO), prostacyclin and endothelium-derived hyperpolarizing factor, and vasoconstrictor substances such as prostaglandins, angiotensin II and endothelin (Fontaine *et al.* 2006; Vane *et al.* 1990). Therefore, an intact functional endothelium is a prerequisite for the regulation of

microcirculatory blood flow. As endothelial function cannot be separated from vascular smooth muscle cell function, evaluations of endothelial function usually include an assessment of both endothelial-dependent vasodilation (vascular endothelial function) and endothelial-independent vasodilation (vascular smooth muscle function). Table 1.2 summarizes some common endothelial-dependent and endothelial-independent vasodilators /vasoconstrictors.

Table 1.2. Examples of endothelium-dependent and endothelial-independent vasodilators/ vasoconstrictors

Types of vasodilatory system	Neurotransmitters
Endothelium-dependent vasodilation	Acetylcholine (Ach)
	Eugenol
	Histamine
	Bradykinin
Endothelium-independent vasodilation	Sodium nitroprusside (SNP)
	Propofol

Endothelial dysfunction has been demonstrated in both type 1 and type 2 diabetes mellitus (Arora *et al.* 2002; Arora *et al.* 1998; Elhadd *et al.* 1999; Johnstone *et al.* 1993; Khan *et al.* 2000; Morris *et al.* 1995; Singh *et al.* 2003; Veves *et al.* 1998; Williams *et al.* 1996). A defect in the synthesis and function of NO has also been observed in the early course of diabetes (Khan *et al.* 2000; Veves *et al.* 1998; Vinik *et al.* 2000). It has been reported that changes in the endothelial function precedes the development of diabetes and is present in the prediabetic stage (Elhadd *et al.* 1999). Although it is well established that endothelium-dependent vasodilation is impaired in both micro- and macrocirculation for patients with type 1 diabetes (Caballero *et al.* 1999; Elhadd *et al.* 1999; Jaap *et al.* 1997), whether there is impairment of endothelium-independent vasodilation in type 1 diabetic patients is a

controversial issue. Singh *et al.* (2003) and Koïtka *et al.* (2004) found impaired endothelial-dependent vasodilation but not endothelial-independent vasodilation in those with type 1 diabetes without secondary complications. The conflicting results that have been observed may be due to differences in the methodological design of those studies and to their small sample sizes.

1.4.5 Structural Microvascular Changes in Diabetes

Various structural abnormalities can be detected in microvasculature as diabetes progresses. The most obvious structural changes are the thickening of the capillary basement membrane, diminished capillary size, and pericyte degeneration (Cameron *et al.* 2001; Jaap *et al.* 1996; Malik *et al.* 1989; Rayman *et al.* 1995; Tesfaye *et al.* 1994; Yasuda and Dyck 1987). Increased hydrostatic pressure and shear forces in the microcirculation are the initial steps in developing thickening in the capillary basement membrane. The postural regulation of blood flow is impaired in diabetes. In particular, the capability of effective precapillary vasoconstriction on standing is reduced. This exposes the capillary bed to a high hydrostatic load, producing oedema and thickening of the capillary basement membrane (Belcaro *et al.* 1992). These stresses are thought to evoke an inflammatory response in the microvascular endothelium, with a subsequent release of extravascular matrix proteins. Over time, this process results in thickening of the basement membrane with arteriolar hyalinosis (Tilton *et al.* 1985). In diabetic foot, thickening of basement membrane is also found in skeletal muscle capillaries (Raskin *et al.* 1983). Thickening of the basement membrane may impair normal transport across capillary walls. Furthermore, the elastic properties of the capillary walls are also reduced, limiting the capacity for vasodilation and hence impairing the normal hyperemic

response to injury (Parving *et al.* 1983; Tooke 1995). The secretory functions of the endothelium are lost in diabetic patients as the disease advances. This, together with a sclerosed basement membrane, physically limits vasodilation, resulting in a failure to meet the metabolic demands of the tissue when the body parts come under stress or injury (Singleton *et al.* 2003).

1.4.6 Functional Microvascular Changes in Patients with Diabetes

Besides structural changes, there are also functional changes in microcirculation in the skin of those with diabetes. These include altered microvascular blood flow, vascular resistance, tissue PO_2, and vascular permeability characteristics. Functional ischaemia is expressed as an impaired ability in the microcirculation function of diabetics to vasodilate in response to stress or injury (Arora *et al.* 2002; Veves *et al.* 1998). Much work has been done in the past decade to investigate why the microvasculature of diabetics fails to respond appropriately to stress and injury. The proposed mechanism is that blood is shunted away from the nutritional capillaries via subpapillary arteriovenous shunts, which give a much lower resistance compared to capillaries. The subpapillary arteriovenous shunts are innervated by sympathetic nerves; therefore the presence of diabetic autonomic neuropathy with sympathetic denervation may lead to the opening of these shunts and result in a mal-distribution of blood flow between the nutritional capillaries and subpapillary vessels. This leads to a reduction in the flow of blood to the capillaries and to the development of diabetic peripheral neuropathy and other diabetic foot complications (Flynn and Tooke 1995b; Malik *et al.* 1989a; Veves *et al.* 1998; Yasuda and Dyck 1987).

A number of different functional disturbances are found in the microvasculature on diabetic subjects, including impaired reactive hyperemia, thermal hyperaemia, and occlusive hyperaemia (Arora *et al.* 2002; Golster *et al.* 2005; Jörneskog *et al.* 1998; Jörneskog and Fagrell 1996; Jaap *et al.* 1994; Jaffer *et al.* 2008; Kasalová *et al.* 2006; Meyer and Schatz 1998; Rayman *et al.* 1995; Rayman *et al.* 1986b; Rendell and Bamisedun 1992; Shore *et al.* 1991; Walmsley *et al.* 1989; Wilson *et al.* 1992). In the case of injury or infection, it is important for hyperaemia response to increase the local blood flow sufficiently to cope with the increase in metabolic waste products. Interruptions of this increased blood flow response coupled with an increase in metabolic demands could create a pathological environment within the microvasculature, especially when the skin is either abnormally stressed or injured. This leads to in an increase risk for diabetics to develop foot ulcerations and delayed healing of ulcers that have already developed.

1.4.7 Haemodynamic Hypothesis

The haemodynamic hypothesis is a possible explanation for microvascular dysfunction in the neuropathic foot (Table 1.3). This hypothesis was first introduced by Parving *et al.* (1983), who suggested that blood flow dysregulation is mediated by hyperglycaemia in the early stage of diabetes. This process is known to stimulate the polyl pathway, which ultimately limits the production of nitric oxide. The result is an increase in microvascular flow and capillary pressure (Sandeman *et al.* 1992), which subsequently induces an endothelial injury response. In a later stage, microvascular sclerosis does occur as a result of the thickening of capillary basement membrane due to long-term structural adaptation and remodeling (Chittenden and Shami 1993; Pham *et al.* 1998). The elasticity of the vessel walls is reduced, which physically

limits vasodilation. This leads to a loss of reactive hyperemia and impaired autoregulation when faced with the demands of changes in the environment. An increase in vascular endothelium permeability was also found, which leads to oedema and a reduction in the supply of nutrients to tissues (Deckert *et al.* 1989).

Table 1.3. Proposed mechanisms for microvascular dysfunction in diabetic foot

Haemodynamic hypothesis	Capillary steal syndrome
● Blood flow dysregulation is mediated by hyperglycaemia ● Stimulate polyl pathway and limits production of nitric oxide ● Increase in microvascular flow and capillary pressure ● Induce endothelial injury response ● Thickening of capillary basement membrane ● Microvascular sclerosis & reduce vascular elasticity which physically limits vasodilation ● Loss of reactive hyperemia ● Increase in vascular endothelial permeability ● Oedema and reduction in nutrient supply	● Sympathetic denervation in autonomic neuropathy ● Loss of vasoconstriction ● Capillary steal: blood is shunt away from capillaries through arteriovenous shunt ● Increase in total peripheral blood flow though arteriovenous anastomosis

Several studies have provided evidences that are supportive to the haemodynamic hypothesis and shown that capillary blood flow and pressure increases in diabetic persons (Flynn *et al.* 1988; Netten *et al.* 1996; Sandeman *et al.* 1992). However, several studies have shown contradictory results. Fagrell *et al.* (1984) demonstrate there was no increase in capillary blood flow among diabetic patients as compared to healthy control subjects, but they reported a delay in the post-occlusive hyperaemia response in the skin capillaries of the diabetic patients.

Similarly, Shore *et al.* (1994) found no difference in nailfold capillary flow pressure between people with type 2 diabetes and healthy control subjects. No correlation was found between capillary pressure and glycemic control, while a negative association between capillary pressure and the duration of diabetes was demonstrated. Therefore, whether there is an increase or decrease in capillary blood flow and pressure in diabetic patients is a question that remains controversial.

1.4.8 Increased Arteriovenous Shunt Flow and Capillary Steal Syndrome

Capillary steal syndrome is another hypothesis that has been put forward to explain microvascular dysfunction in the diabetic foot (Table 1.3). An increase in total peripheral blood flow has been demonstrated in the diabetic neuropathic foot. This is thought to be secondary to peripheral sympathetic denervation with loss of vasoconstriction, which results in an increase in the flow of blood through the arteriovenous shunts. It has been suggested that what then develops is "capillary steal," in which blood is shunted away from the capillaries through these vessels, resulting in reduced nutrition to the skin (Krishnan and Rayman 2006; Uccioli *et al.* 1992). Several studies have been conducted to investigate the arteriovenous shunt flow in patients with diabetes (Boulton *et al.* 1982; Houben *et al.* 1993; Jörneskog *et al.* 1995a; b; Nabuurs-Franssen *et al.* 2002; Rendell *et al.* 1989; Stevens *et al.* 1993). Boulton *et al.* (1982) found an increase in venous oxygenation in the diabetic neuropathic foot, which is consistent with arteriovenous shunting. Uccioli *et al.* (1992) reported an increase in arteriovenous shunt flow in diabetic patients with neuropathy and foot ulcerations, followed by those with neuropathy alone, and then by those without neuropathy. This finding provides evidence that autonomic neuropathy exerts an influence on arteriovenous shunts, which may play a role in the

pathogenesis of the diabetic foot. Houben *et al.* (1993) investigated both the nutritive capillary blood flow and arteriovenous shunt flow in patients with type 1 diabetes. They found an increase in the arteriovenous shunt flow, but the capillary blood flow remained unchanged. Therefore, the majority of these studies demonstrated a reduction in capillary blood flow, but their findings on arteriovenous shunt flow are controversial. Rendell *et al.* (1989) found that the arteriovenous shunt flow was reduced in patients with type 1 diabetes as compared with healthy control subjects, which is contradictory to capillary steal syndrome. Jörneskog *et al.* (1995b) also reported that total skin microcirculation in people with type 1 diabetes was similar to that of the control subjects, whereas the capillary circulation was markedly reduced in diabetic patients both with and without late complications. Further, Jörneskog *et al.* (1995a) found a reduction in capillary blood flow but not in arteriovenous shunt flow in diabetic patients with peripheral vascular disease, indicating that a sufficient amount of blood reaches the area but does not enter the capillaries. Nabuurs-Franssen *et al.* (2002) demonstrated a reduction in capillary blood flow in type 2 diabetic with polyneuropathy, with the reduction being most severe in those with a history of foot ulceration. It is non-conclusive that whether the microvascular impairments observed in diabetic patients can be explained by the haemodynamic hypothesis or are a result of the capillary steal syndrome.

1.4.9 Clinical Implications and Management of Diabetic Foot

The microvascular complications of diabetes such as neuropathy can lead to a loss of sensation and results in developing foot ulceration due to the reduced perception of foot trauma. Neuropathy is the main factor associated with the impaired endothelium-dependent and endothelium-independent vasodilations and

subsequently predisposed to foot ulceration (Veves *et al.* 1998). The reduction or absence of the nerve-axon reflex together with the presence of neuropathy further renders the diabetic foot unable to mount a vasodilatory response when body parts come under stress or injury. It has been proven that glycemic control may prevent or partially reverse neuropathy and modulate neuropathy (Veves *et al.* 2008). So, in the prevention of microvascular disease, good control of hyperglycemia is the most effective intervention. It is recommended that people with diabetes should receive annual somatosensory examination on their level of sensation in the feet. A simple screening examination of the neurological, vascular, dermatological, and musculoskeletal systems are important to identify changes occurring in the high risk foot (Mayfield *et al.* 2004). Prevention and management of the high risk conditions are of utmost importance to manage the high risk foot. Once foot ulceration is developed, it should follow the main principle of good wound care, debridement, adequate off-loading and prompt treatment of infection and moist wound dressings (Dinh and Veves 2006).

1.4.10 Methods for Studying Skin Microcirculation

The concept of measuring skin microcirculation is based on quantifying the optical and thermal properties of the skin. Four measurement parameters including blood flow, blood volume, intracellular oxygenation, and cellular respiration are of particular interest in determining microvascular status (Cobb and Claremont 2002). Blood flow has been considered a primary indicator of haemodynamic status. The light reflected by the skin is due to the multiple scattering of photos by the various elements of the skin structure. Therefore, there is considerable interest in determining the flows of haemoglobin distributed in different plexuses of cutaneous

microcirculation (Berardesca *et al.* 2002). Several techniques can be used in an in vivo study to investigate SBF. They include transcutaneous oxygen tension measurement ($TcPO_2$), photoplethysmosgraphy (PPG), capillaroscopy, laser Doppler flowmetry (LDF), laser Doppler imaging (LDI), and orthogonal polarization spectral imaging (OPS). Table 1.4 summarizes the advantages and disadvantages of each measurement tool.

1.4.10.1 Transcutaneous oxygen tension measurement ($TcpO_2$)

$TcpO_2$ is a non-invasive method that quantifies the oxygen molecules that are transferred out through the skin. These oxygen molecules probably come from skin capillaries as well as subpapillary plexus (Fagrell 1995). Oxygen is capable of diffusing throughout the tissues of the body and skin, although the diffusion rate is very low under normal body temperatures. The application of heat to a localized area of skin in the range of 37^{o}C to 45^{o}C can sufficiently enhance the flow of oxygen through the dermis to allow for non-invasive measurements of capillary oxygen levels (Cobb and Claremont 2002). This is an indirect measure of the amount of oxygen that is delivered from the blood in the total process of skin microcirculation out of the surface of the skin. However, these measurements can be inaccurate because they appear to fluctuate with skin temperature and room temperature. In principle, the measured $TcPO_2$ values can be compared with normal ranges to provide an indication of the nutritional status of the tissues.

1.4.10.2 Photo-plethysmography (PPG)

Photo-plethysmography records the changes in optical intensity in response to changes in the volume of blood in sampled tissue (Challoner 1979; Wright *et al.*

2006). It allows for the measurement of venous blood volume or pulsatile arterial blood volume (Schultz-Ehrenburg and Blazek 2001). This technique requires a light source (red or near infrared light) and a photodetector. When the light enters the skin, it is scattered, reflected, refracted, and absorbed before again reaching the photodetector (Swain and Grant 1989). The degree of absorption is dependent on the volume of blood in the tissue; tissue volume is inversely related to the recorded signal (Allen *et al.* 2002).

1.4.10.3 Capillaroscopy

Capillaroscopy is one of the most sensitive methods for estimating the nutritional status of skin tissues at the microscopic level in vivo. This technique allows for a real-time 2D visualization of the capillaries to evaluate the capillary morphology, capillary density, and capillary blood cell velocity (videocapillaroscopy) (Shore 2000; Wright *et al.* 2006). The capillaries can be studied better in the nailfold than in other parts of the body (Yvonne-Tee *et al.* 2006). In the nailfold, the capillaries may appear as lines, and the whole capillary loop can be visualized as the capillaries are lying parallel to the surface of the skin. In other regions of the skin, the capillaries may appear as black dots as they are lying perpendicular to the surface of the skin (Yvonne-Tee *et al.* 2006).

1.4.10.4 Laser Doppler flowmetry and imaging

Laser Doppler flowmetry (LDF) is a non-invasive continuous measurement of skin microvascular perfusion based on the effect of light on moving red blood cells (RBC) in a restricted volume of tissue (Wright *et al.* 2006; Yvonne-Tee *et al.* 2006). It is the most widely used form of measuring microcirculation. LDF mainly measures

the flow of blood in arterioles, venous plexuses, and arteriovenous anastomises, but only a small part is contributed by the motion of blood cells in the nutritional capillaries (Bongard and Fagrell 1990; Fagrell 1990). It uses a monochromatic low-powered red laser light that is transmitted to the skin through a fiberoptic cable. RBCs moving in the skin backscatter light from the laser, producing a Doppler frequency shift that is sensed by the photodetector (Yvonne-Tee *et al.* 2006). The output from the LDF is referred to a perfusion flux instead of flow, and the response is proportional to RBC velocity and RBC concentration. The extraction of RBC velocity and volume requires mathematical models. It is assumed that the blood cell velocities are randomly distributed in direction. The microvascular perfusion flux is then expressed in terms of arbitrary units and is calculated as the product of the number of RBCs in the sampling volume times the mean velocity, which is proportional to blood flow (Cobb and Claremont 2002; Yvonne-Tee *et al.* 2006).

Laser Doppler Imaging (LDI) is a non-contact device used to map the skin blood flow perfusion (Picart *et al.* 1998). This device is comprised of a helium neon laser, a scanner, an optical detector system, and a computer (Picart *et al.* 1998). It is based on the same principle as LDF, but can be used for scanning tissue areas of various sizes instead of just measuring a single point as in LDF. Once the region of interest is defined, it can be used to monitor and capture blood flow images in minutes. The device can then generate a color-coded perfusion map profile of the defined region, with dark blue representing the lowest and red the highest blood perfusion area (Humeau *et al.* 2007).

1.4.10.5 Orthogonal Polarization Spectral (OPS) Imaging

OPS imaging is a non-invasive technique developed in the last decade to visualize the microcirculation in both the skin and internal organs (Groner *et al.* 1999). It is a handheld device that uses a polarized light technique to examine the absorption of haemoglobin (Groner *et al.* 1999). OPS imaging allows for the in vivo assessment of the microcirculation up to a depth of 3 mm in the skin (Lupi *et al.* 2008). The tissue being investigated is illuminated with linearly polarized light at a wavelength of 538 nm. A large part of the radiation is reflected through a polarizer oriented orthogonally to the plane of the light. Only a small amount of light is able to penetrate to deeper layers, where it undergo multiple dispersion, depolarization, or reissues to the surface (Lupi *et al.* 2008). The degree of penetration of polarized light depends on the density of the examined object and the power of the polarized radiation used. As polarization is preserved in reflection, only photons scattered from relatively deep in the tissue contribute to the images. The microcirculatory parameters obtained by OPS imaging have been demonstrated to be comparable to those obtained by capillaroscopy (Mathura *et al.* 2001).

1.4.10.6 Indocyanin Green Fluorescence Angiography (ICGFA)

ICGFA is a technique for providing evaluation of tissue viability. It uses a low-power laser that incorporated with a charge-coupled camera and indocyanine green to sequence perfusion at the surface of the skin. It is capable to provide real-time capillary fill of tissue perfusion, and hence appears to distinguish between viable and nonviable tissue. Such information could aid to provide information on the extent of viable tissue and the subsequent surgical intervention needed in a diabetic foot. However, the technique is limited by its penetration depth for up to

5mm only. Therefore, examination of deep tissue perfusion may not be applicable with this technology (Perry et al., 2012).

Table 1.4. The advantages and disadvantages of common techniques for measuring skin microcirculation.

Techniques	Advantages	Disadvantages
$TcpO_2$	Cheap and easy to use Relatively free of operator bias	The area and depth of tissue are difficult to characterize Poor spatial resolution Time is required to obtain a stable measure of resting value
PPG	Relatively cheap and easy to use Experienced operators not required Has good prognostic value	Limited spatial resolution Dependent on the optical geometry of the sensor Uncertainty regarding the physiological origin (e.g., skin or muscle)
Capillaroscopy	Has good spatial resolution Can direct to a single capillary Allows for both morphological and blood flow measurement Can measure capillary diameter, length, and density in a specified area	Limited to the peripheral regions Restricted to measurements of nutritive capillaries only Requires high image quality for valid measurements Relatively complex and time consuming
LDF	Relatively cheap and easy to use Does not require specialist training Instrument is well validated	Limited spatial resolution Penetration depth is limited by its wavelength Direct contact between the probe and skin is required Restricted to single point measurement Inability to measure absolute flow values
LDI	Non-contact method Can measure skin blood perfusion in a larger area with better spatial resolution Provides color mapping on skin perfusion	Time is needed to produce the perfusion imaging
OPS imaging	Non-invasive and convenient Allows for blood flow measurement in both the skin and internal organs	It is a contact method technique, which may impede the microcirculation
ICGFA	A minimally invasive assessement tool to measure tissue perfusion, and to determine the extent of viable and nonviable tissue Provide real-time perfusion on capillary fill	Provide perfusion information at a depth of 5mm only. Incapable of assessing deep tissue perfusion

$TcpO_2$: transcutaneous oxygen tension measurement
PPG: photoplethysmosgraphy
LDF: laser Doppler flowmetry
LDI: laser Doppler imaging
OPS: orthogonal polarization spectral
ICGFA: indocyanin green fluorescence angiography

1.5 Classification of Diabetic Foot Ulcers

A number of scoring systems have been developed to describe ulcer characteristics such as the size, depth, and appearance. In addition, parameters such as extent of infection, neuropathy, ischemia, extent of tissue loss and location are also to be included (Karthikesalingam *et al.* 2010). A validated classification system might help in monitoring treatment effectiveness and facilitate accurate and standard documentation that improves communication. Several wound classification systems have been created for diabetes but that there is no universal accepted system for classifying foot ulcers. However, among all, Wagner ulcer classification system and the University of Texas diabetic wound classification system are the most well known one (Frykberg 2002). Wagner ulcer classification system uses six grades (0 to 5) to describes details of the depth of penetration, the presence of osteomyelitis or gangrene, and the extent of tissue necrosis in a wound (Wagner FW 1981). The drawback of the Wagner classification system is that it does not specifically address information on ischemia and infection, which are thought to be important parameters to be mentioned (Karthikesalingam *et al.* 2010). Another scoring system, the University of Texas diabetic wound classification system, uses four grades of ulcer depth (0 to 3) and four stages (A to D), based on depth, ischemia and/or infection to provide information on the depth of ulcer penetration, the presence of wound infection, and the presence of clinical signs of lower-extremity ischemia (Lavery *et al.* 1996). In addition, it also provides information on the presence of devitalized tissue, granulation tissue, exudates and odor. This system benefits from objective criteria for the definition of ischaemia, and improving its reproducibility (Karthikesalingam *et al.* 2010).

1.6 Wound Assessment

Accurate evaluation of the physiologic status of a wound bed and wound edges provides important information to guide for appropriate treatment decisions and for optimal monitoring of treatment effectiveness. So far, there is lack of accurate quantitative methods to examine wound healing. The present documentation of wound healing status mainly restricts to measure wound appearance or dimensions. The most commonly used dimensional methods are the linear measurement, area measurement, and volume measurements. Wound appearance documentation most commonly to be assessed by using digital images or incorporated with some computer software such as Computerized photogrammetry (VERG System, VERG Technologies, Ltd, Winnipeg, Manitoba, Canada) to capture the appearance of a wound (Goldman and Salcido 2002). However, the status of wound healing should not be judged solely by the appearance of the wound. The restoration of the mechanical properties of tissue strength is a potential quantitative outcome of the process of repairing the wound (Gamelli and He 2003). In order to prevent contaminating the wound, it is of the utmost importance to be able to assess the mechanical properties of wound tissues quantitatively in a non-contact way. Thus far, there is a lack of non-contact assessment method for examining the mechanical property of wound tissue.

1.7 Indentation Methods for Characterizing the Biomechanical Properties of Soft Tissues

Several indentation methods have been developed in the past decades to characterize the mechanical properties of the thickness or stiffness of plantar soft tissue. The main difference of these methods comes from the techniques to measure

the displacement of the indenter or the deformation of the tissues. Measurements with three-dimensional position sensors or different indentation speeds through a driving motor have been reported (Klaesner *et al.* 2002; Pathak *et al.* 1998). Imaging techniques such as magnetic resonance imaging system and ultrasound have also been proposed for this purpose (Gefen *et al.* 2001; Hsu *et al.* 2000). However, system portability is always a problem for *in vivo* operations with a bulk measurement device. In order to solve this problem, Zheng *et al.* (2000); Zheng and Mak (1996) have developed a hand-held tissue ultrasound palpation system (TUPS) that utilizes single ultrasound transducer in tandem with a load cell to measure the stiffness of plantar soft tissues in healthy and people with diabetes. The thickness of the tissue can also be obtained with ultrasound measurement (Abouaesha *et al.* 2001; Hsu *et al.* 2002; Zheng *et al.* 2000). Therefore, ultrasound indentation system is one of the most popular indentation techniques used to measure the mechanical properties of plantar soft tissues with its capability of real-time imaging function in recent decades.

1.8 Optical Coherence Tomography (OCT)-based Air-jet Indentation System

In laboratory research, the evaluation of biomechanical properties of soft tissues can be achieved by testing the excised soft tissue specimen using traditional methods of Material Testing Systems such as assessed by tensiometer *in vitro* (Doillon *et al.* 1985; Doillon *et al.* 1988; Hamilton *et al.* 1970; Ksander *et al.* 1977; McGuire 1980; Pickett *et al.* 1996; Williams and Harrison 1977). However, the testing procedure is invasive and disruptive that cannot be performed in clinical studies. With the advances in medical technology, an optical coherence tomography (OCT) based air-jet indentation system has recently been developed (Huang *et al.* 2009). OCT is a

fast developing imaging modality that provides high resolution two-dimensional imaging of internal biological structures by measuring their optical reflection (Huang *et al.* 1991). The air-jet system can be used for the characterization of the biomechanical properties of wound tissue in a non-contact way *in vivo*. This system is comprised of an OCT component to capture the displacement of soft tissue, as well as its stiffness under indentation (Huang *et al.* 2009). The concept of the system is to utilize the optical interferometric technique for obtaining the deformation of the tested tissue during indentation. By using a pressure controlled air-jet as an indenter and OCT-signals to extract the deformation that is induced, it would then be possible to objectively and quantitatively deduce the biomechanical properties of soft tissue.

1.9 Rationale and Objectives of the Studies

Although diabetic foot ulcer can lead to devastating outcome of leg amputation, up to 80% of ulcers are potentially preventable (Reiber *et al.* 1999). The prevention of ulcers among people with diabetes can be achieved by early identifying of risk factors for foot ulcers and underlying pathology involved. Microangiopathy may play a significant role in the pathogenesis of tissue breakdown in a diabetic foot. However, the precise mechanisms of this process remain unclear and poorly understood. Apart from the microvascular dysfunction, pathological changes may take place in plantar skin morphology, plantar soft tissues properties and foot swelling may further increase the risk of foot ulceration in people with diabetes. In order to develop strategies to prevent or manage diabetic ulcer, it is vital to have better understanding on the underlying pathophysiology of the diabetic ulcer. Also, a precise and quantitative evaluation method for ulcer healing is essential in making appropriate treatment decision and monitoring the treatment efficacy. Thus far, there

is a lack of precise quantitative method to assess wound healing or ulcer tissue properties. The restoration of the mechanical properties of wound tissue is an important indicator on the quality of wound healing. Nonetheless, no study has provided quantitative assessment of the biomechanical properties of wound tissue of diabetic foot ulcers *in vivo*. Moreover, the changes in the biomechanical properties of skin wound tissues throughout different phases of wound healing process has not been explored. In laboratory work, the evaluation of wound tissue properties can be achieved by testing the excised wound specimen using the Material Testing Systems *in vitro*. An optical coherence tomography (OCT)-based air-jet indentation system is a novel non-contact method that has been recently developed for characterizing the biomechanical properties of soft tissues in a non-contact way and can potentially be used for assessing wound tissues properties *in vivo*. Therefore, the objectives of the present studies are as follows:

Study 1: To examine the morphological changes in plantar epidermal thickness and soft tissues properties of the diabetic foot

Study 2: To examine the association of skin blood flow and oedema on epidermal thickness in the feet of people with and without diabetes

Study 3: To examine the stiffness of diabetic foot ulcer tissues and to evaluate the test/retest reliability of the newly developed optical-coherence tomography (OCT)-based air-jet indentation system on characterizing the biomechanical properties of wound tissues *in vivo*

Study 4: To examine the biomechanical properties of healing skin wounds *in vivo* using the air-jet indentation system and *in vitro* using the conventional material testing system in a rat model.

Chapter 2 describes the instrumentations used in this study and the results of the pilot study. Chapter 3 compares the epidermal thickness of plantar skin and the total thickness and stiffness of the plantar soft tissues in diabetes with or without complications. Chapter 4 examines the association of skin blood flow and oedema on epidermal thickness in the feet of people with and without diabetes. Chapter 5 reports a novel non-contact air-jet indentation system to assess the biomechanical properties of wound tissue in humans and the test-retest reliability of measuring it. Chapter 6 evaluates the biomechanical properties of healing skin wounds *in vivo* using the air-jet indentation system and *in vitro* using the conventional material testing system in a rat model. Chapter 7 concludes the summary of the findings.

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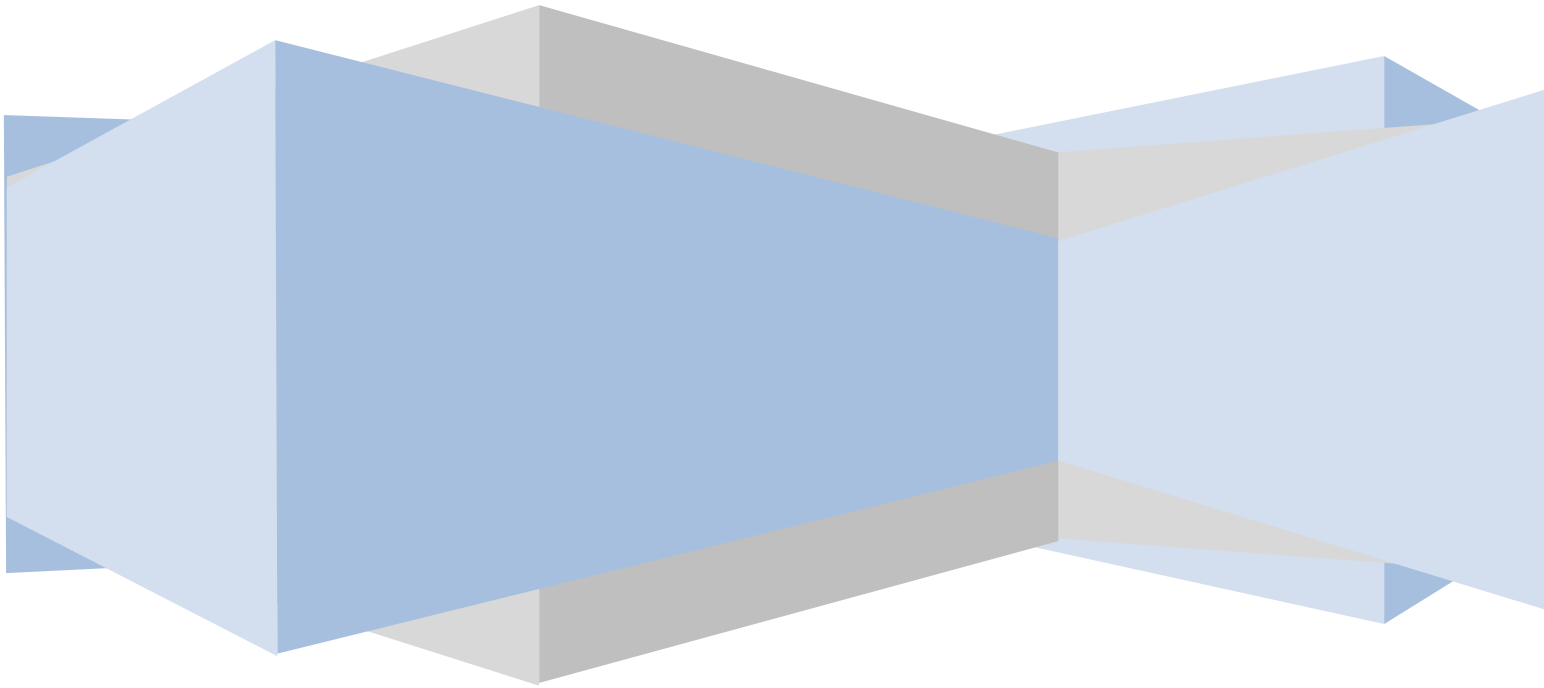
CHAPTER TWO

Instrumentation and Pilot Study

Clare YL Chao



This chapter describes the instrumentation used in this study and the results of the pilot study. Biomechanical properties of plantar soft tissues/ wound tissues were assessed by either the Tissue Ultrasound Palpation System (TUPS), the optical coherence tomography (OCT)-based air-jet indentation system or the material testing system. Skin morphology was measured by using the high frequency ultrasonography. Skin microcirculation was measured by using the laser Doppler flowmetry and the video capillaroscopy. The testing procedures describe below are identical to the studies presented in the following chapters of this thesis.

2.1 Measurements of Biomechanical Properties of Soft Tissues

2.1.1 *Tissue Ultrasound Palpation System*

2.1.1.1 *The thickness and Young's modulus of soft tissues*

An ultrasound (US) indentation system comprised a pen-size, hand-held indentation probe (Zheng 2000) was used for measuring the thickness and Young's modulus of plantar tissue (Figure 2.1). It was shown to have high reliability and validity in previous studies done in the same laboratory (Zheng and Mak 1999; Zheng and Mak 1996). A 5 MHz US transducer with diameter of 9 mm at the tip of the probe served as the indentor. A 10N compressive load cell was connected in series with the US transducer to record the corresponding force response. The thickness and indentation depth of the soft tissue layer were determined from the flight time of the ultrasound echo signal that reflected from soft tissue-bone interface. The deflection of US echo wave due to the tissue deformation was determined with the reference of the echo peak. The sound speed in soft tissues was assumed uniformly to be 1540 m/s (Goss *et al.* 1980). The load signal and US signal were digitized and collected by a computer. A program displayed in real time the response

of load and indentation depth, as well as US signals. The effective Young's modulus was calculated by equation described by Hayes *et al*. (1972).

$$E = \frac{1-\nu^2}{2a\kappa(\nu, a/h)}\frac{p}{W}$$

where P is the applied force, w is the indentation depth, a is the radius of the indenter, v is the Poisson's ratio, h is the tissue thickness, and κ is a scaling factor which is a function of ν and a/h.

With the same design and working mechanism, another small TUPS with a diameter of 3 mm at the tip of the probe that incorporated with a 10 MHz ultrasound transducer was also used for measuring the thickness and stiffness of soft tissues, depends on the measuring sites, in the present study. Details of the procedures were discussed in the validation study in later part of this chapter.

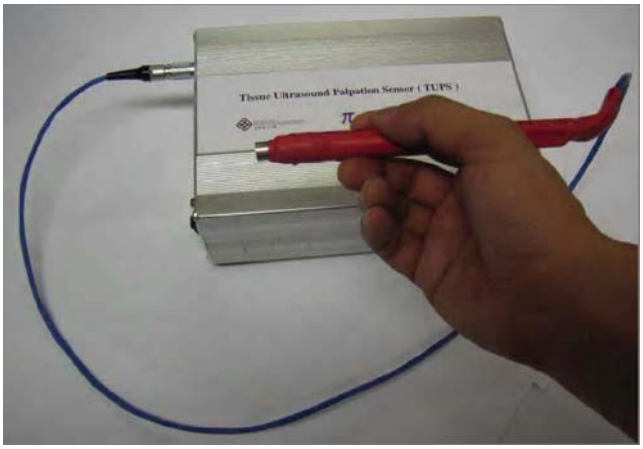


Figure 2.1. The tissue ultrasound palpation system

2.1.2 *Optical coherence tomography (OCT)-based air-jet indentation system*

2.1.2.1 *The stiffness of soft tissues*

A novel non-contact OCT-based air-jet indentation system was used to measure the biomechanical properties of the plantar soft tissues. Details of this system can be found in a previous publication (Huang *et al*. 2009). The system comprised of two parts: the testing probe and the data collection part (Figure 2.2). The probe consisted of a time domain OCT system (developed by the Lab of Optical Imaging and Sensing, Graduate School at Shenzhen, Tsinghua University, China) and an air-jet bubbler. This OCT system provided an axial resolution of 18 μm and an imaging depth of approximately 2 to 3 mm in highly scattered material. The fiber-based OCT probe was modified to allow for the installation of an air-jet bubbler for producing the air-jet. The diameter of the orifice of the air-jet bubbler was 1 mm. The probe was fixed on a rigid stand with an adjustable height. The fine control knobs allowed fine adjustments in the anteroposterior or mediolateral direction so that the laser beam could be accurately adjusted to focus vertically on the skin surface which was around 5 mm below the surface of the bubbler during *in vivo* measurements.

With an advancement of the system as compared to the previous prototype (Huang *et al*. 2009), an electronic proportional valve with pressure feedback (ITV 1030-311L-Q, SMC Corporation, Tokyo, Japan) at a measurement range of 0.5 MPa was installed before the bubbler to continuously control and monitor the pressure of the air-jet by voltage control. Calibration was performed to evaluate the relationship between the force on the tested specimen and the pressure. The relationship was expressed as y = 0.1864x - 0.191 with r^2 equal to 0.9997, where x was the pressure

value read from the software, and y was the force (N). A transparent plate was installed at the top of the bubbler to seal the pressurized air from the OCT components while laser beams could pass through. The data collection part of the system involved the use of in house PC software to collect the OCT and pressure signals. The second function of the software was also to control the operation of the electronic valve. The deformation of the plantar soft tissue during indentation was extracted by a cross-correlation technique to track the displacement of the skin surface.

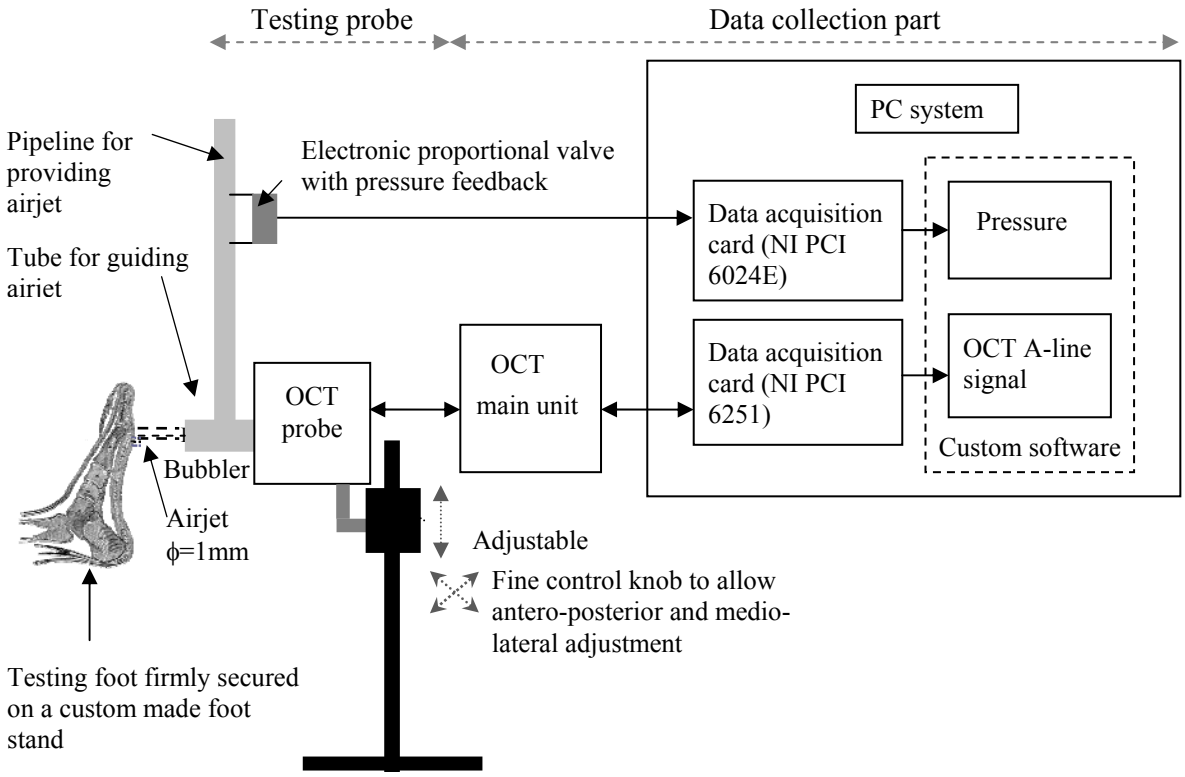


Figure 2.2. The OCT-based air-jet indentation system

Four cycles of loading and unloading with a total duration of approximately 36s was carried out for each indentation trial loading cycle. The stiffness *k* (N/mm) for the force/deformation ratio was then calculated from each test and it was used to

represent the biomechanical properties of the soft tissue. Only the loading phase at the 2nd to 4th cycle was utilized to calculate the stiffness.

$$k = F / d$$

where F was the indentation force and d was the deformation of the tissue. This was obtained by a regression of the force/deformation curve of the indentation.

2.1.2.2 *Test-retest and inter-rater reliability*

An *in-vivo* study was conducted for 10 healthy normal subjects (aged 24 to 34 years) for the measurement of biomechanical properties of the plantar tissues by using the air-jet indentation system. In order to examine the test-retest reliability of this measurement, assessment was conducted by the first operator twice with a time lapse of 1 hour. To evaluate the inter-rater reliability, measurement was first performed by the first assessor, then repeated by the second assessor. The two assessors have received formal training for 2 weeks on the principles and operation procedures of the measurement.

The selected testing site at the plantar tissue was the first metatarsal head. Subjects were asked to sit on a chair with the hip flexed at around 70 degrees and with the knee in slight flexion and ankle in neutral position and supported on a foot stand. Measurement made at the testing site was repeated and the mean of the two trials was recorded.

The ICC and the Pearson correlation were used to evaluate the reliability of the measurements. In this pilot study, the test-retest and intra-rater reliability was, assessed by ICC(1,3) and ICC(3,3) respectively.

A high test-retest reliability (ICC: 0.904; Pearson's correlation: r=0.825, p<0.001) and inter-rater reliability (ICC: 0.870; Pearson's correlation: r=0.770, p=0.002) were demonstrated, indicating that the air-jet system was a reliability tool for the present experimental procedures.

2.1.3 *Material Testing System*

2.1.3.1 *The load relaxation, ultimate tensile strength, ultimate tensile stress, stiffness and energy absorption capacity of soft tissues*

The biomechanical properties of healing skin wound tissues in terms of load relaxation, ultimate tensile strength, ultimate tensile stress, stiffness and energy absorption capacity of soft tissues were evaluated *in vitro* using a material testing system (MTS Synergie 200 machine manufactured by MTS Systems Corporation, Minnesota, USA) (Figure 2.3). Rats were euthanized by intra-peritoneal double dose injection of Ketamine and Xylazine (Alfasan International, Woerden, Netherlands) in a mixture of 100/20 mg per kg of body weight intraperitoneally. The dead bodies were stored in a freezer at -80^{o}C prior to biomechanical testing. At least 6 hours before testing, the dead bodies were thawed in room temperature and the skin wound specimens on both legs were carefully dissected from the wound site to remove all surrounding soft tissues. The remaining skin layer was cut into strips measuring 6.0 mm width × 18.0 mm parallel to the long axis of the tibia. The specimen geometry, including the length, width and thickness was measured using Vernier digital calipers before mechanical testing. The skin at two ends was secured between two pieces of white adhesive labeling tape using quick-setting gel (Henkel Pattex Super Glue), leaving the testing wound tissue (6.0 mm × 6.0 mm) in the middle. Extra care was taken to prevent the glue from running down the skin wound. The glued strips of

skin were then mounted on the clamps of the material testing system from top to bottom, with the distance between each clamps being 6.0 mm. An extensiometer (MTS model no. 634, 13F-24, MTS Systems Corporation) was attached to the interface of the clamps to measure the local displacement of the wound tissue. The room temperature was controlled at around 25^{o}C and the wound portion of the specimen was kept moist with a normal saline solution throughout testing to prevent the skin from drying.

Figure 2.3. The material testing system.

Each specimen was preconditioned with 10 oscillation cycles of 2.5% strain at a rate of 10mm per minute to minimize the deep freeze effect on the wound tissue. After preconditioning, the specimen was elongated to 2.5% strain for 5 minutes and visoelasticity testing was conducted according to Ng *et al.* (2004). Throughout these 5 minutes the loads were recorded at a sampling rate of 5Hz. Unloading then took place for another 5 minutes to allow the specimen to return to its original length. The load relaxation property was measured by subtracting the final load by the initial

load and dividing by the initial load times 100 percent. The failure test was conducted according to the protocol of Ng *et al.* (2011), the specimen was elongated at a rate of 500 mm/min until failure occurred, while the load and displacement data were recorded at a sampling rate of 50 Hz. The load-displacement curve was plotted. The linear portion immediately beyond the toe region represented the structural stiffness of the specimen, while the recorded breaking load divided by the cross-sectional area of the specimen represented the ultimate tensile stress, and the area under the force deformation curve before the breaking point represented the energy absorption capacity.

2.2 Measurement of Skin Morphology

2.2.1 *High frequency ultrasound*

2.2.1.1 *The epidermal thickness and subepidermal low echogenic band*

A high-frequency ultrasound scanner operated at a center frequency of 55MHz scanhead interfaced with a *Vevo* 708 (VisualSonics Inc, Toronto, Canada) was used for imaging skin morphology (Figure 2.4). This frequency gives an axial resolution of 30μm and a lateral resolution of approximately 70μm, producing high-resolution images with a maximum depth of 8mm. The system displays the information obtained in the form of a B-scan in a grey scale image. Ultrasound gel was applied over the measuring sites. The *Vevo* 708 probe was then placed perpendicular to the surface of the skin during the capturing of the image, to minimize the pressure of the transducer on the skin's surface so as to avoid compressing the skin. By using fractal geometry, quantitative data on epidermal thickness and subepidermal low echogenic band thickness was measured by analyzing the change in the echogenicity of the ultrasound image on each sonogram.

Figure 2.4. The high frequency ultrasound machine (*Vevo* 708; VisualSonics Inc, Toronto, Canada).

2.3 Measuring of Skin Microcirculation

2.3.1 Laser Doppler Flowmetry

2.3.1.1 *The skin blood flux*

The plantar skin arteriovenous shunt flow was recorded by laser Doppler flowmetry (DRT4; Moore Instruments, Milway, Devon, United Kingdom) (Figure 2.5). The measurement was carried out in a quiet environment with the room temperature controlled at 24±0.2°C. Cautions were taken to minimize external disturbances. Subjects were positioned in supine comfortably for at least 20 mins to acclimatize the room temperature. The testing site of the skin was cleaned with alcohol prep. The laser Doppler probes was then applied gently on the testing areas to avoid vascular compression using an adhesive pad. Baseline resting skin blood perfusion in arbitrary units, calculated as a product of mean blood cell velocity and

the concentration of blood cells in the recording area, was then monitored continuously for 5 mins in each of the measuring points, and was recorded after stabilization of the output signal in the last 2 mins. All data was stored on a computer for off-line analysis.

Figure 2.5. The laser Doppler flowmetry. (DRT4; Moore Instruments, Milway, Devon, United Kingdom)

2.3.2 *Video Capillaroscopy*

2.3.2.1 *The capillary diameter and capillary blood cell velocity*

Capillary blood cell velocity (CBV) representing skin nutritive blood flow was monitored in the big toe nailfold using CapiScope capillaroscopy (KK Technology, England) (Figure 2.6). Subjects was positioned in supine comfortably with the testing foot placed on a custom made foot stand platform. He/she was acclimatized for 20 min while the CapiScope was positioned. A drop of liquid paraffin oil was applied to the testing area to maximize the translucency of the keratin layer and decrease reflection. Images were subsequently videotaped, coded, and stored through CapiScope videocapillaroscopy analysis software for off-line analysis (KK Technology, England). Assessment of capillary diameter and CBV were then measured. CBV was recorded for 1 min in five different capillaries identified at the

measuring point and the mean value was calculated to yield a single value in the testing area.

Figure 2.6 The video capillaroscopy (KK Technology, England)

2.4 Validation of the OCT-based Air-jet Indentation system by Tissue Ultrasound Palpation System

This pilot study aimed to determine the correlation between findings obtained from the newly developed air-jet indentation system and the use of Tissue Ultrasound Palpation System (TUPS).

2.4.1 Methods

2.4.1.1 Subjects

Thirty healthy subjects (17 male, 13 female) without any foot lesions, orthopaedic foot problems, or diabetes participated in the present study. Their age ranged from 19 to 74 years (mean age: 39.97). The averaged body mass index is 23.09 kg/m^2.

2.4.1.2 Experimental Setup

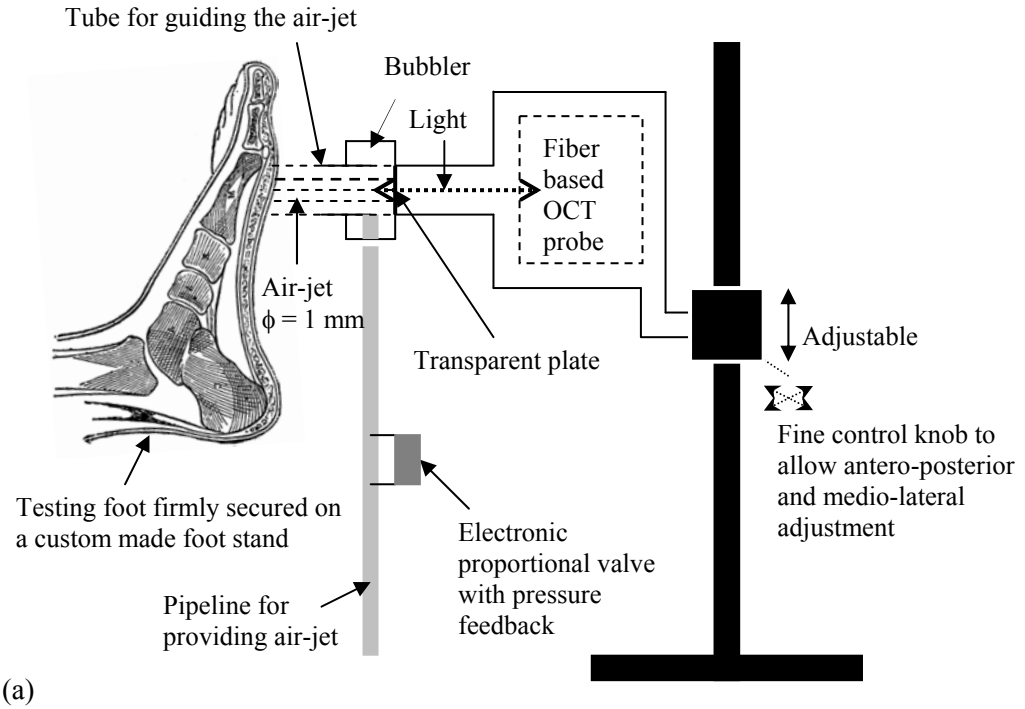
An examination of the biomechanical properties of plantar soft tissue was performed at the 1st and 2nd metatarsal heads (MTH) of the right foot by both the air-jet indentation system and TUPS. All of the participants were positioned comfortably in a sitting position with the testing leg supported on a stool. The testing knee was maintained in a slightly flexed position by a small towel placed underneath the knee, and the ankle was kept in a neutral position by a tailor-made stainless steel ankle foot supporting frame. The location of the testing points of the 1st and 2nd MTHs was marked by a pen to standardize the measurement site for the two measurement tools and between trials. The skin of the tested area was cleaned with alcohol to facilitate ultrasound and penetration of optical signal into the plantar soft tissues. Preconditioning of four loading–unloading trials was performed on each testing point before the actual measurements were taken for each instrument, to ensure that the soft tissue was in a steady state. Two trials of indentation measurements were conducted on each measuring point with each instrument, with a 5-minute rest that allowed for recovery of tested soft tissue between the measurements. The mean of the two measurements was computed for further data analysis. There was 10 minutes rest between the two measurement tools and the sequence of measurement tools was randomized.

2.4.1.3 Instrumentations

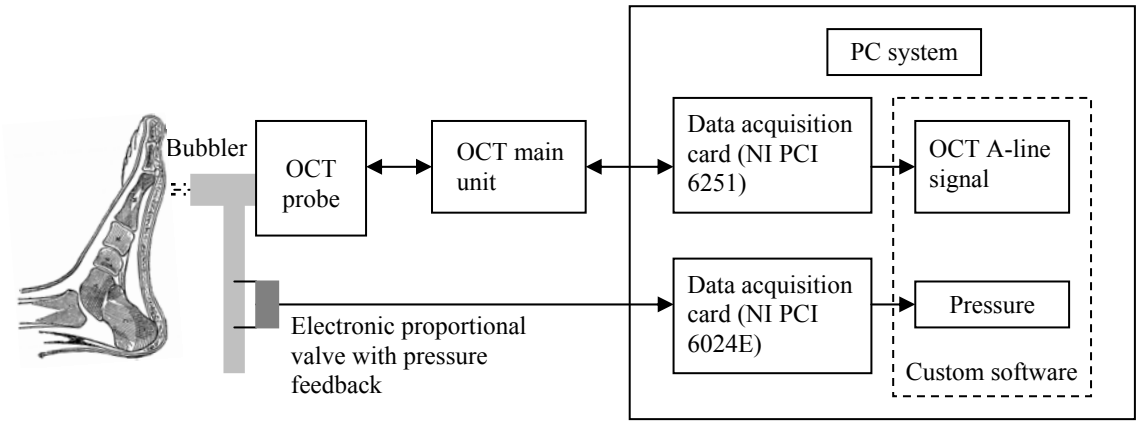
2.4.1.3.1 The OCT-based air-jet indentation system

Detail of the system design was the same as what described above. Figure 2.7 shows a schematics representation of the testing probe and the data collection part of the OCT-based air-jet indentation system. Figure 2.8 shows a picture on the setup of

testing on plantar soft tissue *in vivo* by the air-jet indentation system. An indentation rate of around 0.4 mm/s was carried out for each indentation trial loading cycle. The maximum indentation force used was 1.4 N and it gave a maximum displacement of about 1.6 mm in the softest forefoot plantar tissue being assessed.



(a)



(b)

Figure 2.7. Schematics of the (a) probe and (b) data collection part of the OCT-based air-jet indentation system.

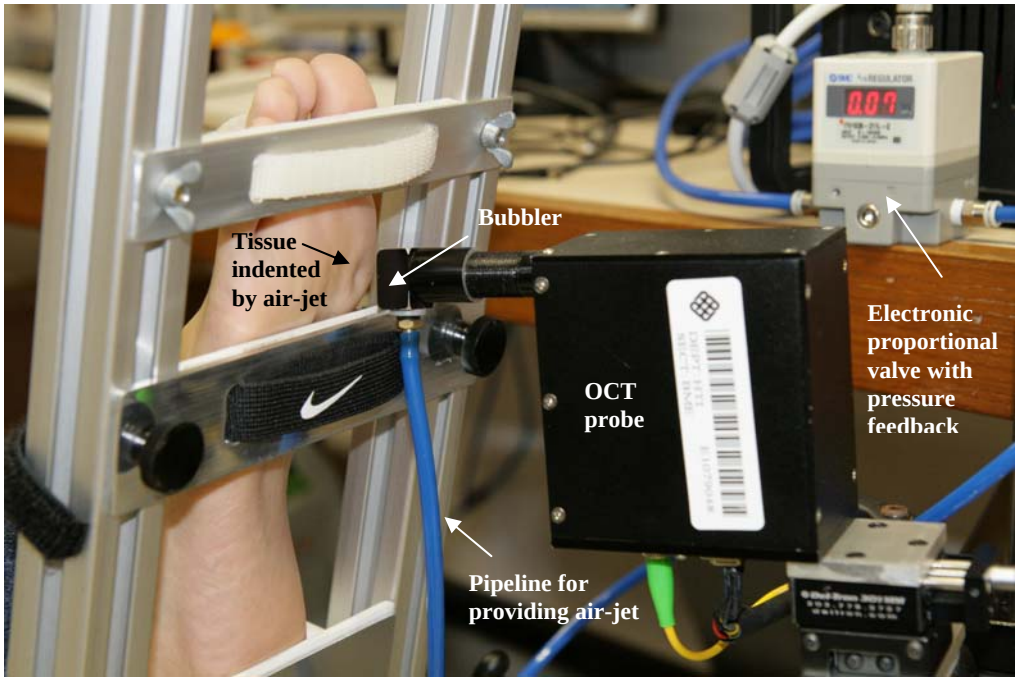


Figure 2.8. A picture shows the setup of testing on plantar soft tissue *in vivo* by the air-jet indentation system.

2.4.1.3.2 Tissue Ultrasound Palpation System

A small TUPS with 3 mm diameter that incorporated with a 10 MHz ultrasound transducer was used to validate the air-jet system. Detail of the system design was aforementioned. Four to five cycles of loading and unloading, with a total duration of approximately 8 s were carried out for each indentation trial to ensure a stable manual indentation rate. The rate was estimated by monitoring the force and deformation curve shown in the computer screen. In comparison with air-jet indentation, the indentation rate used for TUPS measurement was approximately 4 times larger. According to the results of our previous study, the variation of measured modulus was negligible under these indentation rates (Zheng *et al.* 1999). The thickness and deformation of the plantar soft tissue layer were determined from the flight time of the ultrasound echo signal that was reflected from the soft tissue-bone interface.

2.4.1.4 Statistical analysis

Data analyses were conducted using SPSS (version 17.0 for Windows). The Pearson correlation coefficient was used to assess the relationship between the continuous variables of the stiffness *k* (N/mm) as measured by the air-jet indentation system under the 1st and 2nd MTHs and Young's modulus value *E* (kPa) by TUPS under the same sites and also the relationship between plantar tissue thickness and stiffness.

2.4.2 Results

The indentation curve and a typical load-deformation record during the loading and unloading cycle as measured by the air-jet indentation system are shown in Figures 2.9(a) and (b) respectively. The final calculation of the parameter in the loading phases at the 2nd to 4th cycles is shown in Figure 2.9(c). The Pearson's correlation revealed a strong positive correlation (r=0.88, p<0.001) between the stiffness as measured by the air-jet indentation system and the effective Young's modulus as measured by TUPS (Figure 2.10).

2.4.3 Discussion and Conclusion

The present study demonstrated a strong positive correlation of the findings obtained between the air-jet indentation system and TUPS. Therefore, the air-jet indentation system is capable of characterizing the biomechanical properties of plantar soft tissue *in vivo* and can potentially be used in providing quantitative examinations of fine delicate tissue such as wound areas in a non-contact way.

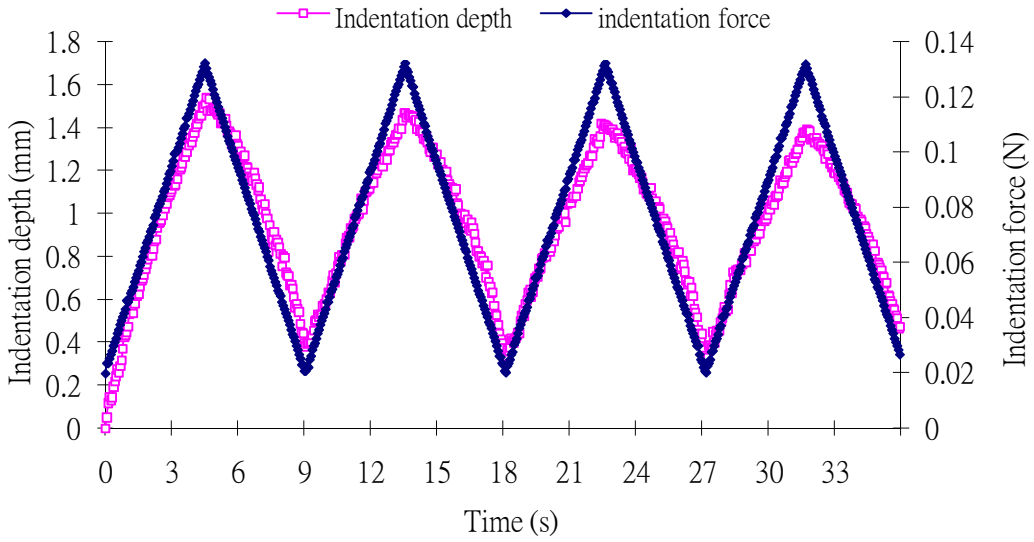


Figure 2.9(a). A typical indentation curve obtained on one subject with four loading and unloading cycles by the air-jet indentation system.

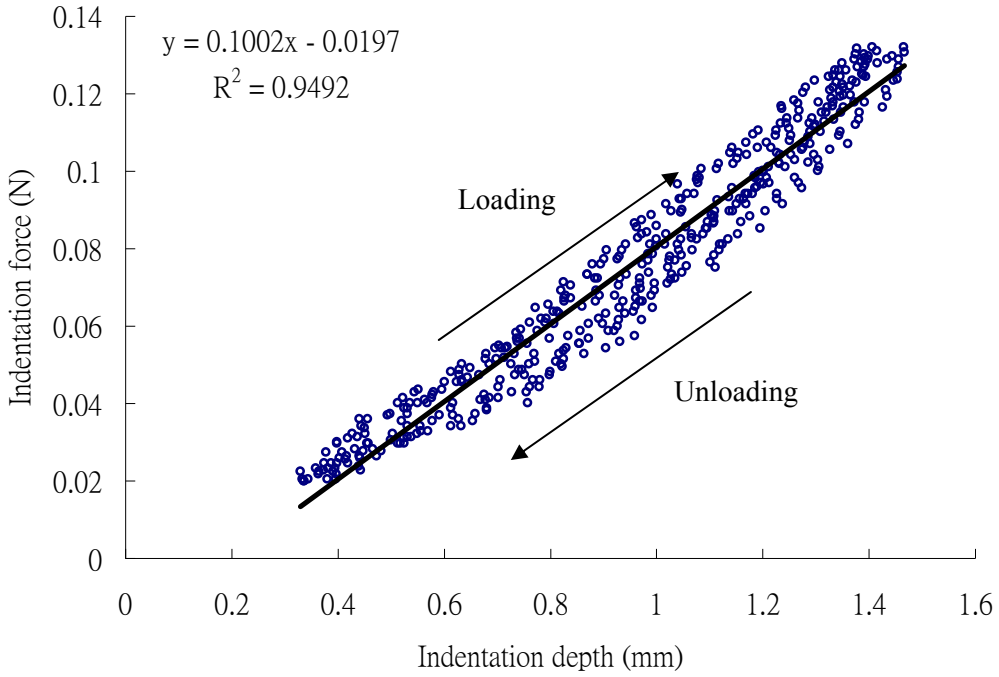


Figure 2.9 (b). A typical load-indentation curve obtained from a subject and the corresponding regression curve by the air-jet indentation system at the 2nd to 4th indentation cycles.

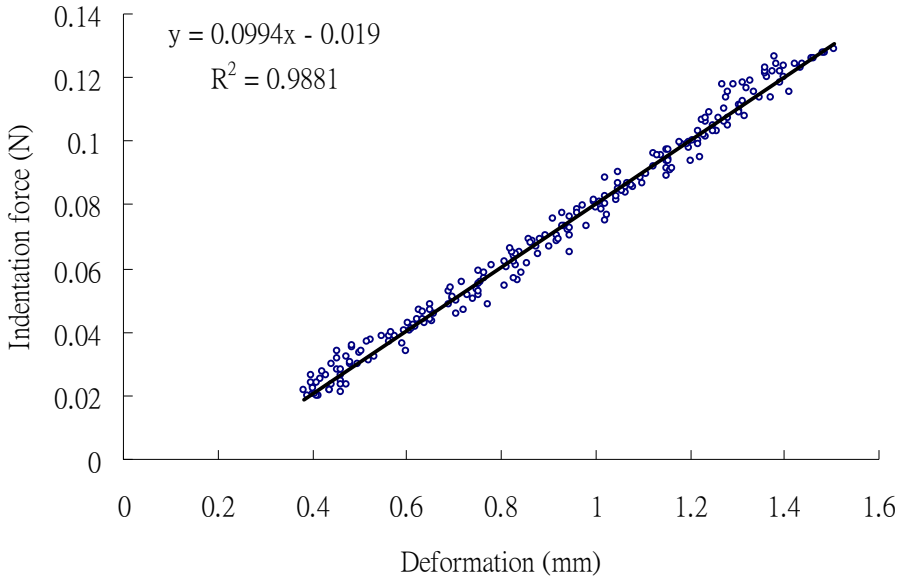


Figure 2.9 (c). The selected data points used for estimation of stiffness *k* (N/mm) of plantar soft tissues. The data in the loading phases at the 2nd to 4th cycle were selected. The relationship between the force measured within the air-jet and the relative deformation of the plantar tissues is shown in the corresponding regression curve.

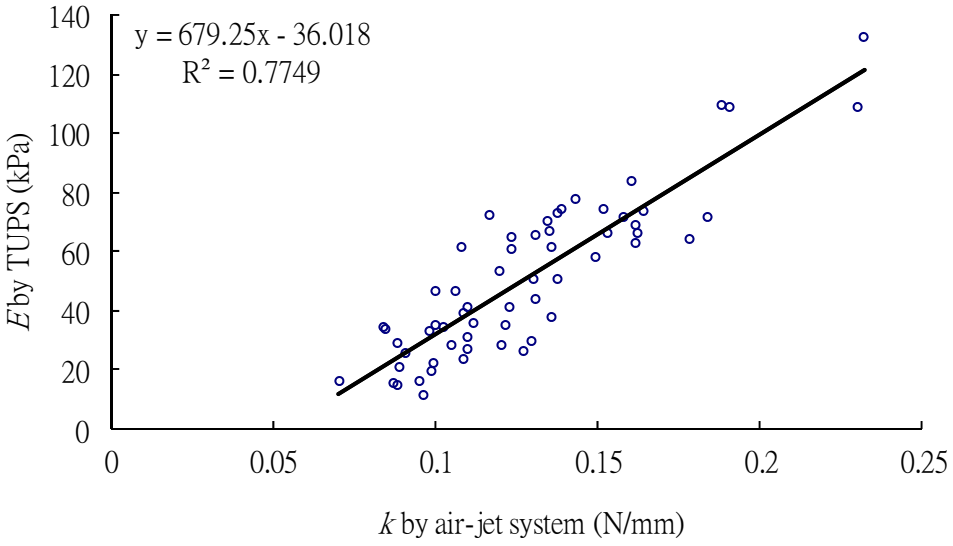


Figure 2.10. The correlation between the Young's moduli of the plantar tissue mechanical properties determined using the tissue ultrasound palpation system and the stiffness measured by the air-jet indentation system.

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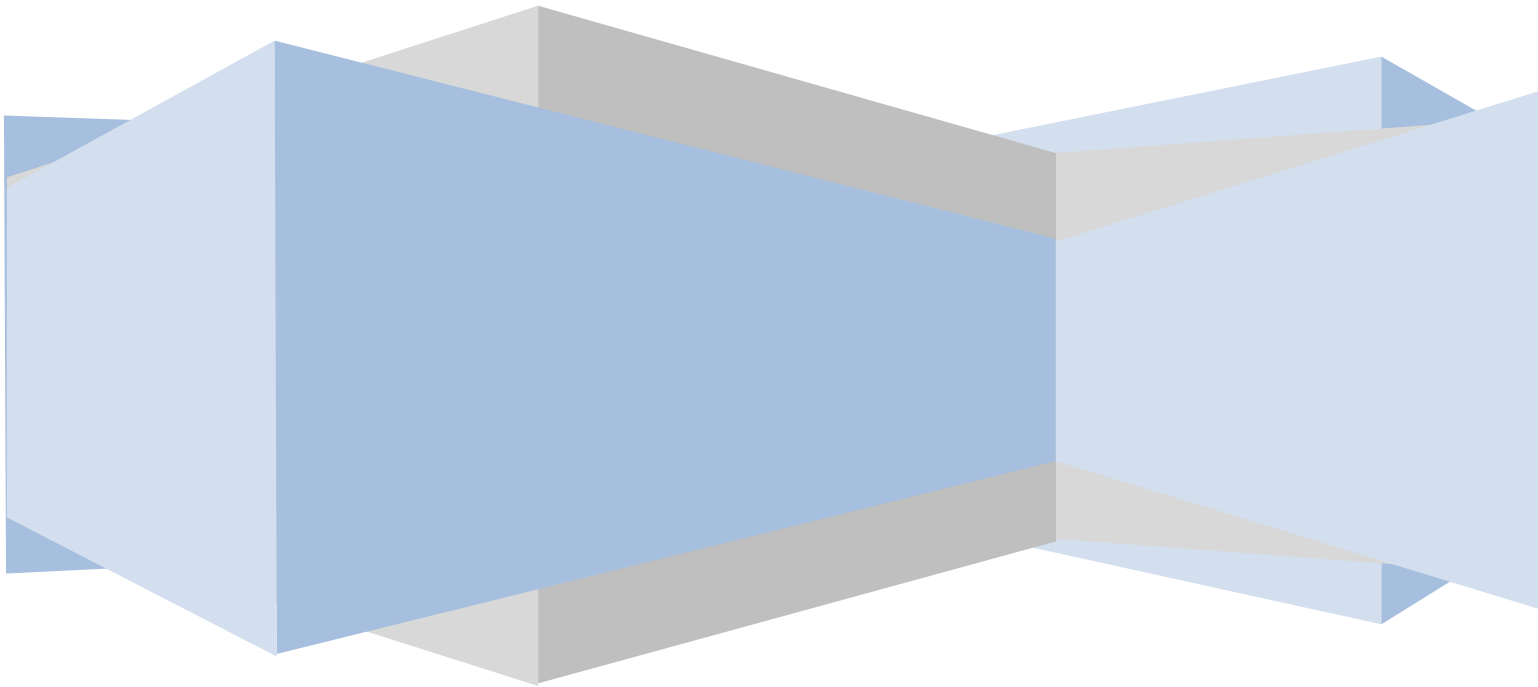
CHAPTER THREE

Epidermal Thickness and Biomechanical Properties of Plantar Tissues in the Diabetic Foot

Clare YL Chao



3.1 Abstract

Diabetic foot is a common complication for people with diabetes but it is unclear whether the change is initiated from the skin surface, or underneath plantar tissues. This chapter compared the thickness of epidermis, the thickness and stiffness of the total plantar soft tissue among people with diabetes with or without complications. Seventy-two people with diabetes, including 22 people with neuropathies, 16 foot ulcerations, 34 pure diabetics without complications; and 40 healthy controls participated in the study. The thickness of the epidermal layer of the plantar skin was examined using high frequency ultrasonography; the thickness and stiffness of the total plantar soft tissue were measured by using Tissue Ultrasound Palpation System at the big toe, first, third, and fifth metatarsal heads, and heel pad. As compared with the control group, the average epidermal thickness of plantar skin was reduced by 15% in people with diabetic foot ulceration and 9% in people with neuropathy, but increased by 6% in pure diabetics. There was a 8% increase in total thickness of plantar soft tissue in the 3 diabetic groups at all testing sites (all $p<0.05$), except the first metatarsal head. The stiffness of plantar soft tissue was increased in all diabetic groups at all testing sites as compared with the control (all $p<0.05$). The epidermal plantar skin is getting thinner and plantar soft tissues are stiffened for people with diabetes, particularly for people who have neuropathy or ulceration, which increases the risk of tissue breakdown and ulceration formation.

3.2 Introduction

Foot disorder is one of the major complications of diabetes mellitus that constitutes to 50% of all diabetes-related hospital admissions (Smith *et al.* 2004). The common foot problems include delayed wound healing, infection, gangrene and ulceration, of which foot ulceration are the most devastating one. It happens in 15% of diabetic people and precedes 85% of all non-traumatic lower limb amputations (Reiber *et al.* 1999). The predisposing factors for diabetic foot ulceration include neuropathy, vascular insufficiency, plantar callus, elevated plantar pressures and limited joint range of motion (Bennett *et al.* 1996; Fernando *et al.* 1991; Mueller *et al.* 2005). Local changes in skin morphology and biomechanical properties of plantar soft tissue may further increase the risk for foot ulcer.

Plantar skin is the primary site of physical interaction between a person and the ground. It undergoes various stresses during activities of daily living and acts as the first line of biological barrier in protecting the foot from mechanical trauma. Any pathological changes of it places an important impact on tissue viability and hence tissue breakdown. However, evidences suggest that the process of injury in diabetic feet may not initiate entirely on the skin surface, but also in deeper plantar soft tissue layers (Gefen 2003; Thomas *et al.* 2003). The whole layer of plantar soft tissues is responsible for withstanding the dynamic loads of the whole body during ambulation. It comprises of a complex framework of skin, fat cells, fascia layers, and muscles. These tissue layers work together and serves as a cushion for optimizing load-bearing during ambulation. How the local changes in skin morphology interact with the changes in biomechanical properties of the plantar soft tissues layers and hence the overall cushioning effects of the whole layers of plantar soft tissues in a diabetic

foot remains unclear. Therefore, it is worthwhile to examine both parameters together in people with diabetes.

Morphologically, skin is composed of an outer thinner epidermal and an inner thicker dermal layer (Boulais and Misery 2008). The epidermis is primarily consists of layers of densely packed keratinocytes that produce the protein keratin, which serve as a barrier and give resistance to physical wear and tear and makes skin waterproof (Hagisawa and Shimada 2005). The dermis consists of papillary and reticular layers of collagen and elastic fibres to provide mechanical strength, extensibility, and elasticity. A basement membrane is present between the epidermal-dermal interfaces (Hagisawa and Shimada 2005). Skin thickening has been observed in people with diabetes and has been postulated to be due to excessive accumulation of advanced glycosylation end products in the collagen of the dermis (Seirafi *et al.* 2009). However, such skin thickening seems to appear only at selected body parts including the fingers, hands and upper back region (Paron and Lambert 2000). Several groups have found an increase in skin thickness of the forearm among people with type 1 diabetes as compared to a control group (Collier *et al.* 1986; Hanna *et al.* 1987). Very few studies have reported the detail morphological changes of the skin in diabetic foot. Using ultrasonography imaging, Huntley and Walter RM (1990) demonstrated an increased total skin thickness on the dorsum of the feet but not at the back of the trunk for people with diabetes. Duffin *et al.* (2002) did not find any increase in total thickness of skin at the plantar surface of the foot among young diabetics. Conversely, Hashmi *et al.* (2006) demonstrated that the plantar epidermal thickness was significantly thicker in type 2 diabetes without neuropathy as

compared to healthy subjects. The morphological changes take place in the plantar skin for people with diabetes remain unclear.

Several studies have investigated the changes in biomechanical properties of plantar soft tissues in diabetic foot but controversial findings on stiffness and thickness of plantar tissues have been reported. Klaesner *et al.* (2002) found that the plantar tissue over the metatarsal head for people with diabetic neuropathy was significantly stiffer than age-matched control subjects. Zheng *et al.* (2000) suggested that the soft tissues on specific plantar pressure points for elderly people with diabetic neuropathy were significantly stiffer and thinner than that of the young healthy subjects. Duffin *et al.* (2002) reported that the plantar aponeurosis was significantly thicker in the people with diabetes. Conversely, Robertson *et al.* (2002) did not demonstrate any significant difference in the plantar soft tissue thickness beneath the metatarsal heads between individual with diabetes and healthy controls.

Prevention of foot problem is an essential goal for people with diabetes. There is a lack of studies that have examined the changes in plantar skin morphology and soft tissue properties in subjects with diabetes. In particular, people who have developed complications in the foot are at higher risk to develop skin breakdown and form foot ulcer. The present study aimed to examine the changes in epidermal thickness and biomechanical properties of plantar soft tissue in people with type 2 diabetes mellitus with or without neuropathy and ulceration.

3.3 Material and Methods

3.3.1 Participants

Seventy-two people with type 2 diabetes with or without complications, and 40 non-diabetic healthy control subjects participated in the present study. All diabetic subjects were recruited from the diabetic clinic of a local hospital. All diabetic subjects had the confirmed medical diagnosis of types 2 diabetes mellitus. The group with diabetic peripheral neuropathy was identified by using the 10g Semmes-Weinstein monofilament (unable to feel for 5 or more out of the 10 testing points) and vibration perception threshold of above 25 volts. The group with foot ulcerations either had history of previous or present diabetic foot ulceration below the malleoli level. Healthy control subjects who had no history of diabetes or any other forms of neuropathy and arterial diseases were recruited from the community. All of them passed the 8-hour fasting glucose test. Subjects were excluded if they have peripheral vascular disease as determined by the absence of both posterior tibial and dorsalis pedis pulses and present of intermittent claudication or symptoms and with ankle brachial index (ABI) smaller than 0.9, unstable cardiac condition, or malignancy.

The foot with history or present foot ulceration was tested. If there was no history of diabetic ulcer, the right foot was tested. If the ulcerated foot had partial amputation or the presence of any skin lesions on the measuring points, the contralateral foot was tested. Ethical approval was obtained from a local university and hospital. A written consent was obtained from each subject.

3.3.2 Epidermal Thickness Measurement

A high frequency Ultrasound scanner operated at a center frequency of 55MHz scanhead interfaced with a Vevo 708 (*VisualSonics* Inc, Toronto, Canada) was used for imaging skin morphology. Details of the system design was same as

what described in Chapter 2. A total of ten ultrasound biomicroscopy scans were performed on the skin of the right foot in five selected sites including the pulp of big toe, first, third, fifth metatarsal heads, and heel pad, with two images captured at each measuring site. By using fractal geometry, quantitative data on epidermal thickness was measured by analyzing the change in echogenicity of the ultrasound image on each sonogram. The mean of the measurements for the two images obtained at each testing point was used for data analysis.

3.3.3 Measurement of Thickness and Stiffness of the Total Plantar Soft Tissue

A Tissue Ultrasound Palpation System (TUPS) comprised of a pen-size, hand-held indentation probe was used for measuring the thickness and stiffness of total plantar soft tissues. Details of the system design was same as what described in Chapter 2.

During the measurement, all subjects were positioned in supine lying position with the knees supported by a small towel. An ankle foot orthosis was used to support the lower leg and ankle of the subjects and maintained the ankle in a neutral position. A coupling gel was applied on the testing site. The selected testing sites included the pulp of big toe, first, third, fifth metatarsal head, and heel pad. Two trials of indentation measurements were done at each measuring site and the mean was obtained for subsequent data analysis.

3.3.4 Statistical analyses

One-way analysis of variance (ANOVA) was used to test for the demographic variables and all testing variables between the study groups. When significant results

were detected, the analysis of variance was then followed by Hochberg's GT2 *post hoc* multiple comparisons if equal variance assumed. If homogeneity of variance was violated, the F-statistics was adjusted by using Brown-Forsythe test. Games-Howell *post hoc* multiple comparisons was used if equal variance not assumed and when significant ANOVA effect was detected. The level of significance was set at 0.05 for all measurements.

3.4 Results

A total of 72 people with type 2 diabetes, including 22 with peripheral neuropathies (DPN) (15 males and 7 females, aged 67.77±8.79 years), 16 with history or present foot ulcerations (DU) (13 males and 3 females, aged 60.29±14.19 years), and 34 pure diabetics without complications (DM) (15 males and 19 females, aged 64.38±9.63 years); and 40 non-diabetic healthy controls (14 males and 26 females, aged 67.07±9.44 years) were included in the study. There were no differences in age, body mass index, diastolic blood pressure, and ankle brachial index found between the groups. As expected, the fasting glucose was significantly higher in the diabetic groups than the non-diabetic counterparts (p=0.002). Subjects in the ulcer group demonstrated a higher systolic blood pressure than pure diabetic and normal groups (p=0.007). In addition, patients with sensory peripheral neuropathy and history or present foot ulceration had a higher vibration perception threshold than pure diabetics and non-diabetic control subjects, (p<0.001). Among the three diabetic groups, no significant differences were observed among the duration of diabetes and glycated haemoglobin. Details of the subject demographics are shown in Table 3.1.

Table 3.1. Subject characteristics

Group characteristics	DU (n=16)	DPN (n=22)	DM (n=34)	Normal (n=40)	*p*-value
Male/Female	13:3	15:7	15:19	14:26	0.004
Age (yr)	60.29±14.19	67.77±8.79	64.38±9.63	67.07±9.44	0.067
Body mass index (kg/m^2)	25.25±3.68	25.29±4.30	24.63±3.98	23.01±3.54	0.059
Systolic blood pressure (mmHg)	152.06±21.91	142.81±24.95	132.55±15.10	133.93±14.14	0.007†
Diastolic blood pressure (mmHg)	83.17±8.35	76.04±14.31	77.33±10.33	78.77±8.97	0.166
Fasting glucose (mmol/L)	6.72±2.70	6.87±1.97	7.33±2.14	5.54±0.67	0.002*
Glycated haemoglobin (HbA_1c) (%)	7.37±1.89	6.93±1.32	7.23±1.14	-	0.618
Duration of diabetes (years)	14.74±12.31	13.58±9.14	8.94±7.28	-	0.106
Vibration threshold	31.93±10.35	35.44±8.37	16.23±5.30	15.75±6.74	<0.001‡
Ankle brachial index (Left)	1.12±0.11	1.08±0.11	1.10±0.11	1.12±0.09	0.420
Ankle brachial index (Right)	1.14±0.14	1.10±0.13	1.15±0.13	1.15±0.11	0.380

Data are mean±SD; *$p<0.05$

DU: diabetic ulcer; DPN: diabetic peripheral neuropathy; DM: diabetes without complications

*Normal group vs ulcer, neuropathy and pure diabetic groups

†Ulcer group vs pure diabetic and normal groups

‡Normal group vs neuropathy and ulcer groups

Table 3.2. The epidermal thickness at different region of plantar foot as measured by high frequency ultrasonography.

Epidermal thickness (mm)	DU (n=16)	DPN (n=22)	DM (n=34)	Normal (n=40)	*F*-ratio	*p*-value
Pulp of big toe	0.43±0.14	0.45±0.11	0.55±0.18	0.51±0.17	3.377	0.022‡
1st MTH	0.51±0.16	0.51±0.17	0.56±0.18	0.55±0.19	0.549	0.650
3rd MTH	0.51±0.17	0.56±0.18	0.66±0.15	0.62±0.15	3.829	0.012*
5th MTH	0.52±0.19	0.58±0.17	0.68±0.14	0.61±0.13	4.765	0.004*
Heel pad	0.53±0.15	0.58±0.14	0.68±0.15	0.66±0.13	5.702	0.001†

Data are mean±SD

DU: diabetic ulcer; DPN: diabetic peripheral neuropathy; DM: diabetes without complications

*Ulcer group vs pure diabetic group

†Ulcer group vs pure diabetic and normal groups

‡Pure diabetic group vs neuropathy and ulcer groups

3.4.1 Epidermal Thickness of Plantar Skin

The epidermal thickness of plantar skin was significantly different among people with or without diabetes or diabetic-related complications at the pulp of big toe, $F(3, 108)=3.38$, $p=0.022$; third metatarsal head $F(3, 108)=3.83$, $p=0.012$; fifth metatarsal head, $F(3, 108)=4.77$, $p=0.004$; and heel pad region, $F(3, 108)=5.80$, $p=0.001$ (Table 3.2). Post-hoc analyses indicated that the average epidermal thickness of plantar skin was significantly thicker in pure diabetes without complications but thinner for those with neuropathy and foot ulceration, as compared to the control (Figure 3.1 and 3.2). No significant difference of epidermal thickness was observed between groups at the first metatarsal head ($F(3, 108)$, $p=0.650$), but still follow the similar trend with the greatest thickness found in the pure diabetes followed by control subjects. On average, the epidermal thickness of plantar skin was reduced in diabetics with foot ulceration by 15% and those with neuropathy by 9% but increased in people with pure diabetics by 6% as compared with healthy control subjects.

3.4.2 Thickness and Stiffness of Total Plantar Soft Tissue

Significant differences in thickness of total plantar soft tissues was found among the study groups at all testing sites except the first metatarsal head. The thickness of plantar soft tissue were increased in all diabetics as compared with healthy control at the pulp of big toe $F(3, 108)=4.66$, $p=0.004$; third metatarsal head $F(3, 108)=4.41$, $p=0.006$; fifth metatarsal head, $F(3, 108)=3.69$, $p=0.014$; and heel pad region, $F(3, 108)=3.40$, $p=0.020$ (Table 3.3). Figure 3.3 showed the post-hoc analyses among the testing sites and groups. On average, the thickness of plantar soft tissue was increased in diabetic groups by 8% at all testing sites. In addition, the stiffness of

plantar soft tissue was greater in all diabetic groups at all testing sites (pulp of big toe $F(3, 108)=3.79$, $p=0.012$; first metatarsal head $F(3, 108)=3.13$, $p=0.029$); third metatarsal head $F(3, 108)=3.98$, $p=0.010$; fifth metatarsal head, $F(3, 108)=5.14$, $p=0.002$; and heel pad region, $F(3, 108)=5.08$, $p=0.003$), to a greater extent to those with foot ulceration who demonstrated an average increase in stiffness by 30%, followed by neuropathy and pure diabetes (20%) as compared with the healthy controls (Figure 3.4).

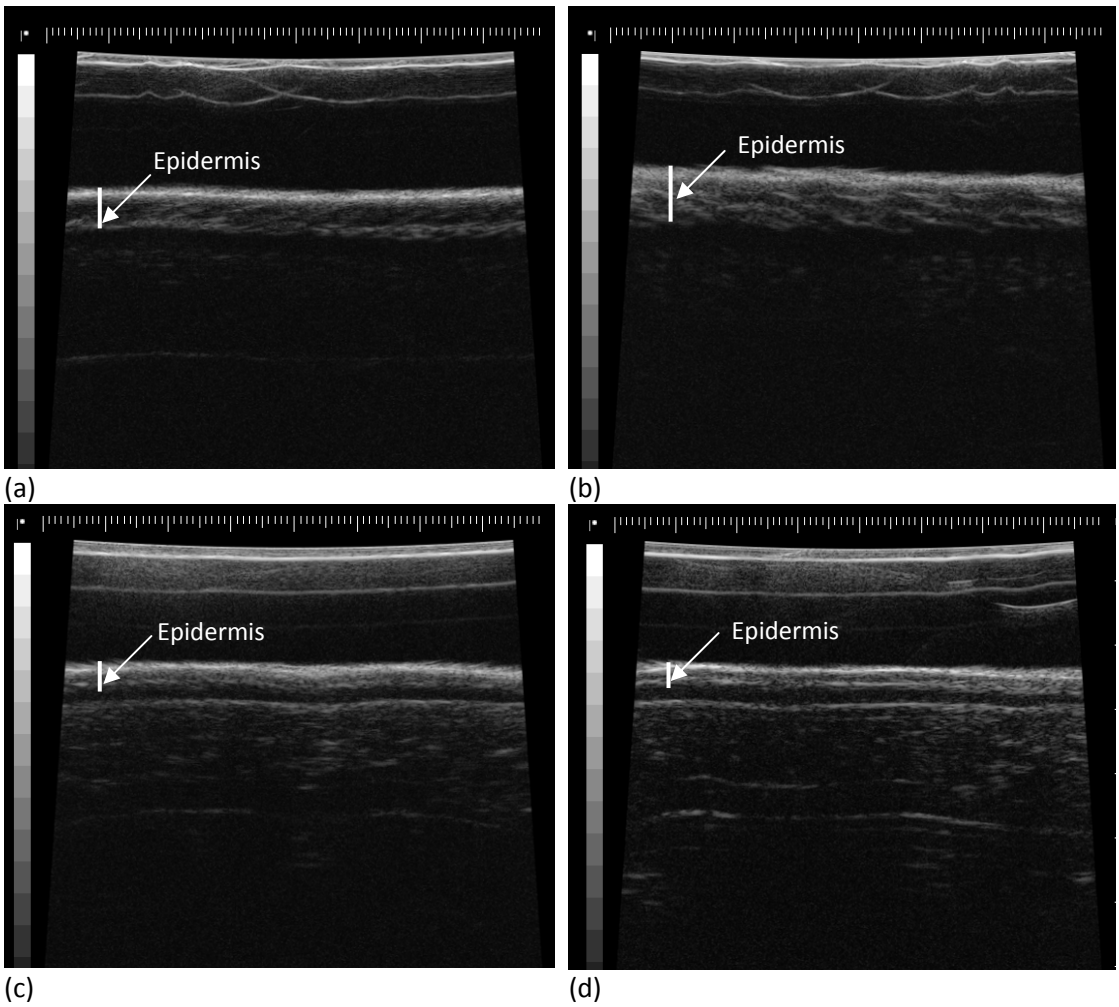


Figure 3.1. Ultrasonic images of human plantar skin under metatarsal head showing different epidermal thickness (a) normal subjects, (b) pure diabetes without complications, (c) diabetes peripheral neuropathy, and (d) diabetes with active or history of foot ulceration. Images are taken by high frequency ultrasound with central frequency at 55MHz (Visualsonic Inc, Vevo 708, Toronto, Canada).

Table 3.3. The total thickness and stiffness of plantar soft tissues at different sites of the foot as measured by Tissue Ultrasound Palpation System.

Tissue properties	DU (n=16)	DPN (n=22)	DM (n=34)	Normal (n=40)	*F*-ratio	*p*-value
Total thickness (mm)						
Pulp of big toe	8.64±1.15	8.04±1.37	7.71±1.58	7.11±1.63	4.663	0.004§
1st MTH	10.07±2.26	10.21±2.67	9.95±1.63	9.43±1.88	0.863	0.463
3rd MTH	10.92±1.52	10.92±1.62	10.51±2.00	9.58±1.55	4.410	0.006*
5th MTH	9.53±2.35	10.02±1.68	8.87±2.08	8.38±2.06	3.689	0.014†
Heel pad	25.12±3.10	25.02±3.19	25.73±2.54	23.65±3.29	3.399	0.020‡
Young's Modulus (kPa)						
Pulp of big toe	84.24±35.77	77.05±18.53	77.50±24.07	62.29±27.85	3.788	0.012§
1st MTH	107.10±33.41	91.40±34.96	99.75±31.61	80.06±34.04	3.125	0.029§
3rd MTH	134.69±44.94	121.45±34.20	121.60±31.80	99.42±46.17	3.982	0.010§
5th MTH	104.30±23.87	99.47±27.26	104.09±26.19	81.81±36.05	5.136	0.002*
Heel pad	87.56±21.36	85.41±15.64	79.37±13.38	69.78±19.74	5.075	0.003*

Data are in mean±SD

DU: diabetic ulcer; DPN: diabetic peripheral neuropathy; DM: diabetes without complications

*Normal group vs neuropathy and ulcer groups

*Normal group vs pure diabetic group and ulcer groups

‡Normal group vs pure diabetic group

†Normal group vs neuropathy group

§Normal group vs ulcer group

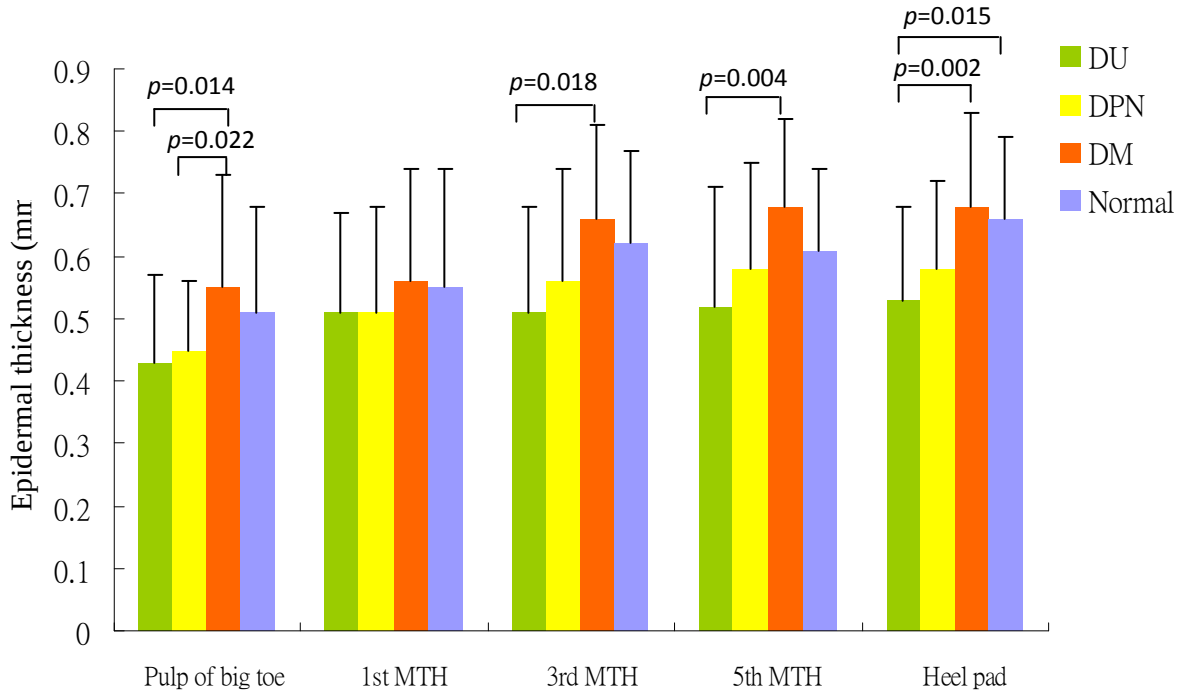


Figure 3.2. The epidermal thickness of plantar skin at different sites of the foot.
DU: diabetic ulcer; DPN: diabetic peripheral neuropathy; DM: diabetes without complications

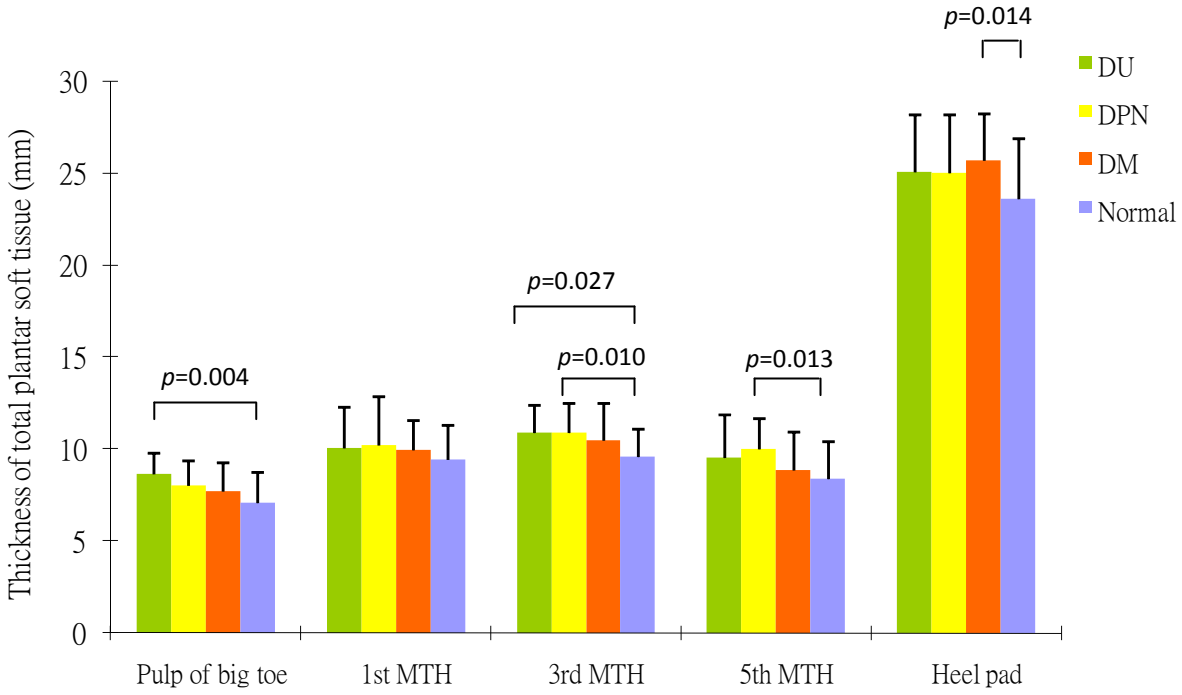


Figure 3.3. The total thickness of plantar soft tissues at different regions of the foot.
DU: diabetic ulcer; DPN: diabetic peripheral neuropathy; DM: diabetes without complications; MTH: metatarsal head

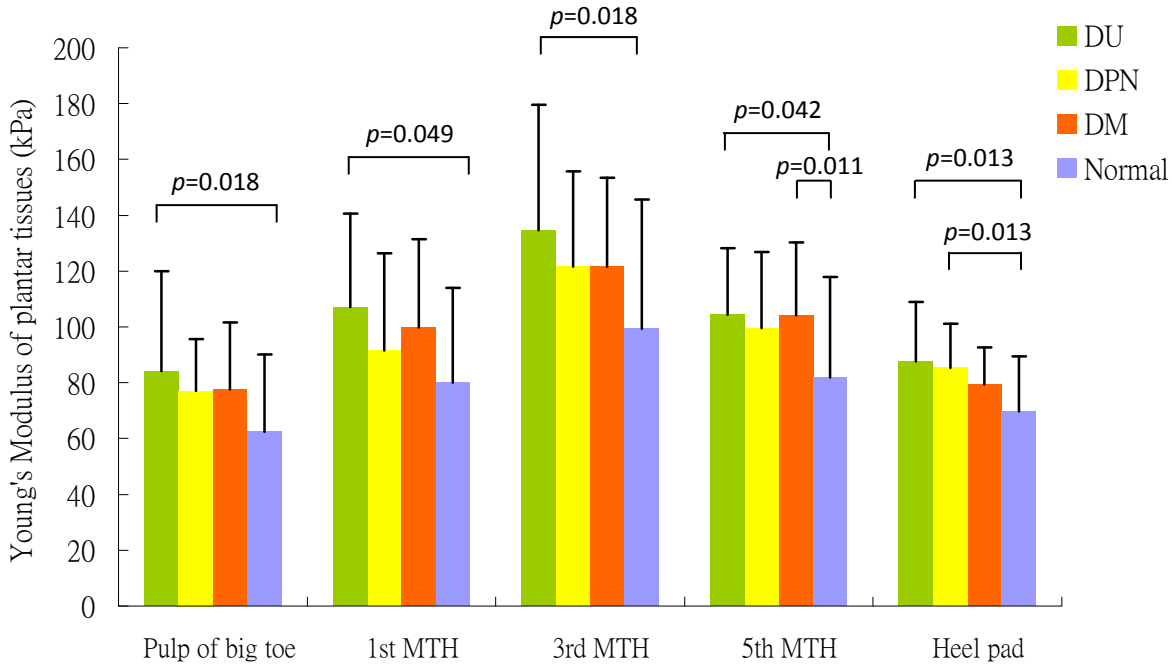


Figure 3.4. The stiffness of plantar soft tissues as different region of the foot.
DU: diabetic ulcer; DPN: diabetic peripheral neuropathy; DM: diabetes without complications; MTH: metatarsal head

3.4.3 Power analysis

A *post hoc* power analysis was performed using the software G*Power. Using the data of epidermal thickness at the 5th MTH, with the sample size 112 for 4 groups and alpha set at 0.05, it yielded an effect size ranging from 0.39 to 0.37 and a power of 0.74 to 0.92 for a one way ANOVA (Table 3.4).

Table 3.4 A *post hoc* power analysis for a one-way ANOVA.

Outcome measures	Epidermal thickness	Plantar soft tissues thickness	Plantar soft tissues stiffness
Alpha level	0.05	0.05	0.05
Effect size	0.3316141	0.2989290	0.3730818
Critical F	2.6886915	2.6886915	2.6886915
Statistical Power	0.8361139	0.7446548	0.9176411

3.5 Discussion

The skin and soft tissues layers of the plantar foot have to undergo various physical stresses during daily activities such as friction, pressure and shearing force. The skin takes an important role to protect individuals from mechanical trauma. Early detection of pathological changes taken place in the skin and soft tissue layers of diabetic foot is therefore extremely important. The present study demonstrated thickening of epidermal layer of plantar skin in people with pure diabetes but thinning of epidermal plantar skin for those diabetes complicated with sensory peripheral neuropathy or foot ulceration. In addition, we found an increase in thickness and stiffness of total plantar soft tissues in people with diabetes but to a greater extent to those complicated with neuropathy or foot ulceration.

3.5.1 Thinning of Epidermal Layer of Plantar Skin Among People with Diabetic Neuropathy and Foot Ulceration

The present study measured the epidermal thickness of plantar skin by the use of two-dimensional B-mode ultrasongraphy image. The current 55MHz high frequency ultrasound imagining allows structures up to a depth of 8 mm below the skin surface to be visualized. However, examination of the dermal thickness of plantar skin from the ultrasound images is difficult to perform because the dermal-hypodermal boundary is unclear. Due to the limitation of penetration depth, it is difficult to identify the interface between dermis and deeper structure. It may due to the thickened stratum corneum, the outermost layer of epidermis, of plantar skin to protect the foot from injury that causes considerable ultrasound beam attenuation. Subsequently, it leads to a decreased signal-to-noise ratio of the ultrasound image. In

contrast, the epidermal-dermal boundary is well defined, which provides great accuracy for epidermal thickness measurements.

We found that the epidermal layer of plantar skin is hyperplastic in people with pure type 2 diabetes without foot complications. In contrast, the epidermis of plantar skin of people with diabetic neuropathy and ulceration become atrophic as compared with non-diabetic healthy controls. Epidermis is known to be a glycolytic tissue and is affected by the insulin level in the body for the regulation of the migration and proliferation of keratinocyte (Chen *et al.* 2009). Keratinocytes are a dynamic population in the epidermis (Huang *et al.* 1999). They proliferate in the basal layer and move upwards to the granular layer. After reaching the top of the granular layer, keratinocytes undergo a specific form of programmed cell death, and finally become cornified (Lippens *et al.* 2005). The keratinized stratified squamous epithelium is being replaced continuously. The thickness of epidermis is maintained by a delicate balance between proliferation, differentiation, and cell death of keratinocytes (Truong and Khavari 2007). The hyperproliferative state of epidermis in plantar skin of pure diabetes is generally regarded to be the consequences of accumulation of advanced glycation products as induced by hyperglycaemia. This enhanced glycosylation of proteins is thought to induce alterations of biochemical and biomechanical properties of the skin changes associated with diabetes (Reihsner *et al.* 2000). Studies have shown an increase in advanced glycosylation end products in the collagen of the dermis in diabetes (Kennedy and Baynes 1984; Sternberg *et al.* 1985), and these end products are postulated to lead to an increase in stiffness (Nikkels-Tassoudji *et al.* 1996) and thickness (Collier *et al.* 1986; Forst *et al.* 1994) of diabetic skin. In addition, Nikkels-Tassoudji *et al.* (1996) demonstrated an increase in

interaction between the fibrous networks of collagen through enriched intermolecular cross-linking in diabetic skin which subsequently results in thickening and stiffening of the skin. Therefore, the increase in cross-linking of collagen among people with diabetes may accounts for the observed skin thickening in diabetes.

However, the present study did not demonstrate an increase in epidermal thickness in plantar skin for all groups with diabetes. Instead, we found that epidermal of plantar skin become thinner when the disease continues to progress up to a point that clinical manifestations of neuropathy and ulceration occur. Apart from being a physical barrier, the epidermis layer is regarded as a sensory organ innervated by Aδ and C fibers connected with free nerve endings (Hsieh *et al.* 1997). It has been suggested that sensory innervation influences epidermal thickness and ultimately the health of the skin (Hsieh *et al.* 1996; Hsieh *et al.* 1997; Huang *et al.* 1999). Sensory nerves interact with keratinocytes and exhibit trophic influences on the epidermis (Chiang *et al.* 1998). Alterations in keratinocytes influence the production of nerve growth factor, and ultimately the maintenance of sensory nerves terminating in the epidermis (Chiang *et al.* 1998). Studies in vitro demonstrated that denervated skin caused epidermal thinning (Chiang *et al.* 1998; Hsieh *et al.* 1996; Hsieh and Lin 1999). Possible explanations may include reduced keratinocyte proliferation, speeding of keratinocyte differentiation, and enhanced programmed cell death. Hsieh and Lin (1999) demonstrated a 30 to 40% reduction in epidermal thickness after depletion of epidermal nerves in rat. This is also supported by the findings of the present study. Our subjects in the diabetic neuropathic or ulceration groups experienced sensory neuropathy as demonstrated in the monofilament examination during the screening test in the inclusion procedures. Comparatively, we

found a 15 and 9% reduction in epidermal thickness in the ulceration group and neuropathy group respectively. It has been reported that in patients with sensory neuropathy as in diabetic peripheral neuropathy, the amount of epidermal nerves is substantially lower than that of normal subjects as shown in the skin biopsies (Hsieh *et al.* 2000; Hsieh *et al.* 1997; Kennedy *et al.* 1996; Umapathi *et al.* 2007). This may explain the observed epidermal thinning in our diabetic groups who complicated with neuropathy or ulceration. In addition, we also demonstrated that the extent of epidermal thinning was more obvious in people with ulceration than neuropathy alone. This may explain why people with diabetic neuropathy or history of ulceration may prone to have higher incidence of ulceration or re-ulceration.

3.5.2 Thickening and Stiffening of Plantar Soft Tissue in People with Diabetes with or without Complications

Plantar soft tissues are rich in collagen and are therefore susceptible to non-enzymatic glycation of the collagenous component. The present study demonstrated an increase in thickness and stiffness of plantar soft tissue for all subjects with diabetes, to a greater extent in those complicated with neuropathy and ulceration as compared with non-diabetic healthy control. The observed increased thickness and stiffness of plantar soft tissue properties in diabetes is thought to be associated with high glucose levels in the tissues, which promotes non-enzymatic glycation of structural proteins in the collagen, elastin and keratin, hence facilitating intermolecular cross linking of the soft tissue and subsequently causing tissue thickening and stiffening (Huntley 1993).

Unlike epidermal thickness of plantar skin, thickness of total plantar soft tissue was increased in diabetic subjects with neuropathy or ulceration. These groups of patients suffered from impaired sensation and may exert more pressure on the insensate plantar skin and soft tissues unwittingly, which in turn would lead to more hardening and leading to formation of ulcers. In fact, the plantar skin and soft tissues needs to be very mobile and stretchable to accommodate different shape and nature of the ground. The changes in soft tissue biomechanical properties of plantar tissues in diabetes results in abnormal loading during ambulation and subsequently leads to breakdown of the tissues (Edsberg *et al.* 2000). Such an increase in stiffness of the plantar soft tissue may cause stress concentration, diminish its ability of the sole to effectively distribute foot-ground contact force during repetitive load bearing; hence causing abnormal stress concentrations which could lead to micro-tears of the tissues and ulcer development. None of the previous studies have reported both the thickness and stiffness of plantar skin and total plantar soft tissue. The present study demonstrated a decrease in epidermal thickness of plantar skin and reduced flexibility of total plantar soft tissues in people with diabetic neuropathy and ulceration. This implies that diabetes associated changes in biomechanical properties of plantar skin and soft tissues may probably be important risk factors leading to foot ulceration in people with diabetes. Therefore, regular examination of the sole of the foot in people with diabetes and proper shoes wear should be reinforced in order to prevent foot complications. We should emphasized that regular exercise could lower blood sugar level and delay the onset of neuropathy (Balducci *et al.* 2006; Thomas *et al.* 2006).

People with pure type 2 diabetes tend to have thicker epidermal plantar skin than their non-diabetic counterparts. In contrast, epidermal thinning of plantar skin occurs in people who have clinical manifestation of diabetic neuropathy and ulceration. On the other hand, the total thickness and stiffness of plantar soft tissue were increased in all subjects with diabetes, in which a greater increase was found in those complicated with neuropathy and ulceration. Such a decrease in epidermal thickness of plantar skin but an increase in stiffness of total plantar soft tissue makes the diabetic foot prone to tissue breakdown and hence ulcer formation.

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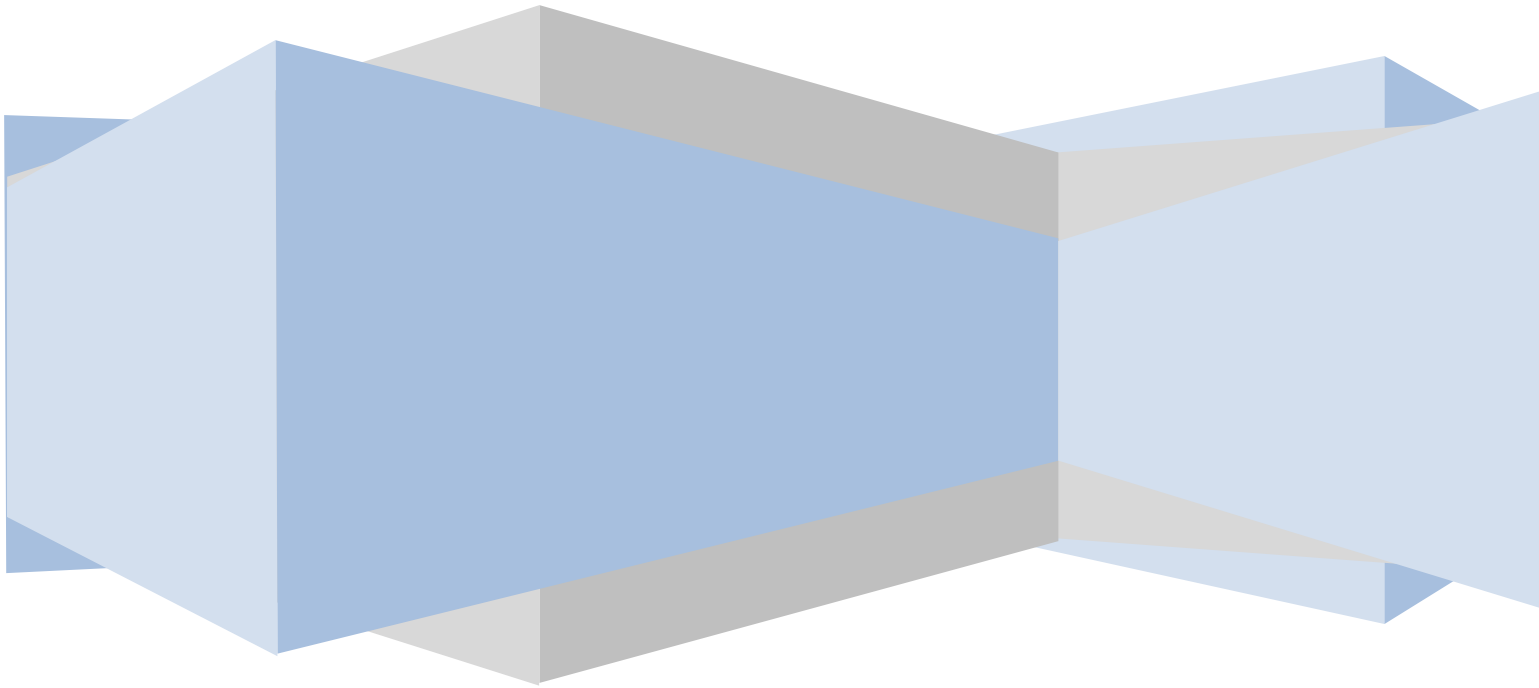
CHAPTER FOUR

The Association Between Skin Blood Flow and Oedema on Epidermal Thickness in the Diabetic Foot

Clare YL Chao



4.1 Abstract

Plantar skin acts as the first line of biological barrier in protecting the foot from mechanical trauma while skin blood flow plays an important role in maintaining the health of the skin. The development of interstitial oedema may impede oxygen diffusion to the skin. Chapter 3 reported the morphological changes in plantar epidermal thickness and in the properties of the soft tissues of the diabetic foot in humans. This chapter described further the association of skin blood flow and oedema and epidermal thickness in the feet of people with and without diabetic neuropathy as compared to a healthy control group. Eighty-seven subjects, including 19 people with diabetic neuropathy and foot ulceration, 35 people with diabetes but without neuropathy, and 33 non-diabetic healthy controls, participated in the study. High frequency ultrasonography was used to measure the epidermal thickness and oedema in papillary skin at the big toe as reflected by the thickness of the subepidermal low echogenic band (SLEB). The capillary nutritive blood flow was measured by the use of video capillaroscopy and skin blood flux was monitored by laser-Doppler flowmetry. There was a 7.2% increase in epidermal thickness in those with diabetes but without neuropathy, and a 16.5% decrease in people with diabetic neuropathy and foot ulceration as compared with the healthy controls (all $p<0.05$). The SLEB thickness increased in all diabetic subjects, to a greater degree in those with neuropathy and ulceration than in those without (64.7% vs. 11.8%, $p<0.001$). Skin blood flux was shown to be higher in the diabetic groups than in the controls (all $p<0.05$), but no significant differences were found in the resting nutritive capillary blood flow ($p>0.05$). A significant fair negative correlation ($p=0.002$, $r=-0.366$) was demonstrated between the SLEB and epidermal thickness at the pulp of the big toe, while no significant correlation was demonstrated between skin blood

flow and epidermal thickness (all $p>0.05$). An increase in subepidermal oedema was demonstrated in people with diabetic neuropathy and ulceration, which may partly contribute to reduced epidermal thickness at the pulp of the big toe. This may subsequently lead to the breaking down of skin in the diabetic foot.

4.2 Introduction

The development of foot ulcers is a common complication seen in people with diabetes mellitus. There are many predisposing factors for diabetic foot ulceration, including impaired blood supply (Bennett *et al.* 1996; Fernando *et al.* 1991; Mueller *et al.* 2005). Skin blood flow provides nourishment and removes waste, which plays a vital role in maintaining the general health of the skin. Thus, any alteration in skin microvascular blood flow may have a significant impact on the general health of the skin. Any pathogenesis that takes place in the skin may result in a change in the thickness of the skin. An example is the breakdown of skin that may occur in a diabetic foot, leading to foot ulcerations. Indeed, it has been suggested that the earliest manifestation of a microcirculatory disorder is oedema (Zimny *et al.* 2001). Yet the relationship among skin blood flow, oedema, and skin thickness in a diabetic foot remains unclear.

Human skin is composed of an outer thinner epidermis and an inner thicker dermis. The epidermis consists of keratin with no blood supply, and nutrition is provided by the papillary layer of the dermis. The microvascular network located in the dermal layer of the skin is composed of both nutritive capillary blood flow and thermoregulatory arteriovenous (AV) shunt flow. The microvascular network varies considerably from one area of the body to another and is regulated through the complex interaction of neurogenic and neurovascular control (Chao and Cheing 2009). The glabrous (hairless) skin is mainly involved in thermoregulation, in which a large number of AV shunts are maintained in the constricted state by sympathetic tone. In contrast, the main function of non-glabrous (hairy) skin is primarily to provide nutrition. Its regulation involves the intrinsic myogenic, sympathetic, and

endothelial control (Urbancic-Rovan *et al.* 2004). In people with diabetes especially if complicated by neuropathy, an increase in foot swelling is a commonly observed feature that precedes the development of noticeable skin lesions. However, the impact of swelling on skin thickness and on the risk of skin breakdown is unclear. Under normal circumstances, the development of distal interstitial oedema is prevented by the venoarteriolar reflex by limiting the rise of capillary hydrostatic pressure during leg dependency (Flynn and Tooke 1995). Such a vasoconstriction reflex response is impaired in people with diabetes, especially in those whose condition is complicated by neuropathy and ulceration (Belcaro and Nicolaides 1991; Belcaro *et al.* 1992; Iwase *et al.* 2007). The reduction or absence of effective precapillary vasoconstriction upon standing will expose the capillary bed to a high hydrostatic load, leading to oedema and thickening of the capillary basement membrane. Very few studies have been conducted that have provided a quantitative assessment of the degree of foot swelling that occurs in a diabetic foot. It is unclear whether foot oedema could lead to a change in skin thickness, a key factor contributing to foot ulceration.

Given that a rich microvascular network is located in the dermal layer of the skin, any change in skin blood flow or in the formation of interstitial oedema would definitely affect the local tissue tension inside, and hence the tissue properties of skin layers in response to external pressure. This is particularly true for plantar skin that is located in weight-bearing areas. The evidence suggests that oedema is an extremely important factor affecting the oxygenation of tissue in that it increases the intercellular spaces, hence causing an increase in the distance for oxygen to diffuse to the most distant cell (Silver 1977). It is believed that skin oedema promotes the

development of ulcers, but there have been very few studies examining the presence of oedema in the skin of the diabetic foot.

Contradictory findings on morphological changes associated with diabetes in the skin of the diabetic foot have been reported (Duffin *et al.* 2002; Hashmi *et al.* 2006; Huntley and Walter RM 1990). Some studies have found thickening of the skin and others thinning of the skin occurring in the feet of people with diabetes. A study suggested that such thickening of the skin may be due to an excessive accumulation of advanced glycosylation end products in the collagen of the dermis (Seirafi *et al.* 2009). We postulate that skin blood flow and oedema may influence the thickness of the skin. In the past decade, ultrasound technology has become one of the most popular techniques for assessing skin morphology (Mofid *et al.* 2006; Vogt and Ermert 2005). It has been proposed that the echogenicity of the skin tissues is inversely related to the amount of fluid contained in the ultrasound images, and that the subepidermal low echogenic band (SLEB) represents either the oedema in the papillary dermis or the photodamage of the skin (El-Zawahry *et al.* 2007). High frequency ultrasonography is a commonly used instrument to measure skin thickness and oedema in the papillary skin layer (Gniadecka 1995; 1996).

Skin health is reliant on an adequate perfusion of blood supply. Oedema may disrupt the microcirculation system, thereby impairing the supply of nutrients to the skin. This can eventually lead to changes in skin morphology and to the breakdown of the skin. The aim of this study was to evaluate the association between skin blood flow and oedema and epidermal thickness in the feet of people with and without diabetes.

4.3 Materials and Methods

4.3.1 Participants

Eighty-seven subjects, comprised of 35 diabetic people without neuropathy, 19 with neuropathy and a history or present condition of foot ulceration, and 33 non-diabetic healthy controls participated in the present study. All diabetic subjects were recruited from the diabetic clinic of a local hospital and had the confirmed medical diagnosis of type 2 diabetes mellitus. Diabetic peripheral neuropathy was identified using the 10g Semmes-Weinstein monofilament (lack of feeling in at least 5 of the following 10 testing points: the pulp of the first, third, and fifth toes; the plantar aspects of the first, third, and fifth metatarsal heads; the plantar medial and lateral sides of the midfoot; the plantar area of the heel; and the dorsal aspect of the midfoot) and a vibration perception threshold of above 25 volts. The group with foot ulcerations all had diabetic neuropathy; they either had a history of diabetic foot ulceration below the malleoli level or were currently suffering from that condition. Healthy control subjects who had no history of diabetes or any other form of neuropathy or arterial disease were recruited from the community by convenience sampling. All of them passed the 8-hour fasting glucose test. Subjects were excluded if they had peripheral vascular disease as determined by the absence of both posterior tibial and dorsalis pedis pulses and the presence or symptoms of intermittent claudication and with an ankle brachial index smaller than 0.9, unstable cardiac condition, or malignancy.

Testing was undertaken on those with a history or present condition of foot ulceration. For subjects who had no history of diabetic ulcers, the right foot was tested. If the ulcerated foot had been partially amputated or if any skin lesions were

found on the measuring sites, the contralateral foot was tested. Ethical approval for the test was obtained from a local university and hospital. Written consent to participate in the test was obtained from each subject.

4.3.2 Measurement of the Thickness of the Epidermal and Subepidermal Low Echogenic Band

A high-frequency ultrasound scanner with a *Vevo* 708 scanhead operating at a center frequency of 55MHz (VisualSonics Inc, Toronto, Canada) was used for imaging skin morphology. This frequency gives an axial resolution of 30μm and a lateral resolution of approximately 70μm, producing high-resolution images to a maximum depth of 8mm. The system displays the information obtained in the form of a B-scan in a grey scale image. A total of four ultrasound biomicroscopy scans were performed on the skin of the foot being tested, at the nailfold and pulp of the big toe. After applying ultrasound gel over the measuring sites, the *Vevo* 708 probe was placed perpendicular to the surface of the skin during the capturing of the image. The pressure of the transducer on the surface of the skin was minimized to avoid compressing the surface of the skin. By using fractal geometry, quantitative data on epidermal thickness was measured by analyzing the change in the echogenicity of the ultrasound image on each sonogram. The first entry echo as shown in the sonograms corresponds to the interface between the coupling gel and surface of the skin, followed by a broad echorich band underneath corresponding to the epidermis. This is followed by a thin echolucent band, the so-called subepidermal low echogenic band, corresponding to the upper dermis (Figure 4.1). After identifying the boundaries of different layers, the thickness of the skin at various layers as defined as the distance between the demarcation echo lines, was then calculated by

the in-house *Vevo* image analysis software. The mean of the measurements for the two images obtained at each testing point was used to analyze the data

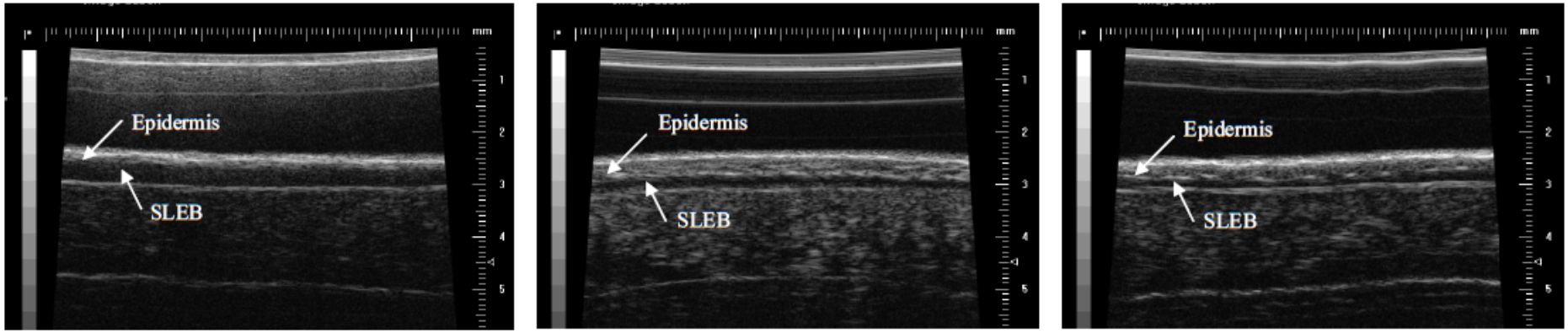
4.3.3 Measurement of Skin Blood Flow

4.3.3.1 The capillary diameter and capillary blood cell velocity

Using CapiScope capillaroscopy (KK Technology, England), capillary blood cell velocity (mm/s) representing skin nutritive blood flow was monitored in the nailfold of the big toe while the subjects were in a supine position. Details of the system design was same as what described in Chapter 2. The subjects were positioned comfortably in a supine position with the foot being tested placed on the platform of a custom-made foot stand. Assessment of capillary diameter and capillary blood cell velocity were measured.

4.3.3.2 The skin blood flux

The skin blood flux over the skin of the nailfold and the pulp of the big toe was recorded using laser Doppler flowmetry (DRT4; Moore Instruments, Milway, Devon, United Kingdom). This instrument mainly measures the flow of blood in arteriovenous anastomises, arterioles, and venous plexuses (Chao and Cheing 2009). The measurements were carried out in a quiet environment with the room temperature controlled at 24±0.2°C. Caution was taken to minimize external disturbances. Details of the system design was same as what described in Chapter 2.



(a) (b) (c)

Figure 4.1. Ultrasound images showing the epidermal and subepidermal low echogenic band (SLEB) thickness at the pulp of big toe among the study groups (a) diabetic neuropathy and ulceration, (b) diabetics without neuropathy, (c) normal healthy controls. Images are taken by high frequency ultrasound with central frequency at 55MHz (Visualsonic Inc, Vevo 708, Toronto, Canda).

4.3.4 Statistical analyses

One-way analysis of variance (ANOVA) was used to test for group difference in the demographic variables and all testing variables. When a significant group difference was detected, Hochberg's GT2 or Games-Howell *post hoc* multiple comparisons were performed to identify pairwise group differences. Correlations among epidermal thickness, the thickness of the subepidermal low echogenic band, and skin blood flow were calculated using Pearson's correlation analysis. The level of significance was set at 0.05 for all measurements.

4.4 Results

Details of the demographics of the subjects are shown in Table 4.1. Nineteen subjects with a history or present condition of diabetic foot ulcerations (DU group) (14 males and 5 females, aged 63.3±12.7 years), 35 diabetics without neuropathy or ulceration (DM group) (16 males and 19 females, aged 65.2±9.0 years); and 33 non-diabetic healthy controls (11 males and 22 females, aged 69.0±4.7 years) participated in the study. No significant differences in demographic data, diastolic blood pressure, and ankle brachial index were found among the groups. In line with the group definition, the fasting glucose was significantly higher in the diabetic groups than in the non-diabetic healthy controls ($p<0.001$). Subjects in the DU group demonstrated a longer duration of diabetes and higher vibration perception threshold than did the DM group and healthy control subjects ($p<0.001$). No significant difference in the glycated haemoglobin level was found between the two diabetic groups.

Table 4.1. Subject characteristics.

Group characteristics	DU (n=19)	DM (n=35)	Normal (n=33)	*p*-value
Male/Female	14:5	16:19	11:22	0.019
Age (yr)	63.3±12.7	65.2±9.0	69.0±4.7	0.103
Body mass index (kg/m^2)	25.75±4.05	24.55±4.15	23.29±3.62	0.111
Systolic blood pressure (mmHg)	144.11±20.43	133.90±14.69	136.18±14.03	0.079
Diastolic blood pressure (mmHg)	80.23±9.49	77.31±10.84	78.83±9.25	0.587
Fasting glucose (mmol/L)	6.90±3.11	7.25±2.13	5.55±0.61	<0.001*
Glycated haemoglobin (HbA_1c) (%)	8.07±2.32	7.27±1.12	-	0.323
Duration of diabetes (years)	13.67±12.09	9.29±7.48	-	<0.001†
Vibration threshold (Hz)	34.06±10.59	16.46±5.35	15.62±6.69	<0.001*
Ankle brachial index (Left)	1.12±0.11	1.10±0.12	1.11±0.08	0.789
Ankle brachial index (Right)	1.15±0.14	1.14±0.14	1.11±0.10	0.695

Data are mean±SD
DU: diabetic foot ulcer; DM: diabetes without neuropathy or ulceration
*Normal group vs diabetes groups
†Ulcer group vs pure diabetic group

4.4.1 Epidermal Thickness and Oedema in Papillary Dermal Skin

The epidermal thickness of plantar skin was significantly different between the study groups at both measuring sites (Table 4.2). *Post-hoc* analyses indicated that the average epidermal of plantar skin was significantly thicker in the DM group but thinner in DU group, as compared to the control (Figure 4.2). Specifically, the epidermal thickness was reduced by 16.5% in the DU group but increased by 7.2% in the DM group as compared with healthy controls. The SLEB was observed only at the pulp of big toe but not at the nailfold. We demonstrated that the SLEB thickness was increased in both diabetic groups ($p<0.001$). As compared with the control group, a 12% increase was found in the DM group and 65% increase was observed in DU group (Figure 4.2). A significant negative correlation ($p=0.002$, $r=-0.366$) was demonstrated between SLEB and epidermal thickness at the pulp of big toe (Figure 4.3).

4.4.2 Skin Blood Flow

At the nailfold of the big toe, the resting capillary diameter and nutritive blood flow and skin blood flux were similar among all the study groups (all $p>0.05$) (Table 4.3, Figure 4.4 and 4.5). At the pulp of big toe, the resting skin blood flux was significantly higher in the DM group, followed by DU group and controls respectively (161.08±100.51 vs 139.46±79.23 vs 93.53±63.26 au respectively, $p=0.009$). The skin blood flux was significantly higher at the pulp than at the nailfold. No significant correlation were demonstrated between skin blood flow and epidermal thickness (all $p>0.05$).

Table 4.2. The epidermal thickness and SLEB thickness at different region of plantar foot as measured by high frequency ultrasonography.

Skin thickness (mm)	DU (n=19)	DM (n=35)	Normal (n=33)	*F*-ratio	*p*-value
Epidermal thickness					
Big toe nailfold	0.19±0.06	0.25±0.08	0.22±0.07	3.993	0.022†
Pulp of big toe	0.40±0.13	0.54±0.18	0.50±0.16	5.246	0.009‡
SLEB thickness					
Pulp of big toe	0.28±0.09	0.19±0.07	0.17±0.08	9.544	<0.001*

Data are mean±SD

DU: diabetic foot ulcer; DM: diabetes without neuropathy and ulceration

SLEB: subepidermal low echogenic band

*Ulcer group vs pure diabetic and normal groups

†Pure diabetic group vs ulcer group

‡Pure diabetic group vs ulcer and normal groups

Table 4.3. The skin blood flow over the big toe nailfold as measured by capillaroscopy

Skin blood flow	DU (n=19)	DM (n=35)	Normal (n=33)	*F*-ratio	*p*-value
Nutritive capillary blood flow					
Capillary blood cell diameter (mm)	8.58±1.38	9.07±1.93	8.90±1.77	0.438	0.647
Capillary blood cell velocity (mm/s)	42.78±10.16	46.24±12.12	42.54±11.76	0.971	0.383
Arteriovenous shunt flow (au)					
Big toe nailfold	45.22±24.37	46.66±26.26	44.93±26.70	0.038	0.963
Pulp of big toe	139.46±79.23	161.08±100.51	93.53±63.26	5.069	0.009*

Data are mean±SD

DU: diabetic foot ulcer; DM: diabetes without neuropathy and ulceration; au: arbituary unit

*Pure diabetic group vs normal group

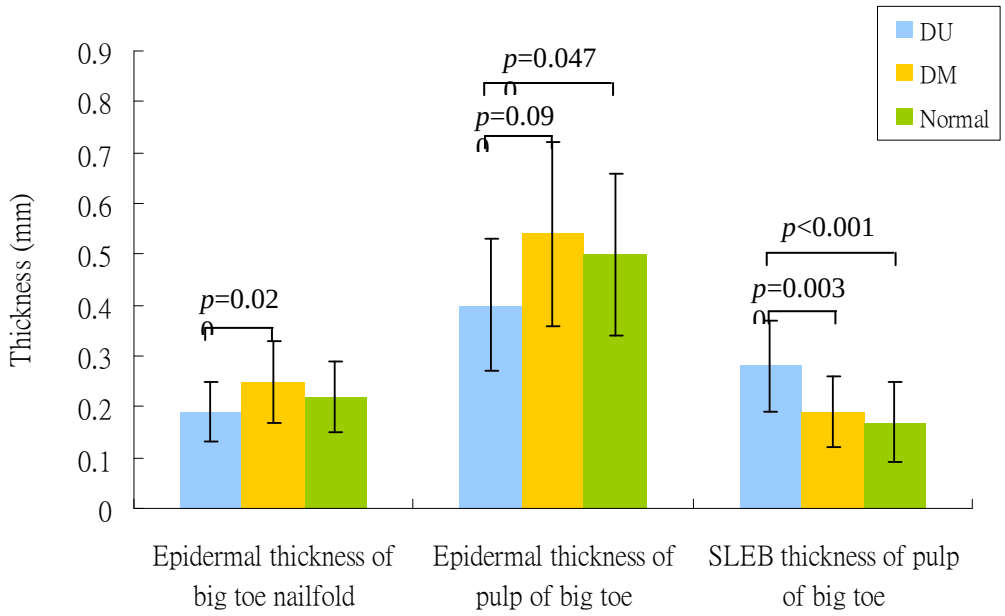


Figure 4.2. The epidermal and subepidermal low echogenic band thickness at different region of the big toe in people with or without diabetes

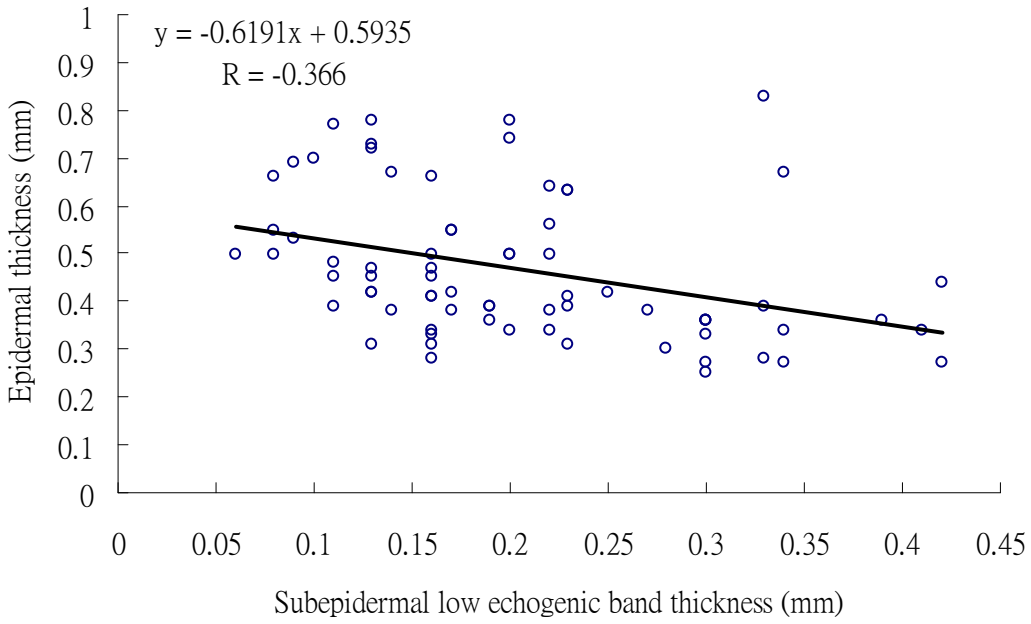


Figure 4.3. The correlation curves between epidermal and subepidermal low echogenic band thickness at the pulp of big toe.

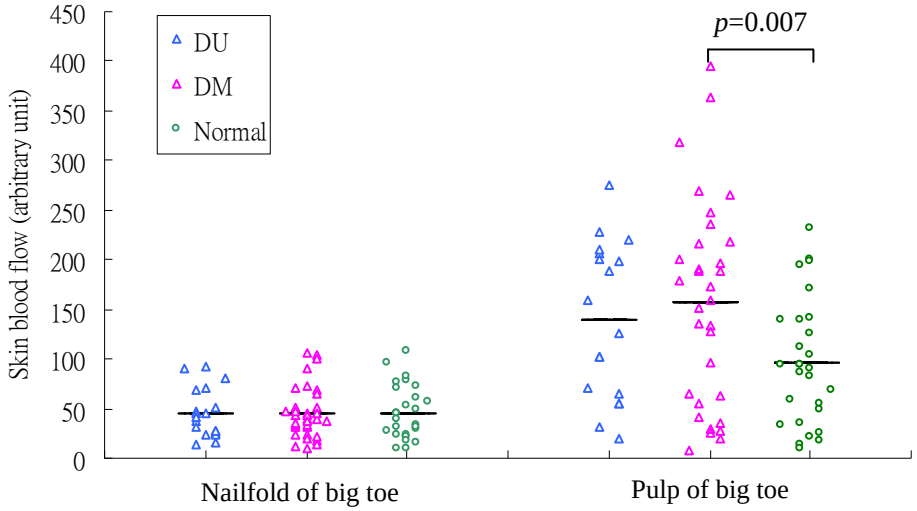


Figure 4.4. The resting arteriovenous shunt flow at nailfold and pulp of big toe as measured by laser Doppler flowmetry. The horizontal bar represents the averaged mean value.

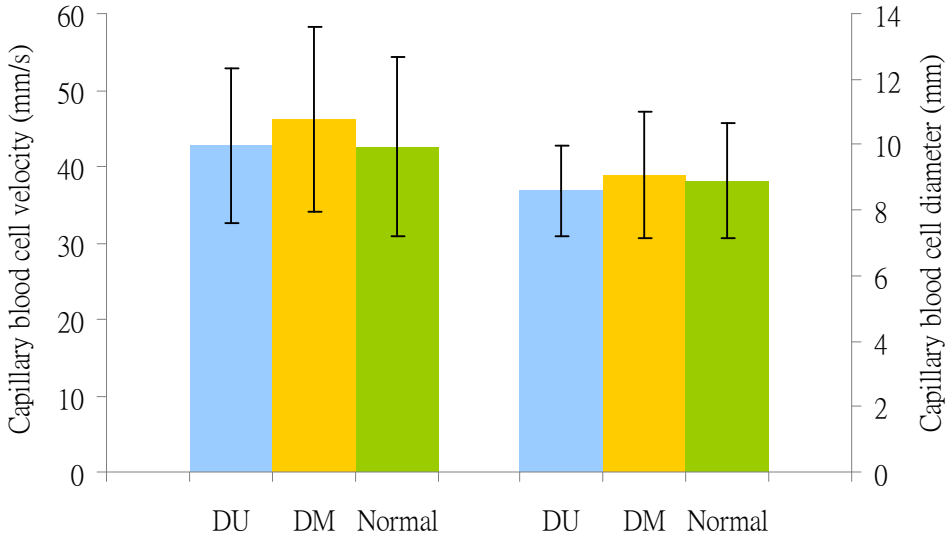


Figure 4.5. The capillary blood cell velocity and diameter at nailfold of big toe.

4.4.3 Power analysis

A *post hoc* power analysis was performed using the software G*Power. Using the data of SLEB thickness at the pulp of big toe, with the sample size 87 for 3 groups and alpha set at 0.05, it yielded an effect size ranging from 0.16 to 0.53 and a power of 0.23 to 0.99 for a one way ANOVA (Table 4.4).

Table 4.4. A *post hoc* power analysis for a one-way ANOVA.

Outcome measures	Epidermal thickness	SLEB thickness	Skin blood flux	Capillary blood cell velocity
Alpha level	0.05	0.05	0.05	0.05
Effect size	0.3524238	0.5266388	0.3811845	0.1562633
Critical F	3.1051566	3.1051566	3.1051566	3.1051566
Statistical Power	0.8320773	0.9941548	0.8884227	0.2303271

4.5 Discussion

We found thickening of the epidermal layer of plantar skin in the DM group, but thinning in the DU group. In addition, there was an increase in papillary dermal oedema over the pulp of the big toe, as reflected by the presence of SLEB in all subjects with diabetes but to a greater degree in those with foot ulceration. We found no difference in capillary diameter and nutritive capillary blood flow among the groups, but we observed an increase in skin blood flux in the diabetic foot. A significant negative correlation was demonstrated between the SLEB and epidermal thickness. However, we did not find any correlation between skin blood flow and the thickness of the epidermal skin.

4.5.1 Thinning of the Epidermal Layer of Plantar Skin and Increased Papillary Dermal Oedema among People with Diabetic Neuropathy and Foot Ulceration

As compared with the healthy control group, the epidermal thickness had decreased by 16.5% in the DU group but increased by 7.2% in the DM group. The epidermis is known to be a glycolytic tissue that is affected by the level of insulin in the body for regulating the migration and proliferation of keratinocyte (Chen *et al.* 2009). The observed hyperproliferative state of the epidermis in the DM group is believed to be the consequence of an accumulation of advanced glycation products induced by hyperglycaemia (Kennedy and Baynes 1984; Sternberg *et al.* 1985) or an increase in the cross-linking of collagen fibers (Nikkels-Tassoudji *et al.* 1996). However, we did not observe an increase in epidermal thickness in any of our subjects with diabetes. We found epidermal atrophy when diabetes is complicated by neuropathy and ulceration. Previous studies using skin biopsies have shown that the amount of epidermal nerves in patients with sensory neuropathy, such as diabetic peripheral neuropathy, is substantially lower than in normal subjects (Hsieh *et al.* 2000; Hsieh *et al.* 1997; Kennedy *et al.* 1996; Umapathi *et al.* 2007). Such a reduction in epidermal nerves may induce a reduction in the proliferation of keratinocyte, speed up keratinocyte differentiation, and enhance programmed cell death, hence causing epidermal thinning (Chiang *et al.* 1998; Hsieh *et al.* 1996; Hsieh and Lin 1999). This may partly explain the epidermal atrophy that was observed in our diabetic group with neuropathy and ulceration. The epidermal layer of plantar skin serves as the front line in the biological barrier protecting the foot from physical wear and tear (Hagisawa and Shimada, 2005). This may explain why people with diabetic neuropathy or a history of ulceration have a higher incidence of ulceration or re-ulceration.

We detected an SLEB at the pulp of big toe but not at the nailfold region. The pulp of the big toe is not an area that is subject to exposure from the sun, so the observed SLEB is not likely to have been due to ultraviolet exposure or photodamage of the skin. Instead, it may represent the degree of oedema in the papillary dermis. We found an increase in SLEB thickness in all subjects with diabetes, to a greater degree in those with foot ulceration. This indicates that people with diabetes develop foot swelling, particularly those with a history or present condition of foot ulceration. In addition, a significant negative correlation was demonstrated between the SLEB and epidermal thickness, meaning that the epidermal layer becomes thinner with an increase in papillary dermal oedema. Therefore, diabetes associated with subepidermal oedema may have pathophysiologic consequences. The efficiency of the delivery of oxygen to the tissues depends on various factors, including the nature of the intercellular matrix, cell density, and whether the intercellular spaces are affected by oedema (Silver 1977). Oedema impairs skin oxygen consumption by increasing the volume of interstitial tissue, hence increasing the distance for oxygen to diffuse between capillaries and target tissues, and leading to less efficient delivery of oxygen to the tissue. Therefore, the presence of oedema in the subepidermal region where the capillary loops are located could significantly impair epidermal nutrition and metabolism. This ultimately leads to epidermal atrophy and predisposes the skin in a diabetic foot to breaking down. This may explain the epidermal atrophy observed in people with diabetic foot ulceration. We found that water tends to accumulate in the subepidermal papillary skin layer. This may due to be the fact that the papillary dermis is rich in collagen fibers, and hence is a site for pronounced collagen damage due to the pathological changes induced by diabetes. With an increase in

compactness and in the degree of the folding of the proteins, the water-binding capacity for fluid accumulation also increases (El-Zawahry *et al.* 2007).

4.5.2 Increased Skin Blood Flux in the Diabetic Foot

Skin blood flow in patients with diabetes mellitus have been studied extensively in the last few decades. However, conflicting results have been found. The present study found no significant differences in the resting nailfold nutritive capillary blood flow and capillary diameters, but a marked increase in the skin blood flux at the pulp of all people with diabetes. LDF provides measures on the flow of blood in both the capillaries and the AV shunts. We demonstrated that the skin blood flux was significantly higher at the pulp (glabrous skin) than in the nailfold (non-glabrous skin), as the pulp contains a large number of AV anastomises. Houben *et al.* (1993) also reported similar findings, namely that the resting skin blood flux increased while the capillary blood cell velocity remained unchanged at the forearm in people with diabetes as compared with healthy controls. However, they did not investigate the change in skin blood flow in the diabetic foot. Two important theories, the haemodyamic hypothesis and capillary steal syndrome, have been proposed to explain the microvascular abnormalities that have been observed in people with diabetes (Chao and Cheing 2009). The haemodyanic hypothesis was first proposed by Parving *et al.* (1983), who proposed that blood flow dysregulation in diabetes is mediated by hyperglycaemia, subsequently causing an increased microvascular flow and capillary pressure. Over time, this causes thickening of the capillary membrane. This, in turn, leads to microvacular sclerosis, which physically limits vasodilation. An increase in vascular endothelial permeability also leads to oedema formation, and hence to a reduction in nutrient supply. We did not measure capillary pressure in the

present study; rather, we found an increase in skin blood flow at the pulp but not at the nailfold. However, an increase in subepidermal oedema was found in all people with diabetes, especially in those whose condition was complicated by neuropathy and ulceration. Capillary steal syndrome is another hypothesis that has been put forward to explain the microvascular dysfunction observed in the diabetic foot. An increase in total peripheral blood flow through AV anastomosis has been demonstrated in the diabetic neuropathic foot. This is thought to be secondary to peripheral sympathetic denervation with loss of vasoconstriction. Blood is then shunted away from capillaries through an AV shunt. Although we found an increase in AV shunt flow as reflected in skin blood flux for all diabetic people with or without neuropathy, we did not find a reduction in capillary blood flow as reported in some of the previous studies (Jörneskog *et al.* 1995; Nabuurs-Franssen *et al.* 2002). Further studies are needed to clarify our controversial findings.

We did not find any significant correlation between skin blood flow and epidermal thickness. Given that the microvascular network is located in only the dermal layer of the skin, and that both an increase in skin blood flux and oedema formation in a restricted volume of the papillary skin layer occurred, it is conceivable that there was a remarkable increase in local tissue tension inside the layers of the skin, especially the plantar skin, in an area subject to repetitive weight bearing during ambulation. This could further impede the skin blood flow during ambulation, ultimately affecting the health of the skin and predisposing it to breaking down and forming ulcers.

4.6 Conclusions

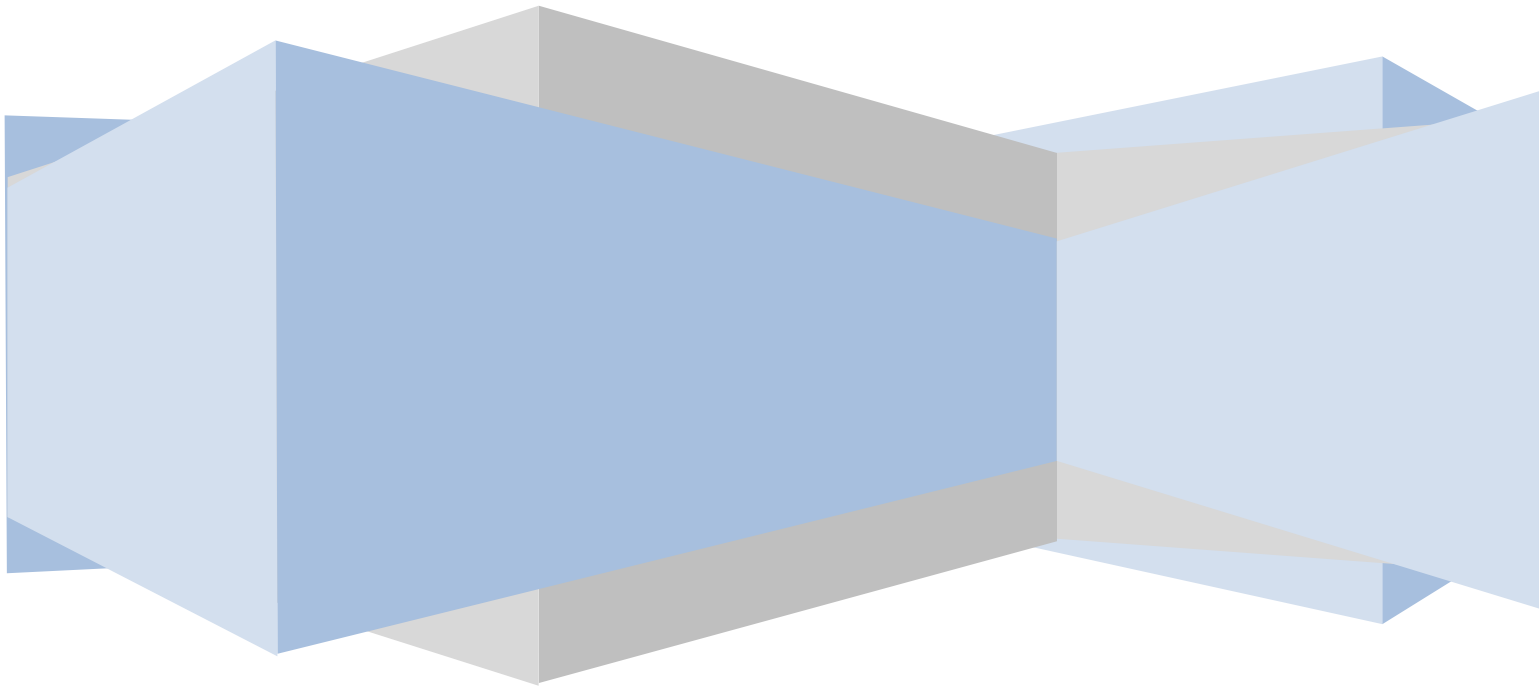
People with diabetes tend to have thicker epidermal skin in the foot. However, if they develop diabetic neuropathy and ulceration, epidermal thinning occurs. Our findings demonstrated that the arteriovenous shunt flow increases in all subjects with diabetes. In addition, we observed an increase in subepidermal oedema in all people with diabetes, with a greater increase occurring in those whose condition was complicated by neuropathy and ulceration. This may contribute to the reduction in epidermal thickness that makes the diabetic foot prone to tissue breakdown and hence ulcer formation.

Note: Part of this chapter was published in *Diabetes Technol Ther* 2012;14(7): 602-9.

CHAPTER FIVE

A Novel Non-contact Method to Assess the Biomechanical Properties of Wound Tissue in Humans

Clare YL Chao



5.1 Abstract

This chapter introduced a novel non-contact optical coherence tomography based air-jet indentation system for characterizing the biomechanical properties of wound tissue in humans. The aims of this study were to measure the stiffness of diabetic foot ulcer tissues by using this air-jet indentation system, and examining the test/retest reliability. Eight subjects with diabetes (7 males, 1 female), with a total of 10 foot ulcers between them, participated in the study. A total of 20 measuring sites located at either the central wound bed (n=10) or peri–ulcer areas (n=10) were evaluated with the air-jet indentation system. Four cycles of loading and unloading, each with a duration of approximately 36 s at an indentation rate of 0.08 mm/s, were carried out for each indentation trial. The test-retest reliability was examined at all measuring points. The average stiffness of the peri-ulcer area (0.47±0.15 N/mm) was significantly larger than that of the central wound bed area (0.35±0.23 N/mm; p=0.042). A high value for test/retest reliability was demonstrated (ICC: 0.986; Pearson's correlation: r=0.972, p<0.001). Our preliminary findings demonstrated that the peri-ulcer area showed greater stiffness than the ulcerated tissues. This greater magnitude of hardness and inelasticity at the peri-ulcer region may scatter part of the contractile forces for wound contraction during the healing process. We found the novel air-jet system to be a reliable tool for characterizing the stiffness of soft tissues around the wound in a non-contact way.

5.2 Introduction

Chronic wounds such as diabetic foot ulcers, pressure ulcers, and venous ulcers are growing challenges for the health care system. They are also a significant cause of morbidity and mortality worldwide. The failure of chronic wounds to heal in a timely and orderly manner leads to a risk of infection, which may have serious health consequences. In the United Kingdom, about 500,000 people are diagnosed with a chronic wound each year, and the total cost of treating these wounds is estimated to amount to 3% of the country's total health care budget (Pollard 2007). The evaluation of wound status and monitoring of wound healing have become a priority from both the research and clinical perspectives. An accurate evaluation of the physiological status of a chronic wound provides important information to guide appropriate treatment decisions and monitor the effectiveness of the treatment. Thus far, there is a lack of accurate quantitative methods to assess the properties of wound tissue. The present clinical documentation of wound healing status only measures the circumference and the color of an ulcer. However, the status of wound healing should not be judged solely by the appearance of the wound. The restoration of the mechanical properties of tissue strength is a potential quantitative outcome of the process of repairing the wound (Gamelli and He 2003). In order to prevent contaminating the wound, it is of the utmost importance to be able to assess the mechanical properties of wound tissues quantitatively in a non-contact way.

5.3 Normal Wound Healing Process

Wound healing is a natural dynamic process of tissue repairing. It involves the reproduction of cells, as well as the recovery of a damaged extracellular matrix that generates resurfacing, reconstitution, and restoration of the mechanical strength of

injured tissue (Mortazavi *et al.* 2009). Under normal circumstances, wound repairing runs into the three overlapping phases of inflammation, proliferation, and maturation (Shakespeare, 2005). The inflammatory phase involves clot formation and phagocytosis. The proliferative phase involves angiogenesis, the formation of an extracellular matrix, and re-epithelialization. Meanwhile, granulation tissue develops and wound contraction begins. The process of re-epithelialization is regarded as generally keratinocyte dependent, whereas the process of wound contraction is fibroblast dependent (McCauley *et al.* 1992). The final maturation or remodeling phase involves continued wound strengthening and contraction. During this phase, the remodeling of connective tissues occurs by the continuous synthesis of new stable collagen and the lyses of old collagen (Shakespeare, 2005). This final wound closure process is both accompanied by and followed by changes in physical and mechanical properties of the wound and peri-ulcer tissues during the biological transformation.

5.4 Biomechanical Properties of the Wound Bed

The biomechanical measurement of the wound healing process is complex, and is partly determined by the thickness and the distribution of the extracellular matrix (Lee and Moon 2003). The physical attributes of the collagen fibers such as the diameter of a fiber, its alignment, and the cross-linking of fibers in a healing wound are thought to be essential factors in characterizing the biomechanical properties of a wound (McCauley *et al.* 1994). It is well known that maturation of the collagen matrix contributes to the mechanical strength of dermal tissue (Lee and Moon 2003), while fibroblast is another type of cell that is thought to have an influence on it. Myofibroblast is a key cell for the remodeling of the connective tissue takes place

during wound healing and fibrosis development (Prakash *et al.* 2007). It is also the main contractile unit for the development of the mechanical force found in wound tissues (Desmoulière *et al.* 2005). Mechanical stresses at the wound site is thought to guide collagen fibrillogenesis (Farahani and Kloth 2008), whereas mechanical tension is considered to control contractile activity of the granulation tissues and myofibroblast differentiation (Hinz *et al.* 2001). Doillon *et al.* (1985) showed a direct relationship between collagen fiber diameters and wound tensile strength. Doillon *et al.* (1988) demonstrated that collagen fiber diameter increases with time post-wounding and is related to tensile strength. As wound healing progresses, the orientation of collagen fibers takes a more uniform pattern and subsequently affects its mechanical strength (Farahani and Kloth 2008). Impaired wound healing due to a decrease in the replication of fibroblast cells may disturb the regeneration of a new dermal matrix, subsequently resulting in a decrease in the mechanical strength of the wound (Lee 2005). It has been suggested that when a wound becomes stiffer, stronger, and more extensible throughout a normal wound healing process, any decrease in mechanical strength may lead to a reduction in the resilience, toughness, and maximum extension of the tissue (Lee and Moon 2003). Therefore, an effective wound management program should promote the optimal mechanical strength of the wound tissue at the various stages of healing (Lee and Moon 2003).

5.5 Evaluation of the Biomechanical Properties of the Wound Bed

Theoretically, the ideal in wound healing is to regenerate tissues in such a way that the structural and functional properties of wounded tissue can be restored to the levels that existed before the wounding (Clark *et al.* 2007). Very few studies so far have examined the biomechanical properties of the wound bed. The majority of these

were *in vitro* animal studies that examined the biomechanical characteristics of excisional wounds during the early phases of healing using such material testing systems as tensiometers (Doillon *et al.* 1985; Doillon *et al.* 1988; Hamilton *et al.* 1970; Ksander *et al.* 1977; McGuire 1980; Pickett *et al.* 1996; Williams and Harrison 1977). This method involves measuring excised strips with given dimensions for uni-axial tensile tests. Standard definitions for axial stress, axial strain, and the modulus of elasticity are usually employed. One of the disadvantages of a tensiometer is that it is invasive and disruptive in nature, in that the tissues being examined must be excised for testing. The biomechanical property measured in the sample tissue can be different from a living *in vivo* wound. With regard to this, compressive indentometry devices have been used to measure the biomechanical behavior of living wound tissue *in vivo* (Edwards and Marks 1995). Perry *et al.* (1993) used the Dimensional Analysis System to perform a biomechanical analysis of linear incisional wounds in rats *in vivo*. In addition, Gingrass *et al.* (1998) described a method for the *in vivo* biomechanical characterization of linear incision wounds in rats using a vacuum pressure chamber for displacement induction and infrared to capture the displacement induced. However, this contact assessment method may potentially contaminate or damage the fragile wound and induce pain during the *in vivo* measurements. Indeed, the majority of previous studies had focused primarily on measuring the biomechanical properties of incisional wounds *in vitro*, little is known about the mechanical properties of an open wound or ulcer in human beings. An optical-coherence-tomography (OCT) based air-jet indentation system has recently been developed. It can be used for the *in vivo* characterization of the biomechanical properties of wound tissue in a non-contact way. This system is comprised of an OCT component to capture the displacement of soft tissue, as well

as its stiffness under indentation (Huang *et al.* 2009). The concept of the system is to utilize the optical interferometric technique for obtaining the deformation of the tested tissue during indentation. By using a pressure controlled air jet as an indenter and OCT-signals to extract the deformation that is induced, it would then be possible to objectively and quantitatively deduce the biomechanical properties of soft tissue. The instrument has been proven to be a useful tool for characterizing the biomechanical properties of soft tissue *in vivo* in humans (Chao *et al.* 2010). As it is non-contact in nature, this device can potentially be used to characterize the biomechanical properties in any type of wound. It would be particularly useful for chronic wounds such as diabetic ulcers, pressure ulcers, and venous ulcers, making it possible to objectively monitor the wound healing process over time.

The present study aimed to characterize and compare the biomechanical properties of diabetic foot ulcers in the central wound bed and the peri-ulcer area using the newly developed non-contact OCT-based air-jet indentation system. The test/retest reliability of the system for measuring the biomechanical properties of diabetic foot ulcer tissues was also examined. The present equipment and testing procedures allow for the non-invasive *in vivo* biomechanical testing of wound tissues, which shed light on the biomechanical properties of the wound bed during the healing process.

5.6 Materials and Methods

5.6.1 Subjects

Eight diabetic subjects (7 males, 1 female) with a total of 10 foot ulcers were evaluated. Their mean age was 59.9±9.5 years, and the mean duration of their

diabetes was 13.1±6.3 years. All of the ulcers were located below the ankle, and the mean duration of the ulcerations was 6.1±7.2 months. The wounds were graded using the Wagner Grading System. None of the subjects suffered from wound infections. Ethical approval for the study was obtained from a local university and written informed consent was obtained from each participant. The demographic characteristics of the subjects are shown in Table 5.1.

Table 5.1. Subject characteristics.

Subjects	Gender	Age	Ulcer location	Ulcer duration (months)	Wound area (cm^2)	Wound grading
1	M	63	Forefoot	24	25.00	2
2	M	48	Big toe	12	0.25	1
3	M	49	Plantar foot	1	1.00	1
4	M	53	Plantar foot	2	1.50	1
			Lateral malleolus	2	4.60	2
5	M	59	Second toe	1	0.64	2
			Lateral heel	6	1.60	1
6	M	68	Heel cord	8	0.48	1
7	F	64	Metatarsal head	3	0.50	1
8	M	75	Lateral heel	2	0.96	1

Wound was graded by Wagner Grading system.

5.6.2 Experimental Procedures

All of the participants were positioned comfortably in a sitting position with the ulcerated foot supported on a stool. The knee of the tested leg was maintained in a slightly flexed position by a small towel placed underneath the knee, and the ankle was kept in a neutral position by a tailor-made stainless steel ankle/foot supporting frame. An examination of the biomechanical properties of diabetic foot ulcer tissue was performed at the central wound bed (n=10) and peri-ulcer area (n=10) by the air-jet indentation system (Figure 5.1). Therefore, a total of 20 measuring sites from

10 wounds were evaluated. Preconditioning of the four loading–unloading trials was performed on each testing point before the actual measurements were taken. This was to ensure that the biomechanical properties of the soft tissue were maintained in a steady state. Two trials of indentation measurements were conducted on each measuring point, with a 5-minute rest period that allowed the tested soft tissue to recover between the measurements. The mean of the two trials was computed for further data analysis. The test/retest reliability was examined by the same assessor at all measuring points, one hour apart.

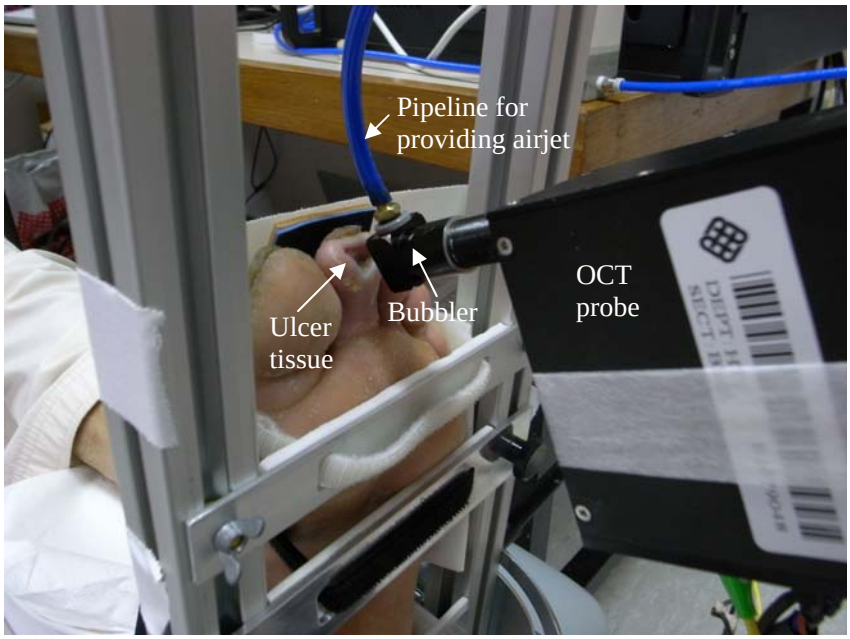


Figure 5.1. A picture shows the setup of testing on diabetic foot ulcer tissue *in vivo* by the air-jet indentation system.

5.6.3 Instrumentation

5.6.3.1 *The OCT-based air-jet indentation system*

The stiffness of the central wound and periwound tissues was evaluted using the OCT-based air-jet indentation system (Figure 5.2). Details of the system design was same as what described in Chapter 2. Four cycles of loading and unloading with a total duration of approximately 36 s at an indentation rate of around 0.08 mm/s was

carried out for each indentation trial loading cycle. The maximum indentation force used was 0.14 N and it gave a maximum displacement of about 0.35 mm. The stiffness *k* (N/mm) of the wound tissues was then calculated.

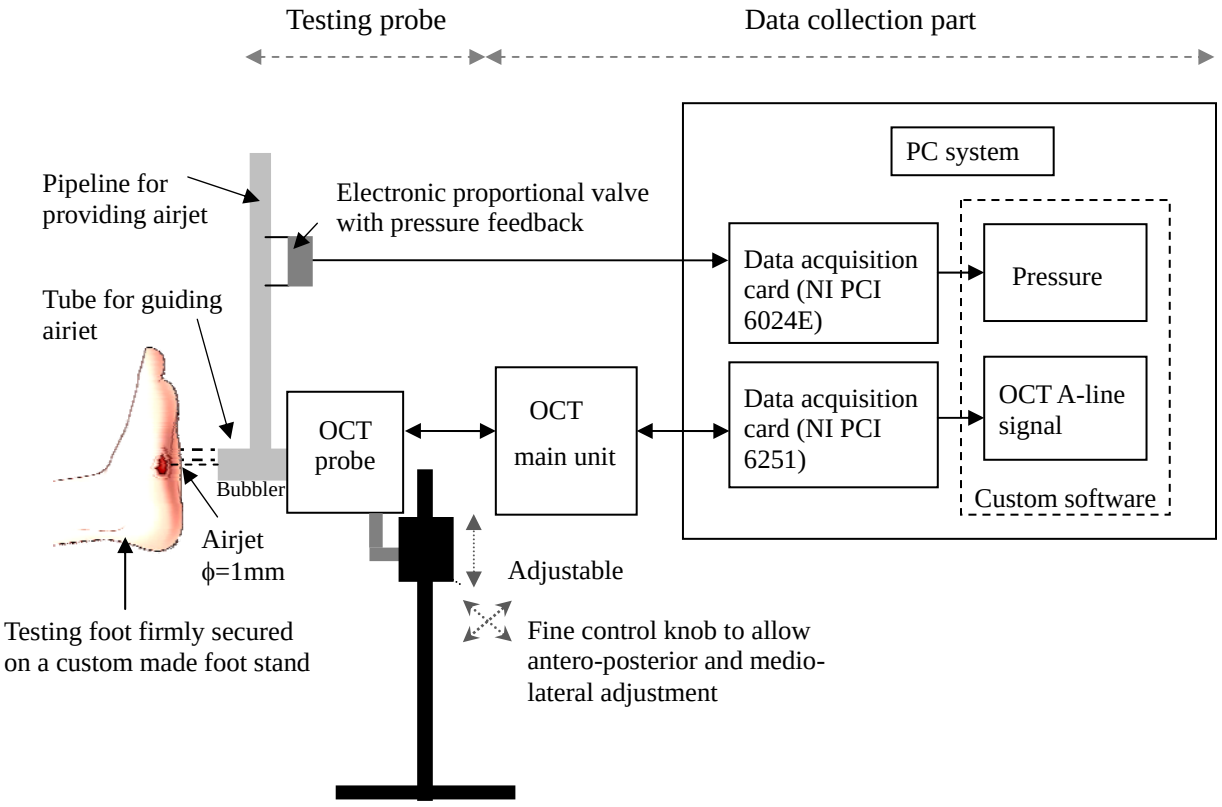


Figure 5.2. Schematics representation of the optical coherence tomography-based air-jet indentation system.

5.6.4 Statistical Analysis

Data analyses were conducted using SPSS (version 15.0 for Windows). A paired sample *t*-test was used to test for the mean difference of the continuous variables of the stiffness *k* (N/mm) between the central wound bed and peri-ulcer tissues. The Pearson correlation coefficient and intra-class coefficient were used to assess the test/retest reliability of the air-jet indentation system. The level of significance was set at 0.05 for all analyses.

5.7 Results

The indentation curve and a typical load-deformation record during the loading and unloading cycle as measured by the air-jet indentation system are shown in Figures 5.3(a) and (b), respectively. The final calculations of the parameter in the loading phases at the second to fourth cycles are shown in Figure 5.3(c). The average stiffness of the peri-ulcer area (0.47±0.15 N/mm) was significantly greater than that of the central wound bed area (0.35±0.23 N/mm; p=0.042). A high value for test/retest reliability was demonstrated (ICC: 0.986; Pearson's correlation: r=0.972, p<0.001) (Figure 5.4).

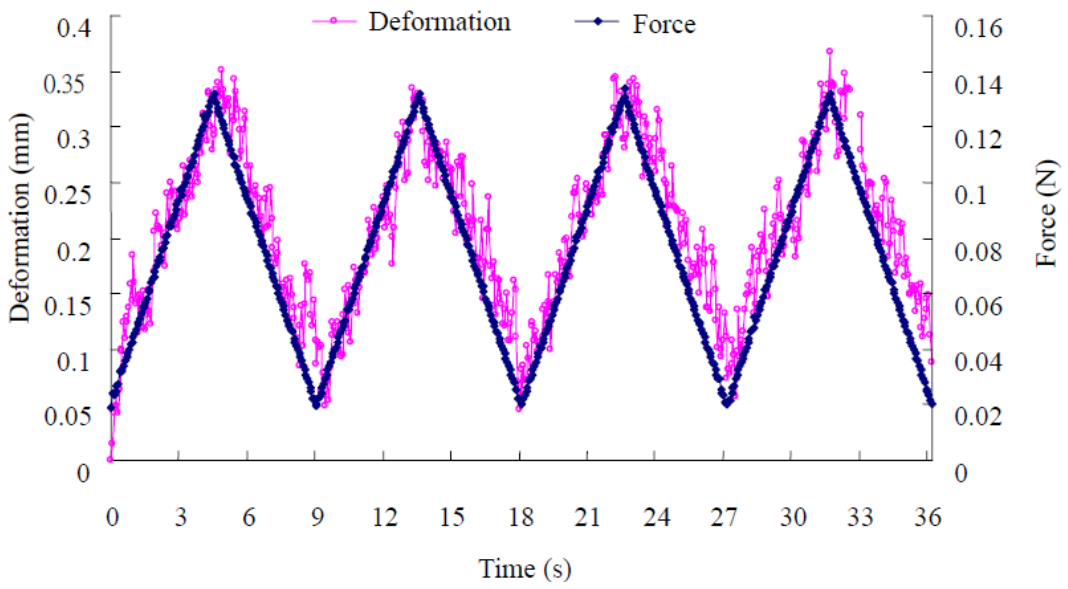


Figure 5.3(a). A typical indentation curve obtained on one subject on the central wound bed tissue with four loading and unloading cycles by the air-jet indentation system.

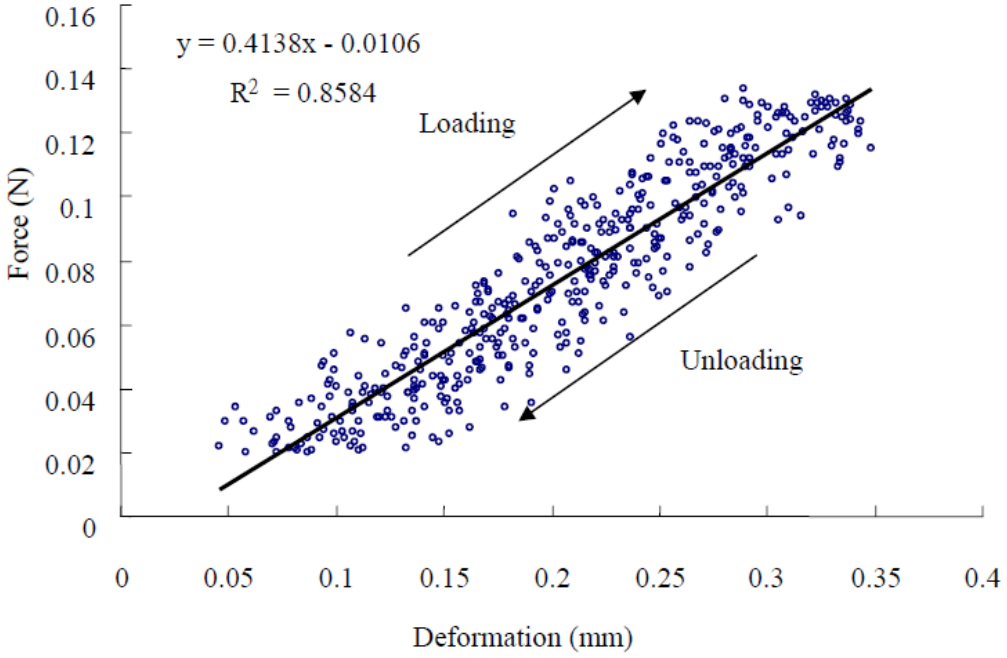


Figure 5.3(b). A typical load-indentation curve obtained from a subject and the corresponding regression curve by the air-jet indentation system at the 2nd to 4th indentation cycles.

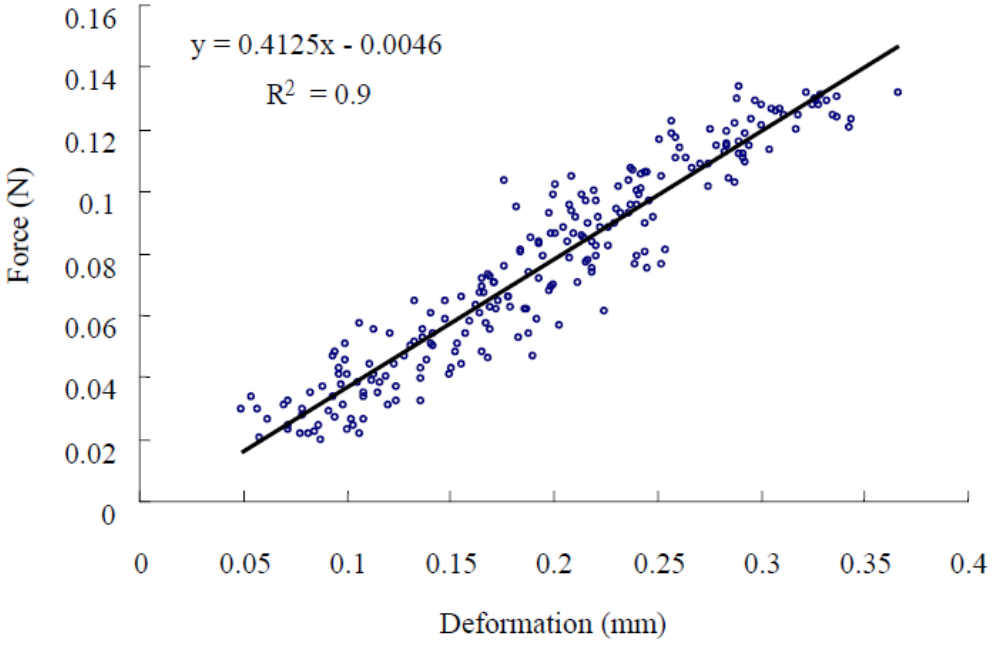


Figure 5.3(c). The selected data points used for estimation of stiffness *k* (N/mm) of the wound tissues. The data in the loading phases at the 2nd to 4th cycle were selected. The relationship between the force measured within the air-jet and the deformation of the wound tissues is shown in the corresponding regression curve.

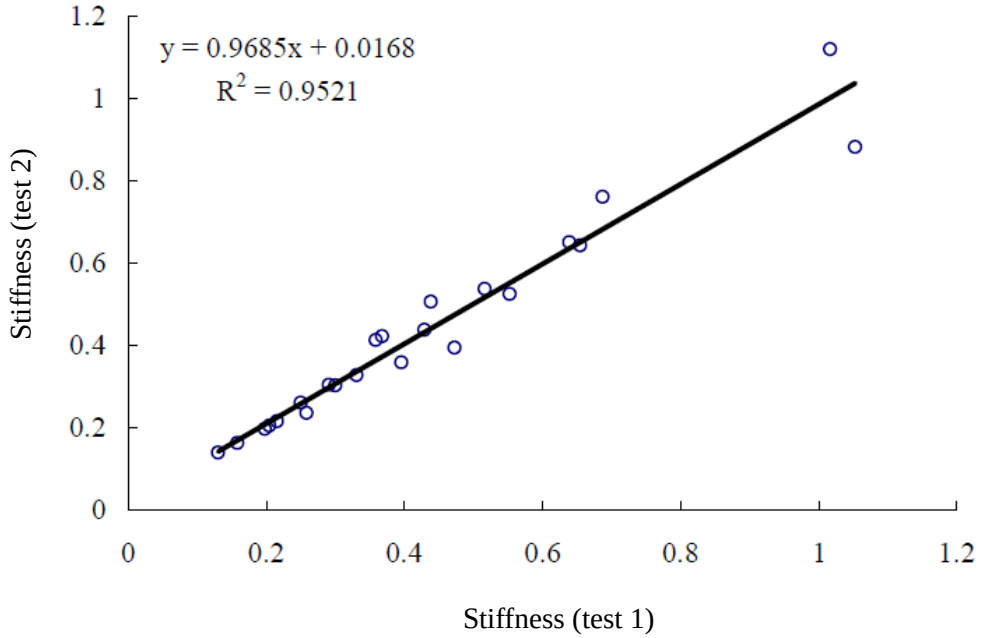


Figure 5.4. The correlation curve between the stiffness obtained by test 1 and test 2 on the same measuring point of the wound or peri-ulcer tissues.

5.7.1 Power analysis

A *post hoc* power analysis was performed using the software G*Power. Using the data of ulcer tissue stiffness at both the central wound bed and peri ulcer area (0.47±0.15 N/mm (peri-ulcer), 0.35±0.23 N/mm (central wound bed)), with the sample size 20 for a paired groups and alpha set at 0.05, it yielded an effect size of 1.50 and a power of 0.99 for a paired sample *t*-test analysis.

5.8 Discussion

In the present study, a novel non-contact OCT-based air-jet indentation system was presented for characterizing the biomechanical properties of wound tissues. To date, no non-contact quantitative measurement tool is available to determinate the biomechanical properties of living tissues. The present air-jet indentation system

incorporated with the fast-scanning OCT signal enables tissue deformations of level of microns to be detected. The system is based on the concept of an optical interferometric technique for extracting the deformation-induced and pressure-controlled air jet as an indenter to stimulate loading and unloading (Huang *et al.* 2009). The force-deformation curves can then be derived from the indentation test, and the stiffness obtained represents the biomechanical properties of the whole tissue layer under the wound surface to be tested. The stiffness of this air-jet indentation system has been shown to have a high correlation with the corresponding Young's moduli obtained by conventional mechanical testing in a phantom model (Huang *et al.* 2009) as well as by a tissue ultrasound palpation system in human plantar soft tissues (Chao *et al.* 2010). Our findings further demonstrated the strong test/retest reliability of this system for characterizing the biomechanical properties of diabetic foot ulcer tissues *in vivo*. Apart from diabetic foot ulcers, this reliable measurement tool could be used in measuring the biomechanical properties of various types of wounds, whether acute or chronic in nature. Better monitoring of the healing process for wound tissues will help to shed light on the pathogenesis of a non-healing wound under certain pathological conditions such as pressure ulcers or diabetic foot ulcers.

The biomechanical properties of connective tissue depend on the extent to which collagen fibers have been laid down, as these are important for the functioning of the tissues. It is thought that such information will provide insights on the degree to which the collagen fibers are cross-linked and the extent of the degradation of the elastic fiber network (Culav *et al.* 1999). In this regard, the mechanical properties of a connective tissue can be interpreted as a reflection of the organization of the tissue

(Ksander *et al.* 1977). However, as there have been very few studies on the biomechanical properties of wound tissues, we postulate that the elastic modulus of wound tissue may differ from those of the surrounding healthy tissues; and the elastic module may change over various phases of the wound healing process. Thus, a quantitative assessment of the biomechanical properties of the wound bed and surrounding tissue is important in understanding the process and factors that may contribute to wound healing. The present study demonstrated that the peri-ulcer area showed a greater magnitude of hardness and inelasticity, as reflected in the average stiffness, than did the ulcerated tissues. This reduced elasticity at the edge of the wound may scatter part of the contractile forces for wound contraction. Hinz (2009) demonstrated that stiffer tissue may modulate the character of the more elastic tissue by driving the differentiation of a variety of precursor cells into fibrogenic myofibroblasts, and stated that this may provide clues to direct cell migration. Wagh *et al.* (2008) investigated the biomechanical properties of wound tissue at different locations of human bronchial epithelial cells. They found that the cells near the edge of the wound undergo localized changes in cellular stiffness, which may provide signals for the spreading and migration of cells. Williams and Harrison (1977) demonstrated different mechanical properties at different parts of a healing wound in pig incisional skin closed either by nylon suture or metal clips. They reported that, in both cases, the mechanical properties varied along the length of the wound. They suggested that this situation could be explained in terms of the modification by the closure method of tension that is transmitted across the wound. Farahani and Kloth (2008) suggested that the mechanical strain of the wound bed regulates the orientation of the collagen fibers and guides the contractile activity towards the restoration of the architecture of the original unwounded tissue and the functional

activity of the previously wounded tissues (Farahani and Kloth 2008). Recent studies have also suggested that tissue stiffness plays a role in controlling the activity of the transforming growth factor-beta1, which subsequently affects wound contraction. Eckes and Krieg (2004) suggested that the mechanical tension can be regarded as an additional important regulatory parameter in modifying the metabolism and phenotype of cells in connective tissue, and that this should be better understood with respect to mechano-sensing cellular structures and specific or shared signaling pathways for the regulation of connective tissue homeostasis. Nevertheless, exactly how different biomechanical properties at different locations of a wound influence wound healing remains uncertain, and further investigations into the matter are needed.

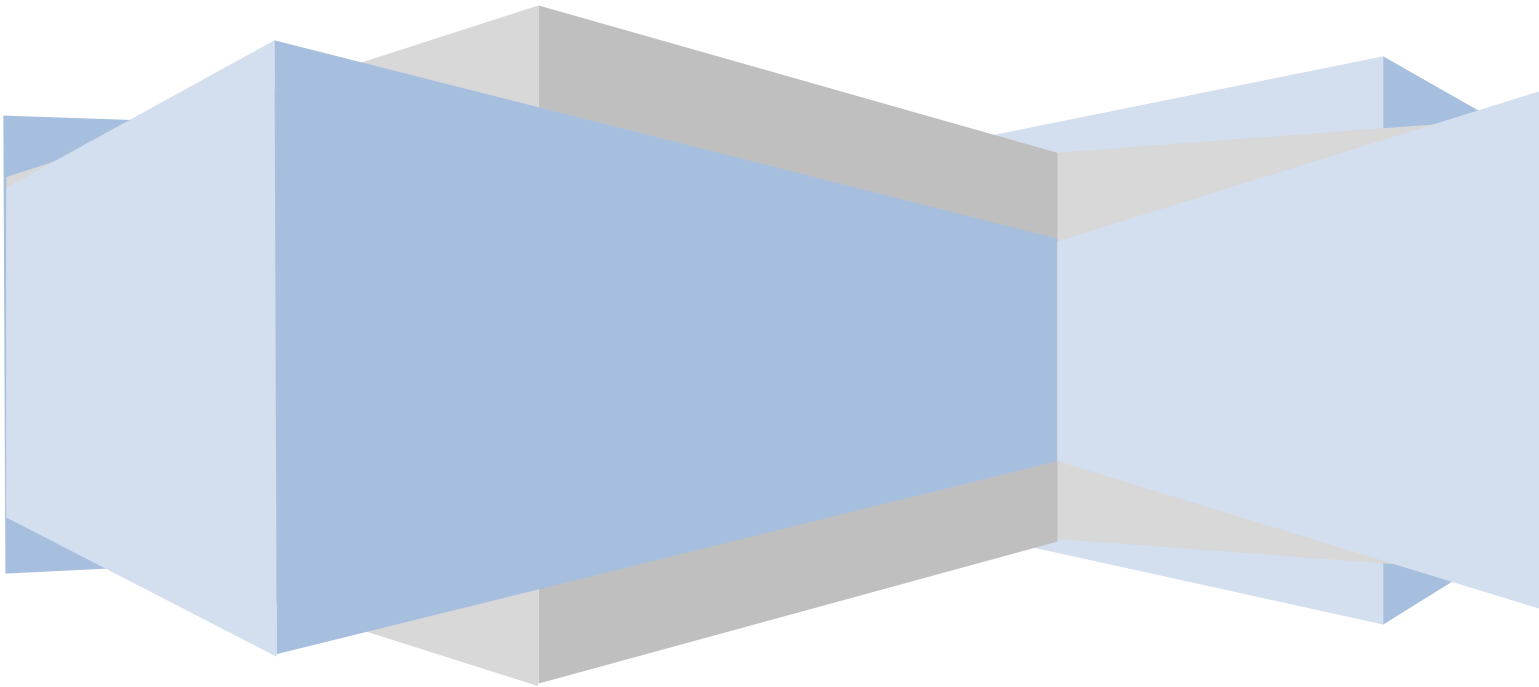
The present study showed that the newly developed air-jet indentation system is a reliable tool for characterizing the biomechanical properties of tissues around the wound in a non-contact way, which allows for better monitoring of wound tissues during the healing process. Information on the stiffness of wound tissue can provide insights for controlling the growth, remodeling, and function of cells. This new quantitative non-contact measurement method is a breakthrough in innovations relating to wound care. It may facilitate the carrying out of more high quality evidence-based research in wound management.

Note: Part of this chapter was published in *Wound Repair Regen* 2011; 19 (3): 324-9 and presented in *The International Conference on the Ultrasonic Measurement and Imaging of Tissue Elasticity*, Zeeland, The Netherlands, September 14-17, 2009 and *Kowloon Central Cluster Convention 2010*.

CHAPTER SIX

In Vivo and *In Vitro* Approaches to Studying the Biomechanical Properties of Healing Wounds in Rat Skin

Clare YL Chao



6.1 Abstract

This chapter evaluates the biomechanical properties of healing skin wounds *in vivo* using the air-jet indentation system and *in vitro* using the conventional material testing system in a rat model. Thirty male Sprague-Dawley rats, each with a 6 mm full-thickness circular punch biopsied wound at both posterior hind limb were used. The mechanical stiffness at both the central and margins of the wound was measured repeatedly in five rats over the same wound sites to monitor the longitudinal changes over time of before wounding, and on Days 0, 3, 7, 10, 14, and 21 after wounding *in vivo* by using an optical coherence tomography-based air-jet indentation system. Five rats were euthanized at each time point, and the biomechanical properties of the wound tissues were assessed *in vitro* using a tensiometer. We found that the size of the wound decreased significantly in the initial few days, and closing almost completely by Day 10. At the central wound bed region, the stiffness measured by the air-jet system was 16.9±2.2 N/m at the baseline pre-wounding stage. This measurement increased significantly from Day 0 (19.8±5.3 N/m, 17.2%), peaked at Day 7 (52.1±20.6 N/m, 208.3%), and then decreased progressively until Day 21 (23.7±3.2 N/m, 40.2%). In contrast, the biomechanical parameters of the skin wound samples measured by the tensiometer showed a marked reduction upon wounding, and then gradually increased with time (all $p<0.05$). On Day 21, the ultimate tensile strength of the skin wound tissue approached 50% of the normal skin; while the tissue recovered its stiffness at a faster rate, reaching 97% of its pre-wounded state. Our results suggested that the stiffness of wound tissues could be an indicator for stimulating wound healing and contraction. It took less time for healing wound tissues to recover their mechanical stiffness than their maximal strength in rat skin. In conclusion, measurements made by the air-jet-indentation system and by material

testing systems involve different principles, but both systems can reflect the biomechanical properties of wound tissue.

6.2 Introduction

Cutaneous wounds can result from a break in the integrity of the skin due to punctures, abrasions, incisions, lacerations, burns, or ulcers. The main function of skin is to act as the body's biological barrier, shielding it from mechanical trauma. The biomechanical properties of skin are important to its normal functions, by allowing repeated reversible extensions and compressions during activities of daily living. The restoration of both the structural and functional properties of wounded tissue back to its pre-wounding state are good indicators of effective wound healing (Clark *et al.* 2007). Therefore, an evaluation of wound mechanics is crucial in assessing the progress made in the wound healing process.

Wound healing involves a series of complex cascading events that generates the resurfacing, reconstitution, and proportionate restoration of the mechanical strength of injured tissue (Mortazavi *et al.* 2009). The process can be divided into defined but overlapping phases: inflammation, proliferation, and maturation (or remodeling) (Shakespeare, 2005). Both the inflammatory phase and the proliferative phase are critical for restoring of the barrier function of the wound and for contraction, while the final maturation or remodeling phase involves continued strengthening and contraction of the wound. During this phase, the remodeling of connective tissues occurs by the continuous synthesis of new stable collagen and the degradation of old collagen (Braiman-Wiksman *et al.* 2007). The proliferation of fibroblast is decreasing and collagen and elastic fibers are being reorganized to increase the strength of the wound tissues. This final wound closure process is both accompanied and followed by changes in the physical and mechanical properties of the wound bed and wound margins (Braiman-Wiksman *et al.* 2007).

Skin is a three-dimensional composite of collagen fibers and elastic fiber bundles that demonstrates viscoelastic behaviour (Meyer *et al.* 1982; Silver *et al.* 2001). The viscous component is associated with the dissipation of energy through the realignment of sliding collagen fibers in response to the application of force from different directions. The elastic component is associated with energy storage and is important for ensuring the recovery of shape after deformation (Dunn and Silver 1983). Such mechanical properties are mainly contributed by the dermis and are related to the structural properties of collagen and elastin fibers (Silver *et al.* 1992). The orientation of collagen fibers of skin tissue is multidirectional with a random arrangement and elastin are twisted filaments interwoven into a rope-like structure that provides the distinct nature of skin (Dunn and Silver 1983). Any damage in skin tissue may significantly interrupt its mechanical function. In this connection, the mechanical properties of a skin tissue can be interpreted as a reflection of the organization of the constituent tissues making up the skin (Ksander *et al.* 1977).

In laboratory research, the evaluation of wound healing stages can be made through the testing of biomechanical properties of the healing tissue with a material testing machine *in vitro* (Doillon *et al.* 1985; Doillon *et al.* 1988; Hamilton *et al.* 1970; Ksander *et al.* 1977; McGuire 1980; Pickett *et al.* 1996; Williams and Harrison 1977). However, such a testing procedure is too invasive and not applicable in clinical studies. Moreover, the biomechanical properties measured in the excised healing tissue can be different from those wound tissues *in vivo*. Although some devices have been developed for *in vivo* mechanical measurements (Edwards and Marks 1995; Perry *et al.* 1993; Gingrass *et al.* 1998), they all involve direct contact that may have high risk of damaging the fragile wound and induce pain during

measurement. Nonetheless, most previous studies focused primarily on measuring the biomechanical properties of incisional wounds during particular phases of healing. Little is known about the change in biomechanical properties of an open wound *in vivo* throughout the entire wound healing process. An optical coherence tomography-based air-jet indentation system is a novel non-contact method that has recently been developed for characterizing the biomechanical properties of soft tissues in a non-contact way (Huang *et al.* 2009). The concept of the system is to utilize the optical interferometric technique for capturing the deformation of the tested tissue during indentation and a pressure controlled air-jet as an indenter for measuring the stiffness of the tissue. The force-deformation curves can then be derived from the indentation test, and the stiffness obtained represents the biomechanical properties of the whole tissue layer under the surface of the wound to be tested (Chao *et al.* 2011; Chao *et al.* 2010). This instrument has been proven to be a reliable tool for examining the stiffness of wound tissues *in vivo* (Chao *et al.* 2011).

The aims of this study were to compare the biomechanical properties of wound tissue between normal skin and in healing wounds following an acute full-thickness incision in a rat model. The comparisons would be made in terms of load relaxation, ultimate tensile strength, ultimate tensile stress, stiffness, and energy absorption capacity as measured by an *in vitro* standard tensiometer and stiffness as measured by an *in vivo* air-jet indentation system. The integration of different aspects of the properties of wound tissues using *in vivo* and *in vitro* approaches allows for a better understanding of the effects of wounding on the biomechanical functions of normal skin wound.

6.3 Materials and Methods

6.3.1 Subjects

Thirty male Sprague-Dawley rats weighting 249 to 419 g (mean: 339.5±50.4 g) were used in this study. The animals were acclimatized to the laboratory conditions for at least 7 days before commencement of the experiment. Ethical approval was obtained from the Animal Subjects Ethics Review Sub-Committee of the administering institution before the study.

6.3.2 Wound creation

The rats were anesthetized with intraperitoneal injection of ketamine and xylazine (Alfasan International, Woerden, Netherlands) in a mixture of 100/20 mg per kg of body weight. The hair on the hind limbs of the rats was shaved and the skin was cleansed using alcohol swabs. A full-thickness round shaped wound with a diameter of 6 mm was excised bilaterally from the middle part of the posterior aspect of the rat's hind limbs using a sterile disposable biopsy punch. After wounding, the rats were kept in cages inside a room with a 12-hour light/dark cycle. They were given food and sterilized water *ad libitum* throughout the study period.

6.3.3 Measuring the biomechanical properties of skin wounds

6.3.3.1 In vivo approach using the optical coherence tomography-based air-jet indentation system

An examination of the elasticity of the healing wound tissues was performed *in vivo* on five sites, namely, the central wound bed and the four margins of the wound, at baseline (pre-wounding), Day 0, Day 3, Day 7, Day 10, Day 14, and Day 21 post-wounding using the optical coherence tomography-based air-jet indentation system.

Before testing, the rats were anesthetized with the above protocol. The tested limb of the rat was supported on a firm small box with the wound area on top. The ankle of the tested limb was manually kept at 90 degrees (Figure 6.1). Two trials of indentation measurements were conducted on each measuring point. The mean of the two trials was computed for further data analysis.

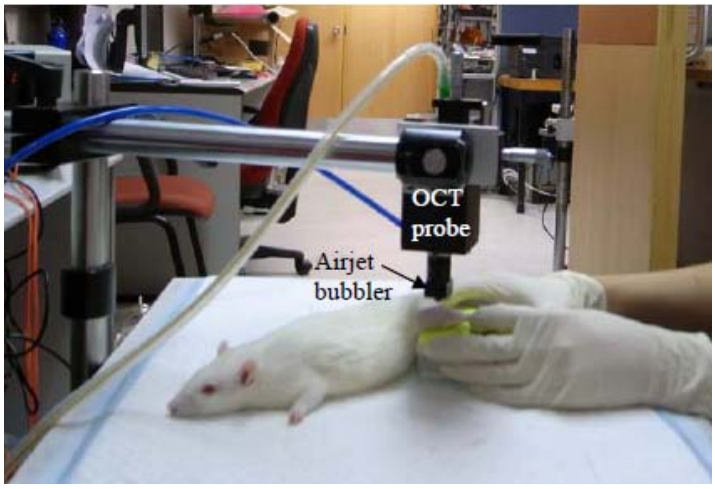


Figure 6.1. The *in vivo* examination of the elasticity of healing wound tissues using optical coherence tomography (OCT)-based air-jet indentation system.

Details of the system design was same as what described in Chapter 2. Three cycles of loading and unloading with approximately 36 s at an indentation rate of 0.13 mm/s were carried out. The maximum indentation force was 0.012 N and it gave a maximum displacement of about 0.8 mm in the softest part of the healing wound tissues being assessed. The stiffness was obtained by performing a regression of the force/deformation curve of the indentation, and it was used to represent the elasticity of the wound tissue. Only data in the loading phase at the 2^{nd} to 3^{rd} cycles was utilized for the subsequent calculation of stiffness.

6.3.3.2 In vitro approach using the Material Testing System

The biomechanical properties of healing skin wound tissues in terms of load relaxation, ultimate tensile strength, ultimate tensile stress, stiffness and energy absorption capacity were evaluated *in vitro* using a material testing system (MTS Synergie 200 machine manufactured by MTS Systems Corporation, Minnesota, USA) (Figure 6.2). Details of the system design was same as what described in Chapter 2. Five of the rats were serially euthanized by intra-peritoneal double dose injection of Ketamine and Xylazine at Day 3, Day 7, Day 10, Day 14, and Day 21 post-wounding for testing.

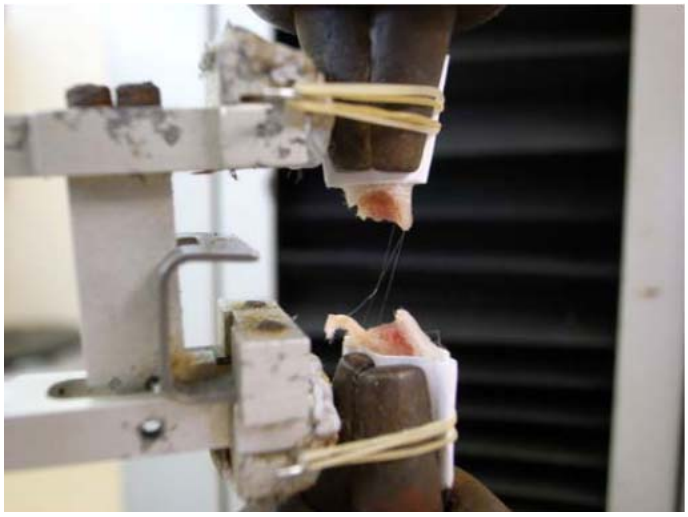


Figure 6.2. The *in vitro* biomechanical evaluation of healing rat skin wound tissues using the material testing system. The skin wound specimen breaks at failure point of maximum load.

6.3.4 Statistical analysis

Data analyses were performed with the Statistical Package for the Social Science (SPSS) software for Windows version 17.0. Repeated measures ANOVA was performed to investigate any change in each outcome measure over time

(within-group). Significant findings were followed up by conducting Bonferroni *post-hoc* tests. The level of significance was set at 0.05 for all data analyses.

6.3.5 Sample sizes estimation

A priori sample size estimation was conducted using the software PASS (NCSS, 2008), with an effect size of 1.81 (Singer *et al.* 2007), alpha level of 0.05 and statistical power of 80% (Cohen 1988). For the measurement at 6 time points, with 5 rats in each group, with about 20% drop out rate estimated, a total of 36 rats were needed (6x5x120%).

6.4 Results

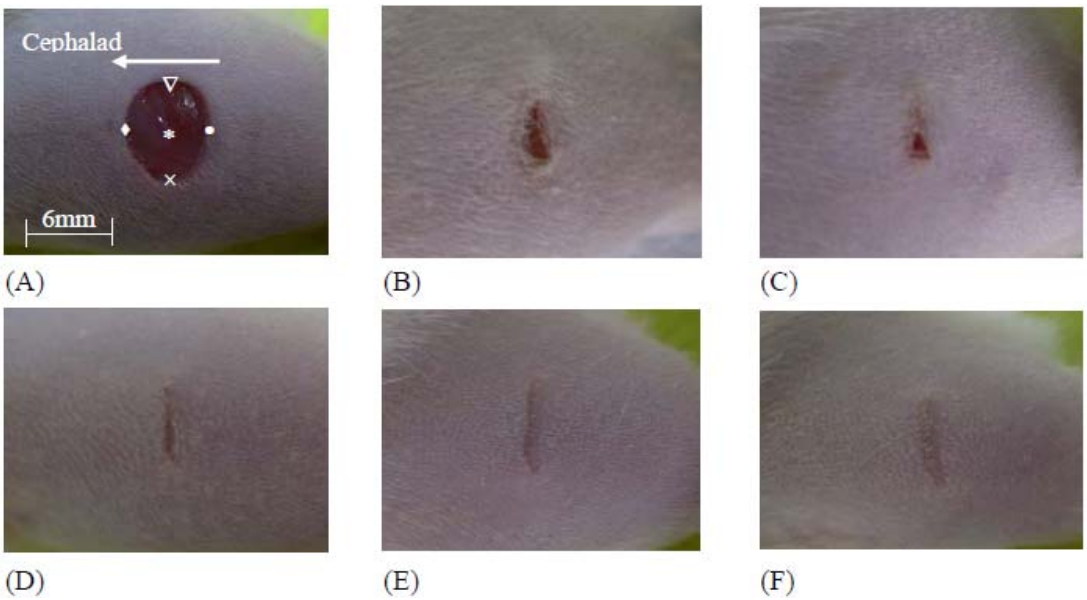
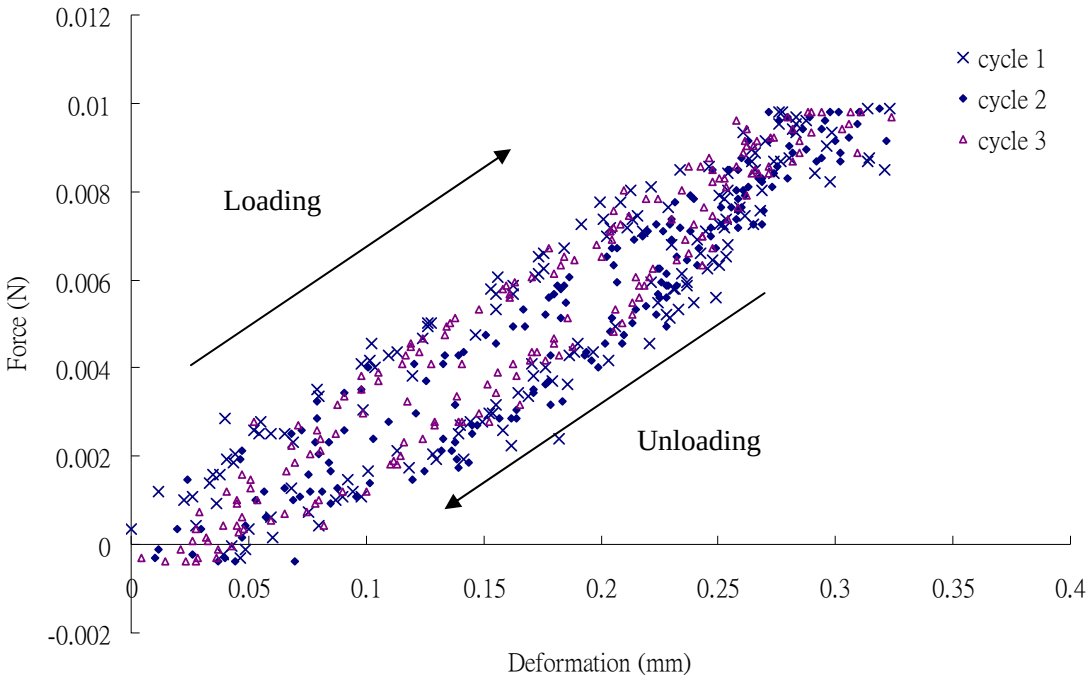


Figure 6.3. Overhead photographs of a 6 mm circular incisional wound on the hind limb of a rat (A) at the time of wounding (post-wounding Day 0), and at (B) Day 3, (C) Day 7, (D) Day 10, (E) Day 14 and (F) Day 21 post-wounding (Arrow indicates cephalad, ∗: wound central, ♦: margin a, ×: margin b, •: margin c, ∇: margin d).

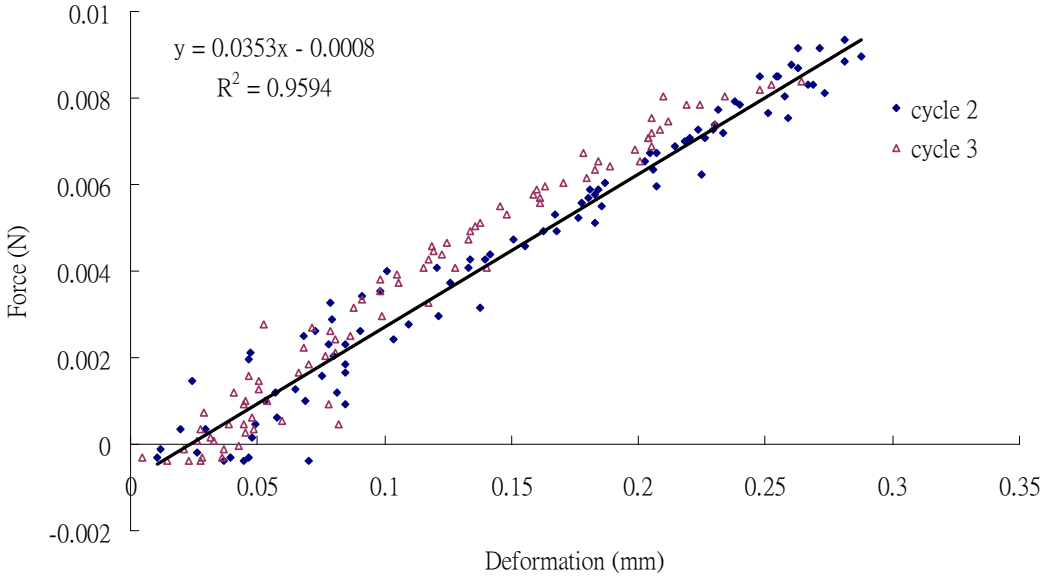
All wounds had significantly decreased in size in the initial few days and reached almost complete closure on Day 10 (Figure 6.3). The load-deformation curve

in Figure 6.4 presents the data points for the stiffness of the wound tissues as measured by the air-jet indentation system. At the centre of the wound region, the stiffness at the baseline (i.e., the pre-wounding stage) was 16.9±2.2 N/m, which increased significantly from the post-wounding stage on Day 0 (19.8±5.3 N/m, 17.16%), reaching its peak on Day 7 (52.1±20.6 N/m, 208.28%), but then gradually decreased untill Day 21 (23.7±3.2 N/m, 40.24%) (Table 6.1, Figure 6.5). As compared to the baseline (pre-wounding), the average stiffness obtained from the four measuring sites on the margins of the wound gradually increased from the baseline (16.0±2.0 N/m) to Day 0 (20.3±4.2 N/m, 27.12%) and to Day 3 (31.9±11.0 N/m, 100%), but then decreased untill Day 7 (22.6±7.6 N/mm, 41.85%), at which point it had reached a plateau.

The findings obtained from the tensiometer test were used to plot a load-deformation curve (Figure 6.6). The biomechanical properties of wound tissue, presented in terms of the load relaxation, ultimate tensile strength, ultimate tensile stress, stiffness and energy absorption capacity at each of the time-points, are summarized in Table 6.2. The load-relaxation values were relatively similar, at around 60%, between the wounded and unwounded tissues measured at the baseline (p=0.954). As compared to unwounded skin, the ultimate tensile strength dropped markedly in the tissues of the healing wound, from 25.2±6.3N to 9.3±2.4N on Day 3 and then recovered to 49.0% of the unwounded skin value on Day 21 (12.3±7.2 N) (Figure 6.7). The ultimate tensile stress of the healing skin wounds, as measured by the breaking force divided by the cross-sectional area of the skin specimen, declined significantly from 6.7±2.2 N/mm^2 in unwounded skin to about 1.7±0.8 N/mm^2 on Day 3 and then increased to 3.2±1.7 N/mm^2 on Day 21, which is only 48.2% of



(A)



(B)

Figure 6.4. The force deformation curve as obtained from the airjet indentation system on healing wound tissues. (A) A scatter plot for data obtained in the 3 indentation cycles. (B) The data in the loading phases at the 2nd-3rd cycle were selected for estimation of stiffness of the healing wound tissues. The relationship between the force measured within the air-jet and the deformation of the wound tissues is shown in the corresponding regression curve.

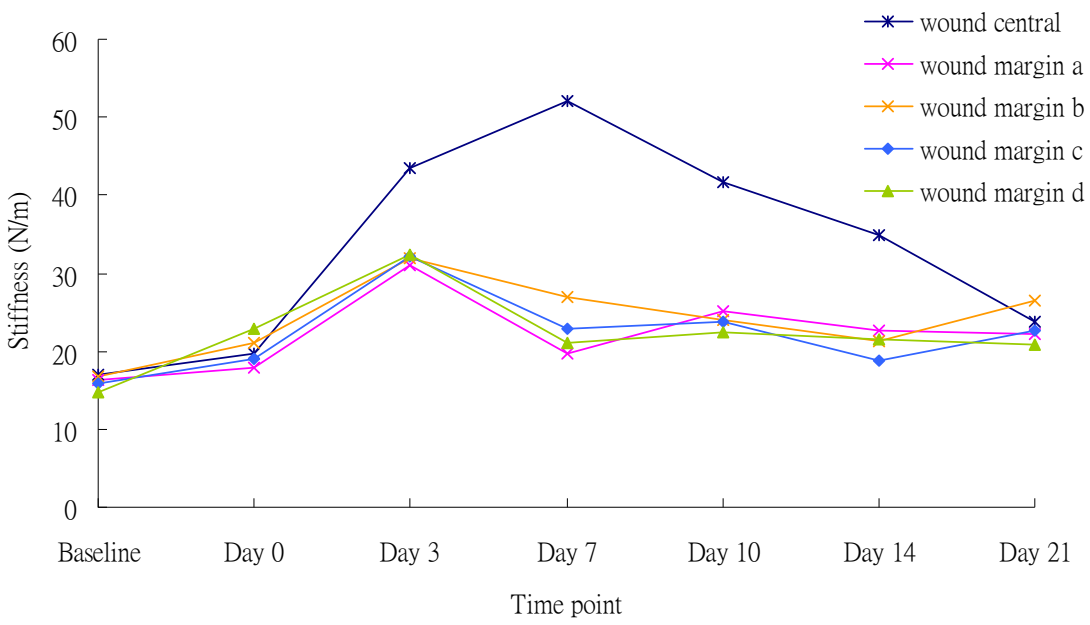


Figure 6.5. The changes in the elasticity of the healing wound tissues monitored longitudinally over a 3-week period as measured by the air-jet indentation system *in vivo*.

unwounded skin value. The stiffness of the wound tissue was calculated from the slope of the load-deformation curve, which increased at a more rapid rate during the healing process, being almost 97.1% of that of the unwounded skin value on Day 21 days (4.5±1.6 kPa vs 4.4±1.8 kPa). The energy absorption capacity, calculated as the area under the curve, increased gradually from 24.7±14.1 on Day 3 to 54.9±50.7 Nmm^2 on Day 21 post-wounding, corresponding to 27.5% and 63.4% of the baseline (89.9±33.5 Nmm^2) value.

Table 6.1. The stiffness (N/m) of the healing wound tissues as measured by the air-jet indentation system *in vivo* (n=10).

Location of wound bed	Pre-wounding	Post-wounding						*F*-ratio	*p*-value
	baseline	Day 0	Day 3	Day 7	Day 10	Day 14	Day 21		
Central	16.9±2.2	19.8±5.3	43.4±12.1*	52.1±20.6*	41.6±12.1*	34.9±12.1*	23.7±3.2*	13.326	<0.001
Margin a	16.3±2.8	17.8±3.4	31.1±11.1*	19.6±4.5	25.1±4.2*	22.7±8.1	22.2±2.1*	6.996	<0.001
Margin b	16.8±2.2	21.2±2.2	31.9±9.6	27.0±9.4	24.0±6.4	21.3±6.0	26.5±6.2*	4.093	0.002
Margin c	15.9±1.9	19.1±5.1	32.2±9.9*	22.9±11.0	23.7±3.7*	18.7±4.0	22.7±4.4*	5.237	0.028
Margin d	14.8±0.9	23.0±6.1	32.4±13.2	21.0±5.5	22.5±4.0*	21.5±7.7	20.8±4.3*	4.689	0.020

Data are in mean±SD

*Values within the same row represent the significant findings obtained in the *post-hoc* tests as compared to the baseline pre-wounding status.

Table 6.2. Biomechanical parameters of the healing rat skin wounds as measured by the material testing system *in vitro* (n=10).

Biomechanical parameters	Pre-wounding	Post-wounding					*F*-ratio	*p*-value
	baseline	Day 3	Day 7	Day 10	Day 14	Day 21		
Load relaxation (%)	60.4±59.1	57.9±66.9	63.7±53.3	64.1±25.0	60.9±58.9	63.9±29.7	0.211	0.954
Ultimate tensile strength (N)	25.2±6.3	9.3±2.4*	9.3±4.0*	10.0±1.6*	10.7±5.8*	12.3±7.2*	14.358	<0.001
Ultimate tensile stress (N/mm^2)	6.7±2.2	1.7±0.8*	1.8±0.5*	2.5±1.1*	2.6±1.4*	3.2±1.7*	17.50	<0.001
Stiffness (kPa)	4.5±1.7	2.3±0.2	2.4±1.2*	2.4±1.1	2.6±2.6	4.4±1.8	2.811	0.038
Energy absorption capacity (Nmm^2)	89.9±33.5	24.7±14.1*	28.0±9.4*	30.8±9.2*	37.9±24.6*	56.9±50.7	8.176	0.006

Data are in mean±SD

*Values within the same row represent the significant findings obtained in the *post-hoc* tests as compared to the baseline pre-wounding status

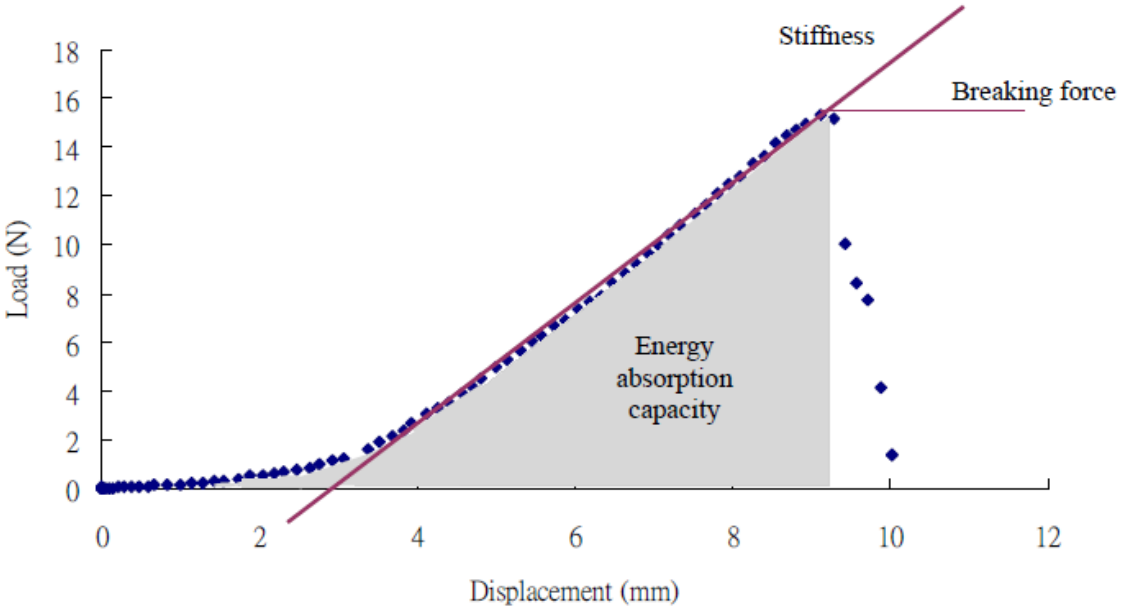


Figure 6.6. The load deformation curve of the healing rat skin wound as measured by the material testing system. The slope of the steepest part represents the stiffness, and the shaped area under the curve till break point represents the energy absorption capacity.

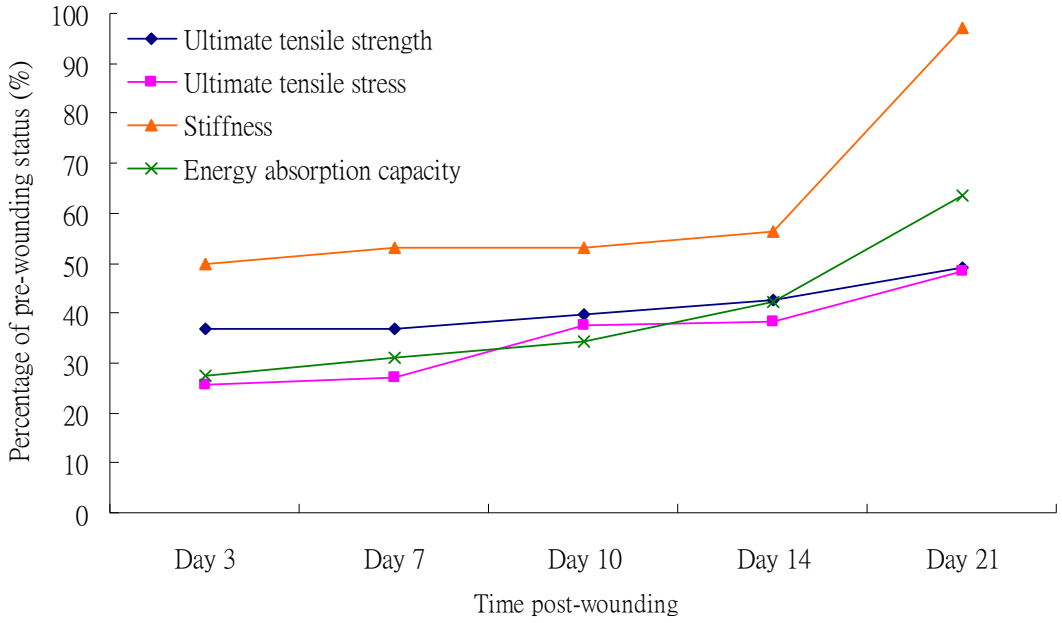


Figure 6.7. Comparisons on the rate of recovery across time on various biomechanical parameters obtained in the *in vitro* test with reference to the value of pre-wounding status.

6.5 Discussion

The healing wound tissues as measured by the *in vivo* air-jet indentation system increased in stiffness in the initial three weeks, with the increase in stiffness being more obvious in the initial 3 to 7 days. In contrast, the biomechanical properties of the tissues as measured by the *in vitro* tensiometer, including the tensile strength, tensile stress, stiffness, and energy absorption capacity, showed a marked reduction upon wounding, and then gradually increased with time. We found that the stiffness recovered at a faster rate than the other properties.

The air-jet indentation system adopted in the present study can measure the biomechanical properties of soft tissue. The air-jet indenter was applied to the skin/wound surface in a non-contact way and compresses the healing skin wound tissue through a loading-unloading process. The stiffness obtained through this indentation process represents the total stiffness contributed by various soft tissue layers from the skin/wound surface to the underneath soft tissue-bone interface. Similarly, the deformation detected is contributed by the change in the total thickness of the soft tissue layers from the skin/wound surface to the underlying bone. Using this air-jet device, we demonstrated that the stiffness of the wound tissue increased immediately after an acute injury on Day 0. The sudden loss in the full thickness skin tissue induced by the biopsy punch definitely affected the localized tension of surrounding tissue and subsequently affects its tissue mechanics. Immediately after wounding on Day 0, inflammatory response was set in motion at wound margins and this may accompany with edema and other associated changes like formation of fibrin clot as induced by the aggregation of thrombocytes at the injury site. All these factors may contribute to the change in stiffness of wound tissues immediately upon

wounding. It has been suggested that the stiffness of wound is important for coordinating cell migration. Previous studies (Farahani and Kloth 2008) had demonstrated that the orientation and contractile activity of the collagen fibers is regulated by the mechanical strain of the wound bed tissues to stimulate the restoration of the architecture of the original unwounded tissue and the functional activity of the previously wounded tissues. Hinz (2009) suggested that stiffer tissue may modulate the characteristic of the more elastic tissue by providing mechanical cue for myofibroblast differentiation. Wagh *et al.* (2008) demonstrated that cells near the edge of the wound undergo localized changes in cellular stiffness, which may regulate the spreading and migration of cells in human bronchial epithelial cells. Our earlier study (Chao *et al.* 2011) suggested that tissue stiffness of a greater magnitude may scatter part of the contractile forces for wound contraction during the healing process. Our present findings further demonstrate that there was an increase in the stiffness wound tissue as compared to the baseline value. This is a result of the healing process but whether such a change in mechanical properties in a continuum of tissue may provide a signal to stimulate wound healing is unknown. We speculate that the change in tissue stiffness immediately after an acute injury could promote cell division and proliferation which is essential for the healing process. Nonetheless, further studies are needed to examine the histochemical and histological patterns with reference to the change in tissue mechanics across the different healing phases.

The present study also examined the longitudinal changes in mechanical stiffness at the center and margins of the wound for 3 weeks. Wound margins were measured at four areas on the day of wounding, namely (a) to (d), as shown in Figure 6.1. Repeated measurements were performed on the same testing sites over time. It appears that area (a) and (c) displayed greater exposure to wound contraction than

did area (d) and (b). However, we did not find a significant regional difference for the stiffness measured at these 4 testing areas of the wound margins. It is well known that wound healing are largely achieved by wound contraction in rats, meaning that the wound shrink in size by pulling the edges of the wound together by contraction. So, it is likely that the measurement area (a) to (d) has become normal skin by Day 3 as it remained at the same position for testing rather than moving along with wound contraction. Although the wound appearance looked pretty normal visually at the original wound margins from Day 3 onwards (Figure 6.1), we did demonstrate changes in tissue stiffness at these sites continuously, indicating that the healing process is still being taken place in the underlining tissues. A remarkable decrease in wound size was demonstrated by Day 3, however it is unclear if the subdermal tethers the dermal margins to the deep fascia under the wound edge, causing the changes in tissue stiffness in the superficial skin that appears normal. The air-jet indentation system is the state-of-art equipment that allows stiffness measurement from the top of the skin surface all the way down to the underneath soft tissue-bone interface. Future studies with histological examination should be performed to measure the migration of wound margins at the dermis-subcutaneous interface so as to have a better understanding on the mechanism of wound healing process in rats. In particular, histological examination should be made with reference to the change in tissue mechanics and wound appearance.

Moreover, we found significantly less stiffness at the margins of the wound of the same area as compared with the center of the wound, and this difference was particularly obvious between Day 3 and Day 14 (Figure 6.5). The wounds had decreased markedly in size in the initial 3 to 7 days, and complete closure mostly

occurred in 10 days (Figure 6.3). This could be interpreted as a progressive increase in distance away from the margins of the wound at the original site on Day 0 as compared to the wound bed. This means that the elasticity of such wound tissues that are close to being healed no longer serves a major role in providing mechanosensing signal processing for cellular migration. This explains the relatively constant stiffness at the margins of the wound from Day 7 to Day 21. At Day 0 post-wounding, there was no skin or healing skin wound tissues at the wound central region. The measured stiffness came from the response of the underlying muscle and deep fascia layers down to the bone-interface. Over time, we found a progressive increase in stiffness at the central wound bed from Day 0 onwards, peaking on Day 7, at 208% of unwounded tissue at the baseline. Such a marked increased in tissue stiffness could be attributed to an increase in the formation of scar tissue, the cross-linking of collagen, and an increase in the contraction of collagen fibers throughout wound healing process. The increase in the cross-linking of collagen fibers acts to stiffen the entire network of collagen fibers, making the wounded tissue less prone to deformation. Wound tissues at this stage are fragile, have reduced elasticity, and can be easily broken by a weak force. However, it was observed that, as the wound continued to heal, the stiffness at the wound center bed gradually decreased (Days 7 to 21), but had still not returned to the baseline value. This recovery of tissue elasticity may be ascribed to a more organized and parallel alignment of collagen fibers. Our results demonstrated a substantial heterogeneity in the stiffness characteristics of healing skin wound tissues, with a coordinated change in stiffness throughout difference phases of wound healing. On Day 21, although the wounded tissues appeared to be almost completely healed, there was still an average increase in stiffness of 43.6% at all testing sites as compared to the baseline. This implies that

wound healing cannot be judged by the gross wound closure alone. The measurement of tissue stiffness is an important indicator of various stages of the wound healing process. Further studies are needed to examine the changes in tissue mechanics with morphology.

Apart from the *in vivo* test, we also examined other biomechanical parameters of the healing wound tissues using a standard material testing system *in vitro*. Assessments were done for the same time points as in the *in vivo* test, except on Day 0, when no wound tissue could be excised for assessment. Our results did not demonstrate any significant effect on the rate of relaxation in wound tissues across time. Load relaxation is a measure of the viscoelastic properties of tissues that is mainly determined by the interaction between small-sized collagen fibrils (Parry *et al.* 1978). The non-significant difference in load relaxation during different phases of wound healing could indicate that wounding has minimal effect on the ultrastructural morphological profile of the small collagen fibrils. However, we demonstrated a significant reduction in the ultimate tensile strength and stress, and energy absorption capacity in healing wound tissues during the initial 3 weeks than the unwounded skin, indicating that the wounded tissues were less resistant to failure. Moreover, we found that wounded tissues recovered their elasticity much more quickly, as reflected in the faster recovery of stiffness to 97% of the normal value by Day 21. In contrast, a slow recovery was observed in the ultimate tensile strength and stress of the wounded skin, with restoration to only about 50% of the normal skin by Day 21. This may be due to the fact that the remodeling phase takes a very long time depending on the size of the wound and other associated factors. These results were in line with those reported by Corr *et al.* (2009), who noted a considerable reduction in the failure properties of

healing skin, but with a similar stiffness, in juvenile pig skin. White *et al.* (1971) also demonstrated a faster return of elasticity than tensile strength in guinea pig skin. Apart from skin wound tissues, previous studies have demonstrated the tissues of ligament and tendon wounds usually recover in stiffness sooner than in strength (Noyes 1977; Yeung *et al.* 2006). Nevertheless, the relatively rapid return of elasticity allows a wound to absorb energy through stretching. This is important for preventing the wound from rupturing with small stresses during the early stages of healing. As for the tensile strength of the healing wounds, this depends not only on the amounts of the collagen fibers, but also the structural orientation of the collagen network, the formation of cross links between the filaments, and the maturation of the collagen fibers (Asadi *et al.* 2010). Our results indicated that the healing process continues even after 21 days in rat skin wounds. There is also apparent prolonged maturation of the wound for the recovery of tensile strength which is a measurement of the maximum strength as opposed to the submaximal loading of elasticity measurement.

The present study presented both *in vivo* and *in vitro* approaches to evaluating the biomechanical properties of healing skin wounds in rats. The two approaches are based on different principles and measurement methods, so it is not surprising that they produce different results. The mechanical properties of skin tissues are dependent on the loading direction (Corr *et al.* 2009), the air-jet machine provides measurements of stiffness perpendicular to the surface of the skin, while the tensiometric assessment provides measurements of tissue properties parallel to the surface of the skin, thus possibly providing measurements of different mechanical responses. The tensiometric machine provides measurements only on the skin layer,

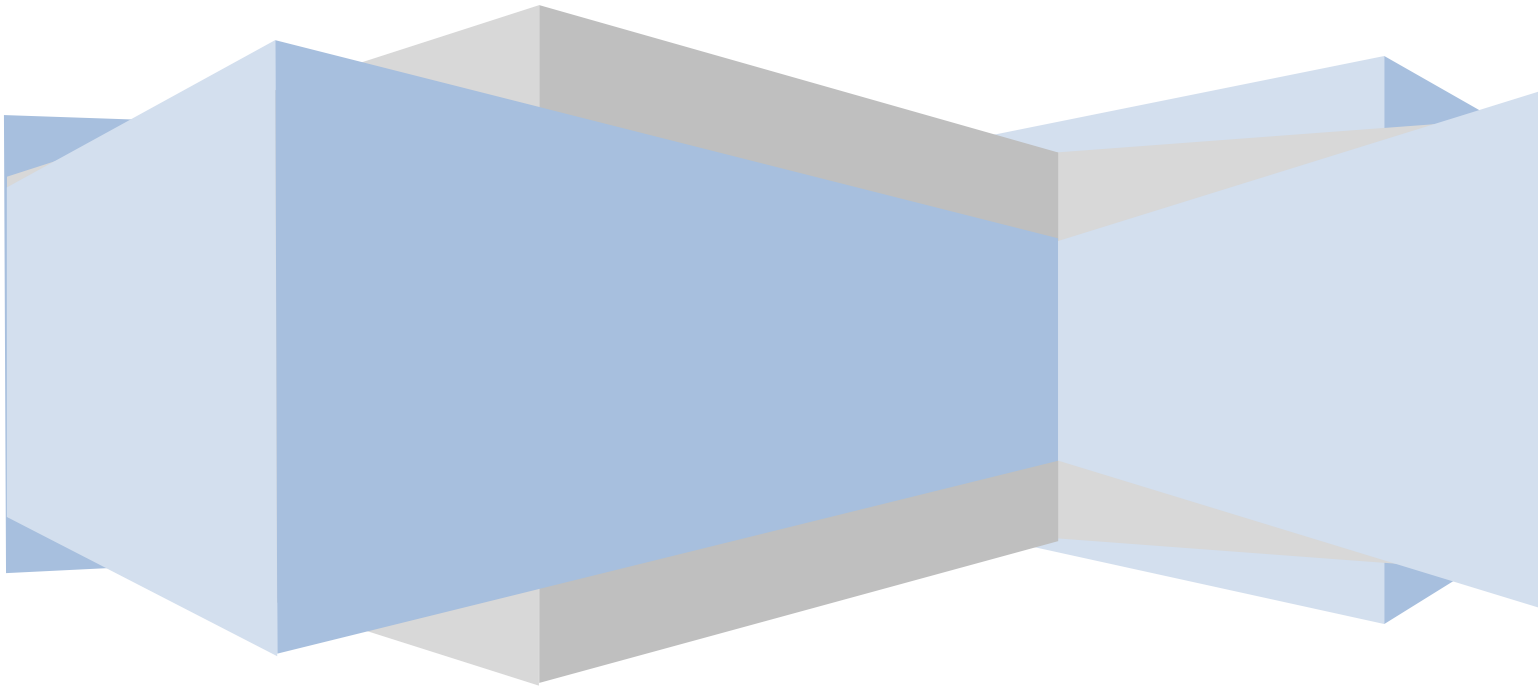
but the air-jet system actually evaluates the combined elasticity property of the various layers of soft tissues being tested. However, one should bear in mind that the measurement made by the air-jet system only focuses on a minute area of about 1 mm^2, so the finding is site specific. In contrast, the tensiometric evaluation represents the gross biomechanical property of a piece of excised skin wound tissue that is possibly contributed by various structures. In addition, the test made by the tensiometric machine is destructive in nature; the excised wound tissues are destroyed and the test cannot be repeated. The air-jet system, on the other hand, is a non-destructive measuring method that allows measurements to be made repeatedly over time, which is preferable in clinical use.

Our findings suggest that the stiffness of wound tissues measured by the air-jet system can provide mechanical signals to stimulate wound healing and contraction. A faster rate of recovery in stiffness than in the failure properties of healing skin wounds was observed in our test model. The air-jet-indentation system and tensiometer are two different methods of evaluation, but they both reflect the biomechanical properties of wound tissue. Previously, we could only rely on the material testing system to study the biomechanical properties of wound tissue in one particular direction i.e., parallel to the surface of the skin; now that with the breakthrough of the optical coherence tomography technology and the development of the air-jet indentation system, the unexplored aspects of the mechanical properties of wound tissues perpendicular to the skin surface can also be evaluated in a non-invasive manner. This will allow for a better understanding of the changes in the biomechanical properties of wound tissue throughout the various phases in the healing of a wound.

CHAPTER SEVEN

Summary and Conclusion

Clare YL Chao



Diabetic foot ulcers are always recalcitrant to treatment. A number of risk factors may predispose a diabetic foot to ulceration including neuropathy, vascular insufficiency, foot swelling, pathological changes in plantar skin and soft tissue properties. In order to develop strategies to prevent or manage diabetic ulcer, it is vital to have better understand of the pathophysiology of the diabetic ulcer. Moreover, a precise and quantitative evaluation method for ulcer healing is essential in making appropriate treatment decision and monitoring the treatment efficacy. Thus far, there is a lack of precise quantitative method to assess wound healing or ulcer tissue properties. The restoration of the mechanical properties of wound tissue is an important indicator on the quality of wound healing. So far, the biomechanical properties of diabetic foot ulcer tissues and changes in the biomechanical properties of skin wound tissues across different phases of wound healing process have not been explored. In laboratory work, the evaluation of wound tissue properties can be achieved by testing the excised wound specimen using Material Testing Systems *in vitro*. However, the testing procedures are disruptive that cannot be performed in clinical studies. An optical coherence tomography (OCT)-based air-jet indentation system is a novel non-contact method that has been recently developed for characterizing the biomechanical properties of soft tissues in a non-contact way and can potentially be used for assessing wound tissues properties *in vivo*. Therefore, the objectives of the four inter-related studies included in the present thesis were:

Study 1. To examine the morphological changes in plantar epidermal thickness and soft tissues properties of diabetic foot

Study 2. To explore the association of skin blood flow and oedema on epidermal thickness in the feet of people with and without diabetes

Study 3. To examine the stiffness of diabetic foot ulcer tissues and to evaluate the test/retest reliability of the newly developed optical-coherence tomography (OCT)-based air-jet indentation system on characterizing the biomechanical properties of wound tissues in human

Study 4. To examine the biomechanical properties of healing skin wounds *in vivo* using an air-jet indentation system and *in vitro* using a conventional material testing system in a rat model

7.1 Study 1 entitled: The epidermal thickness and biomechanical properties of plantar tissues in the diabetic foot

Diabetic foot is a common complication for people with diabetes but it is unclear whether the change is initiated from the skin surface, or underneath plantar tissues. Plantar skin is the primary site of physical interaction between a person and the ground and it acts as the first line of biological barrier in protecting the foot from mechanical trauma. The whole layer of plantar soft tissues is responsible for withstanding the dynamic loads of the whole body during ambulation. It comprises of a complex framework of skin, fat cells, fascia layers, and muscles. These tissue layers work together and serves as a cushion for optimizing load-bearing during ambulation. This study compared the thickness of epidermis, the thickness and stiffness of the total plantar soft tissue among people with diabetes with or without complications. Seventy-two people with diabetes, including 22 people with neuropathies, 16 foot ulcerations, 34 pure diabetics without complications; and 40 healthy controls participated in the study. The thickness of the epidermal layer of the plantar skin was examined using high frequency ultrasonography; the thickness and stiffness of the total plantar soft tissue were measured by using Tissue Ultrasound

Palpation System at the big toe, first, third, and fifth metatarsal heads, and heel pad. As compared with the control group, the average epidermal thickness of plantar skin was reduced by 15% in people with diabetic foot ulceration and 9% in people with neuropathy, but increased by 6% in pure diabetics. There was a 8% increase in total thickness of plantar soft tissue in the 3 diabetic groups at all testing sites (all $p<0.05$), except the first metatarsal head. The stiffness of plantar soft tissue was increased in all diabetic groups at all testing sites as compared with the control (all $p<0.05$). People with pure type 2 diabetes tend to have thicker epidermal plantar skin than their non-diabetic counterparts. In contrast, epidermal thinning of plantar skin occurs in people who have clinical manifestation of diabetic neuropathy and ulceration. On the other hand, the total thickness and stiffness of plantar soft tissue were increased in all subjects with diabetes, in which a greater increase was found in those complicated with neuropathy and ulceration. Such a decrease in epidermal thickness of plantar skin but an increase in stiffness of total plantar soft tissue makes the diabetic foot prone to tissue breakdown and hence ulcer formation.

7.2 Study 2 entitled: The association between skin blood flow and oedema on epidermal thickness in the diabetic foot

Skin blood flow provides nourishment and removes waste, which plays a vital role in maintaining the general health of the skin. Thus, any alteration in skin microvascular blood flow may have a significant impact on the general health of the skin. Indeed, it has been suggested that the earliest manifestation of a microcirculatory disorder is oedema. The development of interstitial oedema may impede oxygen diffusion to the skin. Yet the relationship among skin blood flow, oedema, and skin thickness in a diabetic foot remains unclear. The aim of this study

was to evaluate the association of skin blood flow and oedema and epidermal thickness in the feet of people with and without diabetic neuropathy as compared to a healthy control group. Eighty-seven subjects, including 19 people with diabetic neuropathy and foot ulceration, 35 people with diabetes but without neuropathy, and 33 non-diabetic healthy controls, participated in the study. High frequency ultrasonography was used to measure the epidermal thickness and oedema in papillary skin at the big toe as reflected by the thickness of the subepidermal low echogenic band (SLEB). The capillary nutritive blood flow was measured by the use of video capillaroscopy and skin blood flux was monitored by laser-Doppler flowmetry. There was a 7.2% increase in epidermal thickness in those with diabetes but without neuropathy, and a 16.5% decrease in people with diabetic neuropathy and foot ulceration as compared with the healthy controls (all $p<0.05$). The SLEB thickness increased in all diabetic subjects, to a greater degree in those with neuropathy and ulceration than in those without (64.7% vs. 11.8%, $p<0.001$). Skin blood flux was shown to be higher in the diabetic groups than in the controls (all $p<0.05$), but no significant differences were found in the resting nutritive capillary blood flow ($p>0.05$). A significant fair negative correlation ($p=0.002$, $r=-0.366$) was demonstrated between the SLEB and epidermal thickness at the pulp of the big toe, while no significant correlation was demonstrated between skin blood flow and epidermal thickness (all $p>0.05$). An increase in subepidermal oedema was demonstrated in people with diabetic neuropathy and ulceration, which may partly contribute to reduced epidermal thickness at the pulp of the big toe. This may subsequently lead to the breaking down of skin in the diabetic foot.

7.3 Study 3 entitled: A novel non-contact method to assess the biomechanical properties of wound tissue in humans

Chronic wounds such as diabetic foot ulcers, pressure ulcers, and venous ulcers are growing challenges for the health care system. An accurate evaluation of the physiological status of a chronic wound provides important information to guide appropriate treatment decisions and monitor the effectiveness of the treatment. Thus far, there is a lack of accurate quantitative methods to assess the properties of wound tissue. The present clinical documentation of wound healing status only measures the circumference and the color of an ulcer. However, the status of wound healing should not be judged solely by the appearance of the wound. The restoration of the mechanical properties of tissue strength is a potential quantitative outcome of the process of repairing the wound. Eight subjects with diabetes (7 males, 1 female), with a total of 10 foot ulcers between them, participated in the study. A total of 20 measuring sites located at either the central wound bed (n=10) or peri–ulcer areas (n=10) were evaluated with the air-jet indentation system. Four cycles of loading and unloading, each with the duration of approximately 36 s at an indentation rate of 0.08 mm/s, were carried out for each indentation trial. The test/retest reliability was examined at all measuring points. The average stiffness of the peri-ulcer area (0.47±0.15 N/mm) was significantly larger than that of the central wound bed area (0.35±0.23 N/mm; p=0.042). A high value for test/retest reliability was demonstrated (ICC: 0.986; Pearson's correlation: r=0.972, p<0.001). We demonstrated that the peri-ulcer area showed greater stiffness than the ulcerated tissues. This greater magnitude of hardness and inelasticity at the peri-ulcer region may scatter part of the contractile forces for wound contraction during the healing process.

We found the novel air-jet system is a reliable tool for characterizing the biomechanical properties of tissues around the wound in a non-contact way, which allows for better monitoring of wound tissues during the healing process. Information on the stiffness of wound tissue can provide insights for controlling the growth, remodeling, and function of cells. This new quantitative non-contact measurement method is a breakthrough in innovations relating to wound care. It may facilitate the carrying out of more high quality evidence-based research in wound management.

7.4 Study 4 entitled: *In vivo* and *in vitro* approaches to studying the biomechanical properties of healing wounds in rat skin

In laboratory research, the evaluation of wound healing stages can be achieved by testing the biomechanical property of excised wound tissue using Material Testing Systems such as assessed by tensiometer *in vitro.* However, the testing procedure is invasive and disruptive that cannot be performed in clinical studies. Moreover, the biomechanical properties measured in the excised wound specimen can be different from that of a living wound tissue *in vivo*. An optical coherence tomography-based air-jet indentation system is a novel non-contact method that has been recently developed for characterizing the biomechanical properties of soft tissues in a non-contact way. The aims of this study were to compare the biomechanical properties of wound tissue in terms of the load relaxation, ultimate tensile strength, ultimate tensile stress, stiffness and energy absorption capacity as measured by *in vitro* standard tensiometer and stiffness as measured by *in vivo* air-jet indentation system between normal unwounded skin, and in healing wounds following acute full-thickness incision in a rat model. Thirty male Sprague-

Dawley rats, each with a 6 mm full-thickness circular punch biopsied wound at each of the posterior hind limbs were used. The stiffness at both the wound central and the margins was measured repeatedly in five rats over the same wound sites to monitor the longitudinal change over various wound healing process (i.e., before wounding, and on Days 0, 3, 7, 10, 14 and 21 after wounding respectively) *in vivo* by using an optical coherence tomography-based air-jet indentation system. Five rats were euthanized at each time point and the biomechanical property of the wound tissues were assessed *in vitro* using a tensiometer. We found that the size of the wound shrank significantly in the initial few days, closing almost completely by Day 10. At the central wound bed region, the stiffness measured by the air-jet system was 16.9±2.2 N/m at the baseline pre-wounding stage, which increased significantly from Day 0 (19.8±5.3 N/m, 17.16%), reached its peak on Day 7 (52.1±20.6 N/mm, 208.28%), then decreased progressively until Day 21 (23.7±3.2 N/m, 40.24%). In contrast, the biomechanical parameters of skin wound samples measured by the tensiometer showed a marked reduction upon wounding, which then gradually increased with time (all $p<0.05$). On Day 21, the ultimate tensile strength and stress of the skin wound tissue was approaching 50% that of the unwounded skin; whereas the stiffness of the tissue recovered at a faster rate, reaching 97% of the pre-wounding status. Our results suggested that the stiffness of wound tissues could be an indicator for stimulating wound healing and contraction. It took less time for healing wound tissues to recover their mechanical stiffness than their maximal strength in rat skin. In conclusion, measurements made by the air-jet-indentation system and by material testing systems involve different principles, but both systems can reflect the biomechanical properties of wound tissue.

Overall, the present thesis demonstrated that the epidermal plantar skin is getting thinner and plantar soft tissues are stiffened for people with diabetes, particular for people who have neuropathy or ulceration. In addition, an increase in subepidermal oedema was demonstrated in people with diabetic neuropathy and ulceration, which may partly contribute to the reduced epidermal thickness at the pulp of big toe. All these changes may subsequently lead to skin breaking down in diabetic foot. This implies that diabetes associated changes in biomechanical properties of plantar skin, plantar soft tissues and foot swelling are potential risk factors leading to foot ulceration in people with diabetes. Therefore, regular examination of the sole of the foot in people with diabetes and proper shoes wear should be reinforced in order to prevent foot complications. As for the stiffness of diabetic foot ulcer tissues, we demonstrated that the peri-ulcer area is stiffer than the ulcerated tissues in a diabetic ulcer foot. This greater magnitude of hardness and inelasticity at the peri-ulcer region may scatter part of the contractile forces for wound contraction during the healing process. The newly developed OCT-based air-jet indentation system is a reliable tool for characterizing the stiffness of soft tissues around the wound in a non-contact way *in vivo*. We found that stiffness recovered at a faster rate than did the tensile strength in healing rat skin wounds. Measurement made by the air-jet-indentation system and material testing systems involves different principles, but both systems can reflects the biomechanical behaviour of wound tissues.

7.5 Implications

Overall, the present thesis demonstrated that people with diabetes, particularly for those with neuropathy or ulceration, the epidermal plantar skin become thinner and the plantar soft tissues stiffened. In addition, an increase in subepidermal oedema was demonstrated in people with diabetic neuropathy and ulceration, which may partly contribute to a reduction in epidermal thickness at the pulp of the big toe. All of these changes may subsequently lead to the breaking down of skin in the diabetic foot. This implies that there are diabetes-associated changes in the biomechanical properties of plantar skin, plantar soft tissues, and foot swelling. These changes are potential risk factors for developing foot ulceration in people with diabetes. Therefore, regular examinations of the sole of the foot of people with diabetes including plantar skin and plantar soft tissues, and control of foot swelling and the wearing of proper shoes should be reinforced in order to prevent foot complications.

Moreover, the present thesis presents the use of a novel OCT-based air-jet indentation system for characterizing the biomechanical properties of soft tissues. Because of its non-contact in nature, it is particularly useful on characterizing the biomechanical properties of delicate living tissues that requires for non-contact application such as wound tissues. Currently, there is a lack of quantitative measurement for monitoring chronic wound healing in clinical practice. The present study has demonstrated the reliability and validity of using this new assessment tool in clinical use. Previously, we could only rely on the material testing system to study the biomechanical properties of wound tissue invasively in one particular direction i.e., parallel to the surface of the skin; now that with the breakthrough of the optical coherence tomography technology and the development of the air-jet indentation

system, the unexplored aspects of the mechanical properties of wound tissues perpendicular to the skin surface can also be evaluated in a non-invasive manner. This would allow a better understanding of the changes in the biomechanical properties of wound tissue throughout various phases of wound healing.

7.6 Limitations of the studies

For examining the plantar skin morphology, plantar soft tissues properties, skin microcirculation and foot swelling (study 1 and 2), we used 10g monofilament and vibration perception threshold as a screening tools for neuropathy, subjects in our ulceration and neuropathy group may have relatively severe neuropathy. Therefore, our findings may be more applicable to people with more severe diabetic neuropathy but unable to generalize to people who have mild diabetic neuropathy. Also, our sample size is relatively small. For examining diabetic foot ulcer tissue properties (study 3), we examine the biomechanical properties of diabetic foot ulcers only in patients with mild ulcer grade only (Grade 1-2 according to Wagner Grading System), while we have not yet examined more serious cases (up to grade 5). For the animal study (study 4), we used a SD rat model to examine the changes in mechanical properties throughout different wound healing phases of skin. Our observed results may not be able to completely mimic the situations in human being due to the differences in skin morphology between human and rats. Moreover, histology and histochemical study with references to the changes in tissue mechanics was not conducted in the present study, future study should be conducted to clarify on this.

7.7 Clinical relevance

This study characterizes the morphological and biomechanical changes in diabetic foot ulcer, which certainly helps to understand more about pathophysiology of the disease, and helps to develop strategies to prevent foot ulceration in diabetes. Currently there is a lack of objective quantitative measurement for monitoring wound healing in clinical practice. The present study demonstrated the feasibility of using the OCT-based airjet indentation system for characterizing the biomechanical properties of tissues around the wound in a non-contact way, which allows for better monitoring of wound tissues during the healing process. Information on the stiffness of wound tissue can provide insights for controlling the growth, remodeling, and function of cells. This new quantitative non-contact measurement method is a breakthrough in wound care. It may facilitate more high quality evidence-based research in wound management.

7.8 Feasibility of clinical applications

A more in-depth understanding on the biology of the diseases could definitely aids to develop preventive strategies to manage diabetic foot related complications. In clinical practice, the evaluation of plantar skin morphology, soft tissue properties and foot swelling in a diabetic foot should be added as a routine health check of diabetic foot. The OCT-based air-jet indentation system is a newly developed novel device for characterizing the biomechanical properties of soft tissues such as wound. At the present moment, this system is mainly adopted in research related to wound care. More work is needed and the sample size should be increased to fine-tune the system before applying it to daily clinical practice in the near future.

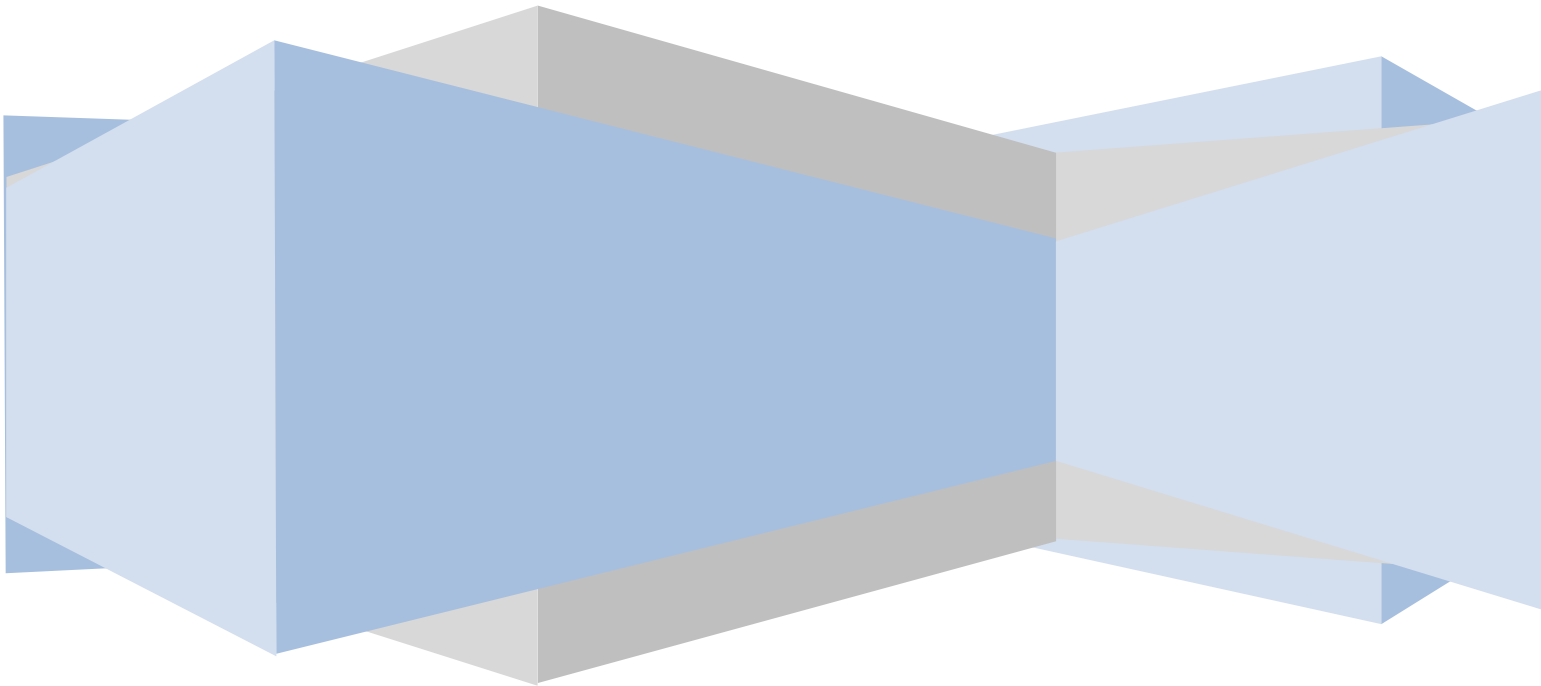
7.9 Recommendation for clinicians

Diabetic foot ulceration is mostly preventable. Therefore, early detection of the at-risk foot is of utmost importance in reducing the rate of foot ulceration. Various screening techniques have been proposed in the past including assessment on the loss of protective sensation, deformity, limited joint mobility, vascular status and callus formation. Our findings suggest that the evaluation of plantar skin morphology, plantar soft tissue properties, and foot swelling in people with diabetes should also be added. For patients who developed epidermal thinning and subepidermal oedema, they are at higher risk of developing foot ulceration. Barefoot walking should be discouraged for people who are at-risk of foor ulcer.

7.10 Suggestions for further study

Further studies can be conducted in diabetic people who have shorter history of diabetes, with or without foot ulceration, who have different severity of neuropathy with a larger sample sizes. It would be of interest to investigate whether the reduction of oedema in a diabetic foot could be a preventative measures for diabetic foot ulcer. In addition, the present study provides preliminary findings to support the use of the airjet indentation system on monitoring wound healing status. Further study with larger sample size and patients with different diabetes disease status should be included. Histology and histochemical examination should be performed in parallel with the wound mechanics should be conducted.

REFERENCES



References:

Abouaesha F, van Schie CH, Griffths GD, Young RJ, Boulton AJ (2001) Plantar tissue thickness is related to peak plantar pressure in the high-risk diabetic foot. *Diabetes Care* **24**, 1270-1274.

Allen J, Frame JR, Murray A (2002) Microvascular blood flow and skin temperature changes in the fingers following a deep nspiratory gasp. *Physiol Meas* **23**, 365-373.

American Diabetes Association (2003) Peripheral arterial disease in people with diabetes: Consensus statement. *Diabetes Care* **26**, 3333-3341.

American Diabetes Association (2004) Diagnosis and classification of diabetes mellitus. *Diabetes Care* **27**, S5-S10.

Amos AF, McCarty DJ, Zimmet P (1997) The rising global burden of diabetes and its complications: estimates and projections to the year 2010. *Diabet Med* **14**, S1-85.

Armstrong DG, Lavery LA, Bushman TR (1998) Peak foot pressures influence the healing time of diabetic foot ulcers treated with total contact casts. *J Rehabil Res Dev* **35**, 1-5.

Arora S, Smakowski P, Frykberg RG, Simeone LR, Freeman R, LoGerfo FW, Veves A (1998) Differences in foot and forearm skin microcirculation in diabetic patients with and without neuropathy. *Diabetes Care* **21**, 1339-1344.

Arora S, Pomposelli F, LoGerfo FW, Veves A (2002) Cutaneous microcirculation in the neuropathic diabetic foot improves significantly but not completely after successful lower extremity revascularization. *J Vasc Surg* **35**, 501-505.

Asadi MR, Torkaman G, Hedayati M (2010) The effect of electrical stimulation intensity on VEGF expression and biomechanical during wound. *World Academy of Science, Engineering and Technology* **45**, 684-686.

Athanasiou A, Karkambounas S, Batistatou A, Lykoudis E, Katsaraki A, Kartsiouni T, Papalois A, Evangelou A (2007). The effect of pulsed electromagnetic fields on secondary skin wound healing: an experimental study. Bioelectromagnetics 28(5): 362-8.

Balducci S, Iacobellis G, Parisi L, Di Biase N, Calandriello E, Leonetti F, *et al* (2006). Exercise training can modify the natural history of diabetic peripheral neuropathy. *J Diabetes Complications* **20**: 216-223.

Belcaro G, Nicolaides AN (1991) The venoarteriolar response in diabetics. *Angiology* **42**, 827-835.

Belcaro G, Nicolaides AN, Volteas N, Leon M (1992) Skin Flow The Venoarteriolar Response and Capillary Filtration in Diabetics. A 3-year Follow-Up. *Angiology* **43**, 490-495.

Bennett PJ, Stocks AE, Whittam DJ (1996) Analysis of risk factors for neuropathic foot ulceration in diabetes mellitus. *J Am Podiatr Med Assoc. 1996 Mar;86(3):112-6.* **86**, 112-116.

Berardesca E, Lévêque JL, Masson P, European Group for Efficacy Measurements on Cosmetics and Other Topical Products (EEMCO Group) (2002) EEMCO guidance for the measurement of skin microcirculation. *Skin Pharmacol Appl Skin Physiol* **15**, 442-456.

Bojsen-Moller F, Flagstad KE (1976) Plantar aponeurosis and internal architecture of the ball of the foot. *J Anat* **121**, 599-611.

Bongard O, Fagrell B (1990) Variations in laser Doppler flux and flow motion patterns in the dorsal skin of the human foot. *Microvasc Res* **39**, 212-222.

Boulais N, Misery L (2008) The epidermis: a sensory tissue. *Eur J Dermatol* **18**,119-127.

Boulton AJ, Scarpello JH, Ward JD (1982) Venous oxygenation in the diabetic neuropathic foot: evidence of arteriovenous shunting? *Diabetologia* **22**, 6-8.

Boulton A The pathway to ulceration: aetiopathogenesis. *In: Boulton A, Connor H, Cavanagh P (editors). The Foot in Diabetes. 2nd ed. Chichester, UK: Wiley; 1994*, pp37-48.

Boulton AJM (2000) The pathway to ulceration: aetiopathogenesis. *In The Foot in Diabetes, Boulton AJM, Connor H, Cavanagh PR (eds). Wiley: Chichester*, 19-31.

Boutayeb A, Boutayeb S (2005) The burden of non-communicable diseases in developing countries. *Int J Equity Health* **4**, 2.

Braiman-Wiksman L, Solomonik I, Spira R, Tennenbaum T (2007) Novel insights into wound healing sequence of events. *Toxicol Pathol* **35**, 767-779.

Brand P (1990) The diabetic foot. In: Rifkin H. Ponte D. editors. Elenberg and Rifkin's diabetes mellitus: theory and practice. *4th ed. New York: Elssevier*, pp829-849.

Brem H, Sheehan P, Rosenberg HJ, Schneider JS, Boulton AJ (2006) Evidence-based protocol for diabetic foot ulcers. *Plast Reconstr Surg* **117**, 193S-209S.

Brownlee M (2005) The pathobiology of diabetic complications: a unifying mechanism. *Diabetes* **54**, 1615-1625.

Buchner A, Erdfelder E and Faul F. How to use G*power. http://www.psycho.uni-duesseldorf.de/aap/projects/gpower/how_to_use_gpower.html

Caballero AE, Arora S, *et al.* (1999) Microvascular and macrovascular reactivity is reduced in subjects at risk for type 2 diabetes. *Diabetes* **48**, 1856-1862.

Cable NT (2006) Unlocking the secrets of skin blood flow. *J Physiol* **572**, 613.

Cacciatori V, Dellera A, Bellavere F, Bongiovanni LG, Teatini F, Gemma ML, Muggeo M (1997) Comparative assessment of peripheral sympathetic function by postural vasoconstriction arteriolar reflex and sympathetic skin response in NIDDM patients. *Am J Med* **102**, 365-370.

Cameron NE, Eaton SE, Cotter MA, Tesfaye S (2001) Vascular factors and metabolic interactions in the pathogenesis of diabetic neuropathy. *Diabetologia* **44**, 1973-1988.

Caselli A, Pham H, Giurini JM, Armstrong DG, Veves A (2002) The forefoot-to-rearfoot plantar pressure ratio is increased in severe diabetic neuropathy and can predict foot ulceration. *Diabetes Care* **25**, 1066-1071.

Caselli A, Rich J, Hanane T, Uccioli L, Veves A (2003) Role of C-nociceptive fibers in the nerve axon reflex-related vasodilation in diabetes. *Neurology* **60**, 297-300.

Cavanagh PR (1999) Plantar soft tissue thickness during ground contact in walking. *J Biomech* **32**, 623-628.

Cevera JJ, Bolton LL, Kerstein MD (1997) Options for diabetic patients with chronic heel ulcers. *J Diabetes Complications* **11**, 358-366.

Challoner A (1979) Photoelectric plethysmography for estimating cutaneous blood flow. *In: Rolfe P, editor. Non-invasive physiological measurement. London: Academic;*, p125-151.

Chao CY and Cheing GL (2009) Microvascular dysfunction in diabetic foot disease and ulceration. *Diabetes Metab Res Rev* **25**, 604-14.

Chao CY, Zheng YP, Cheing GL (2011) A novel noncontact method to assess the biomechanical properties of wound tissue. *Wound Repair Regen* **19**, 324-329.

Chao CY, Zheng YP, Huang YP, Cheing GL (2010) Biomechanical properties of the forefoot plantar soft tissue as measured by an optical coherence tomography-based air-jet indentation system and tissue ultrasound palpation system. *Clin Biomech (Bristol, Avon)* **25**, 594-600.

Charkoudian N (2003) Skin blood flow in adult human thermoregulation: how it works, when it does not, and why. *Mayo Clin Proc* **78**, 603-612.

Chen X, Lin W, Lu S, Shi Y, Zou J, Liu Z, *et al.* (2009) Insulin Prevents Latent Skin Lesions by Inhibiting the Generation of Advanced Glycation End Products in Streptozotocin-Induced Diabetic Rats. *Endocr Pathol* **20**, 163-169.

Chiang HY, Huang IT, Chen WP, Chien HF, Shun CT, Chang YC, *et al.* (1998) Regional difference in epidermal thinning after skin denervation. *Exp Neurol* **154**, 137-145.

Chittenden SJ, Shami SK (1993) Microvascular investigations in diabetes mellitus. *Postgrad Med J* **69**, 419-428.

Clark RA, Ghosh K, Tonnesen MG (2007) Tissue Engineering for Cutaneous Wounds. *J Invest Dermatol* **127**, 1018-1029.

Cobb J, Claremont D (2002) Noninvasive measurement techniques for monitoring of microvascular function in the diabetic foot. *The international journal of lower extremity wounds* **1**, 161-169.

Coffman JD, Cohen RA (1988) Alpha-adrenergic and serotonergic mechanisms in the human digit. *J Cardiovasc Pharmacol* **11**, S49-53.

Cohen, J. (1988) Statistical power analysis for the behavioral sciences. Hillsdale, New Jersey: Lawrence Erlbaum Associates.

Collier A, Matthews DM, Kellett HA, Clarke BF, Hunter JA (1986) Change in skin thickness associated with cheiroarthropathy in insulin dependent diabetes mellitus. *Br Med J*, 292:936.

Consensus statement (1988) Report and recommendations of the San Antonio conference on diabetic neuropathy. American Diabetes Association American Academy of Neurology. *Diabetes Care* **11**, 592-597.

Corr DT, Gallant-Behm CL, Shrive NG, Hart DA (2009) Biomechanical behavior of scar tissue and uninjured skin in a porcine model. *Wound Repair Regen* **17**, 250-259.

Culav EM, Clark CH, Merrilees MJ (1999) Connective Tissues: Matrix Composition and Its Relevance to Physical Therapy. *Phys Ther* **79**, 308-319.

Damci T, Oşar Z, Beyhan S, Ilkova H, Ozyazar M, Gorpe U, Bagriacik N (1999) Does instantaneous blood glucose affect vibration perception threshold measurement using biothesiometer? *Diabetes Res Clin Pract* **46**, 19-22.

Deckert T, Feldt-Rasmussen B, Borch-Johnsen K, Jensen T, Kofoed-Enevoldsen A (1989) Albuminuria reflects widespread vascular damage: the Steno hypothesis. *Diabetologia* **32**, 219-226.

Delmas L (2006) Best practice in the assessment and management of diabetic foot ulcers. *Rehabil Nurs* **31**, 228-234.

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

Dinh T, Veves A (2005) Microcirculation of the diabetic foot. *Curr Pharm Des* **11**, 2301-2309.

Dinh TL, Veves A (2006) Treatment of diabetic ulcers. *Dermatol Ther* **19**, 348-355.

Dobretsov M, Romanovsky D, Stimers JR (2009) Early diabetic neuropathy: triggers and mechanisms. *World J Gastroenterol* **13**, 175-191.

Doillon CJ, Dunn MG, Bender E, Silver FH (1985) Collagen fiber formation in repair tissue: development of strength and toughness. *Coll Relat Res* **5**, 481-492.

Doillon CJ, Dunn MG, Silver FH (1988) Relationship between mechanical properties and collagen structure of closed and open wounds. *J Biomech Eng* **110**, 352-356.

Duffin A C, Kidd R, Chan A, Donaghue K C. High plantar pressure and callus in diabetic adolescents. Incidence and treatment. J Am Podiatr Med Assoc 2003: 93: 214-220.

Duby JJ, Campbell RK, Setter SM, White JR, Rasmussen KA (2004) Diabetic neuropathy: an intensive review. *Am J Health Syst Pharm* **61**, 160-173.

Duckworth T, Boulton AJ, Betts RP, Franks CI, Ward JD (1985) Plantar pressure measurements and the prevention of ulceration in the diabetic foot. *J Bone Joint Surg Br* **67**, 79-85.

Duffin AC, Lam A, Kidd R, Chan AK, Donaghue KC (2002) Ultrasonography of plantar soft tissues thickness in young people with diabetes. *Diabet Med* **19**, 1009-1013.

Dunn MG, Silver FH (1983) Viscoelastic behavior of human connective tissues: relative contribution of viscous and elastic components. *Connect Tissue Res* **12**, 59-70.

Dyck PJ (1988) Detection, characterization, and staging of polyneuropathy: assessed in diabetics. *Muscle Nerve* **11**, 21-32.

Eckes B, Krieg T (2004) Regulation of connective tissue homeostasis in the skin by mechanical forces. *Clin Exp Rheumatol* **22**, S73-76.

Edsberg LE, Cutway R, Anain S, Natiella JR (2000) Microstructural and mechanical characterization of human tissue at and adjacent to pressure ulcers. *J Rehabil Res Dev* **37**: 463-471.

Edwards C, Marks R (1995) Evaluation of biomechanical properties of human skin. *Clin Dermatol* **13**, 375-380.

Elhadd TA, Kennedy G, Hill A, McLaren M, Newton RW, Greene SA, Belch JJ (1999) Abnormal markers of endothelial cell activation and oxidative stress in children, adolescents and young adults with type 1 diabetes with no clinical vascular disease. *Diabetes Metab Res Rev* **15**, 405-411.

Ellenberg M (1961) Absent deep reflexes: a diagnostic clue in unsuspected diabetes. *Am J Med Sci* **242**, 183-188.

El-Zawahry MB, Abdel El-Hameed El-Cheweikh HM, Abd-El-Rahman Ramadan S, Ahmed Bassiouny D, Mohamed Fawzy M (2007) Ultrasound biomicroscopy in the diagnosis of skin diseases. *Eur J Dermatol* **17**, 469-75.

Escoffier C, de Rigal J, Rochefort A, Vasselet R, Lévêque JL, Agache PG (1989) Age-related mechanical properties of human skin: an in vivo study. *J Invest Dermatol* **93**, 353-357.

Fagrell B (1990) Peripheral vascular disease, in Shepherd AP, Öberg Å (eds): Laser. *Doppler Flowmetry. Boston, Kluwer Academic,*, pp201-214.

Fagrell B (1995) Advances in microcirculation network evaluation: an update. *Int J Microcirc Clin Exp* **15**, 34-40.

Fagrell B, Hermansson IL, Karlander SG, Ostergren J (1984) Vital capillary microscopy for assessment of skin viability and microangiopathy in patients with diabetes mellitus. *Acta Med Scand Suppl* **687**, 25-28.

Farahani RM, Kloth LC (2008) The hypothesis of 'biophysical matrix contraction': wound contraction revisited. *Int Wound J* **5**, 477-482.

Fernando DJ, Masson EA, Veves A, Boulton AJ (1991) Relationship of limited joint mobility to abnormal foot pressures and diabetic foot ulceration. *Diabetes Care* **14**, 8-11.

Flynn MD, Edmonds ME, Tooke JE, Watkins PJ (1988) Direct measurement of capillary blood flow in the diabetic neuropathic foot. *Diabetologia* **31**, 652-656.

Flynn MD, Tooke JE (1995) Diabetic neuropathy and the microcirculation. *Diabet Med* **12**, 298-301.

Fontaine JL, Harkless LB, Davis CE, Allen MA, Shireman PK (2006) Current concepts in diabetic microvascular dysfunction. *J Am Podiatr Med Assoc* **96**, 245-252.

Forst T, Kann P, Pfützner A, Lobmann R, Schäfer H, Beyer J (1994) Association between "diabetic thick skin syndrome" and neurological disorders in diabetes mellitus. *Acta Diabetol* **31**, 73-77.

Frykberg RG (2002) Diabetic foot ulcers: Pathogenesis and management. *Am Fam Physician* **66**, 1655-1662.

Frykberg RG, Lavery LA, Pham H, Harvey C, Harkless L, Veves A (1998) Role of neuropathy and high foot pressures in diabetic foot ulceration. *Diabetes Care* **21**, 1714-1719.

Gamelli RL, He LK (2003) Incisional wound healing. Model and analysis of wound breaking strength. *Methods Mol Med* **78**, 37-54.

Gefen A (2003) Plantar soft tissue loading under the medial metatarsals in the standing diabetic foot. *Med Eng Phys* **25**, 491-499.

Gefen A, Megido-Ravid M, Azariah M, Itzchak Y, Arcan M (2001) Integration of plantar soft tissue stiffness measurements in routine MRI of the diabetic foot. *Clin Biomech (Bristol, Avon)* **16**, 921-925.

Giannini C, Dyck PJ (1995) Basement membrane reduplication and pericyte degeneration precede development of diabetic polyneuropathy and are associated with its severity. *Ann Neurol* **37**, 498-504.

Gingrass M, Perry L, Hill D, Wright T, Robson M, Fisher J (1998) Nondisruptive, in vivo method for biomechanical characterization of linear incision wound healing: preliminary report. *Plast Reconstr Surg* **102**, 801-806.

Gniadecka M (1995) Non-invasive methods for determination of oedema and water behavior in the skin. *Skin Res Technol* **1**, 55-60.

Gniadecka M (1996) Localization of dermal edema in lipodermatosclerosis, lymphedema, and cardiac insufficiency. High-frequency ultrasound examination of intradermal echogenicity. *J Am Acad Dermatol* **35**, 37-41.

Goldenberg S, Alex M, Joshi RA, Blumenthal HT (1959) Nonatheromatous peripheral vascular disease of the lower extremity in diabetes mellitus. *Diabetes* **8**, 261-273.

Goldman RJ, Salcido R (2002) More than one way to measure a wound: an overview of tools and techniques. *Adv Skin Wound Care* **15**, 236-243.

Golster H, Hyllienmark L, Ledin T, Ludvigsson J, Sjöberg F (2005) Impaired microvascular function related to poor metabolic control in young patients with diabetes. *Clin Physiol Funct Imaging* **25**, 100-105.

Goss SA, Johnston RL, Dunn F (1980) Compilation of empirical ultrasonic properties of mammalian tissues. II. *J Acoust Soc Am* **68**, 93-108.

Gregg EW, Beckles GL, Williamson DF, Leveille SG, Langlois JA, Engelgau MM, Narayan KM (2000) Diabetes and physical disability among older U.S. adults. *Diabetes Care* **23**, 1272-1277.

Groner W, Winkelman JW, Harris AG, Ince C, Bouma GJ, Messmer K, Nadeau RG (1999) Orthogonal polarization spectral imaging: a new method for study of the microcirculation. *Nat Med* **5**, 1209-1212.

Grunfeld C (1992) Diabetic foot ulcers: etiology, treatment and prevention. *Adv Intern Med* **37**, 103-132.

Hagisawa S, Shimada T (2005) Skin morphology and its mechanical properties associated with loading. In: Bader D, Bouten C, Colin D, Oomens C, eds. Pressure Ulcer Research: Current and Future Prospectives. Berlin, Germany: Springer-Verlag. Berlin, Germany: Springer-Verlag. pp161-185.

Hamdy O, Abou-Elenin K, LoGerfo FW, Horton ES, Veves A (2001) Contribution of nerve-axon reflex-related vasodilation to the total skin vasodilation in diabetic patients with and without neuropathy. *Diabetes Care* **24**, 344-340.

Hamilton R, Apesos J, Korostoff E (1970) Viscoelastic properties of healing wounds. *Plast Reconstr Surg* **45**, 274-278.

Hanna W, Friesen D, Bombardier C, Gladman D, Hanna A (1987) Pathologic features of diabetic thick skin. *J Am Acad Dermatol* **16,** 546-553.

Hashmi F, Malone-Lee J, Hounsell E (2006) Plantar skin in type II diabetes: an investigation of protein glycation and biomechanical properties of plantar epidermis. *Eur J Dermatol* **16**, 23-32.

Hayes WC, Keer LM, Herrmann G, Mockros LF (1972) A mathematical analysis for indentation tests of articular cartilage. *J Biomech* **5**, 541-551.

Henriksen L, Sejrsen P (1976) Local reflex in microcirculation in human cutaneous tissue. *Acta Physiol Scand* **98**, 227-231.

Henriksen O (1991) Sympathetic reflex control of blood flow in human peripheral tissues. *Acta Physiol Scand* **143**, 33-39.

Hinz B (2009) Tissue stiffness, latent TGF-β1 Activation, and mechanical signal transduction: Implications for the pathogenesis and treatment of fibrosis. *Curr Rheumatol Rep* **11**, 120-126.

Hinz B, Mastrangelo D, Iselin CE, Chaponnier C, Gabbiani G (2001) Mechanical tension controls granulation tissue contractile activity and myofibroblast differentiation. *Am J Pathol* **159**, 1009-1020.

Hoffman RP, Sinkey CA, Kienzle MG, Anderson EA (1993) Muscle sympathetic nerve activity is reduced in IDDM before overt autonomic neuropathy. *Diabetes* **42**, 375-380.

Houben AJ, Nieuwenhuijzen Kruseman AC, Bouhouch E, Slaaf DW, Schaper NC (1993) Peripheral macro- and microcirculation in short-term insulin-dependent diabetes mellitus: the role of prostaglandins in early haemodynamic changes. *Eur J Clin Invest* **23**, 662-667.

Hsieh ST, Chiang HY, Lin WM (2000) Pathology of nerve terminal degeneration in the skin. *J Neuropathol Exp Neurol* **59**: 297-307.

Hsieh ST, Choi S, Lin WM, Chang YC, Mcarthur JC, Griffin JW (1996) Epidermal denervation and its effects on keratinocytes and Langerhans cells. *J Neurocytol* **25**, 513-524.

Hsieh ST, Lin WM, Chiang HY, Huang IT, Ko MH, Chang YC, *et al* (1997) Skin innervation and its effects on the epidermis. *J Biomed Sci* **4**, 264-268.

Hsieh ST, Lin WM (1999) Modulation of keratinocyte proliferation by skin innervation. *J Invest Dermatol* **113**: 579-586.

Hsu CC, Tsai WC, Chen CP, Shau YW, Wang CL, Chen MJ, Chang KJ (2005) Effects of aging on the plantar soft tissue properties under the metatarsal heads at different impact velocities. *Ultrasound Med Biol* **31**, 1423-1429.

Hsu TC, Lee YS, Shau YW (2002) Biomechanics of the heel pad for type 2 diabetic patients. *Clin Biomech (Bristol, Avon)* **17**, 291-296.

Hsu TC, Wang CL, Shau YW, Tang FT, Li KL, Chen CY (2000) Altered heel-pad mechanical properties in patients with Type 2 diabetes mellitus. *Diabet Med* **17**, 854-859.

Hsu TC, Wang CL, Tsai WC, Kuo JK, Tang FT (1998) Comparison of the mechanical properties of the heel pad between young and elderly adults. *Arch Phys Med Rehabil* **79**, 1101-1104.

Huang D, Swanson EA, *et al.* (1991) Optical coherence tomography. *Science. 1991 Nov 22;254(5035):1178-81* **254**, 1178-1181.

Huang IT, Lin WM, Shun CT, Hsieh ST (1999) Influence of cutaneous nerves on keratinocyte proliferation and epidermal thickness in mice. *Neuroscience* **94**, 965-973.

Huang YP, Zheng YP, Wang SZ, Chen ZP, Huang QH, He YH (2009) An optical coherence tomography (OCT)-based air jet indentation system for measuring the mechanical properties of soft tissues. *Meas Sci Technol* **20**, 1-11.

Humeau A, Steenbergen W, Nilsson H, Strömberg T (2007) Laser Doppler perfusion monitoring and imaging: novel approaches. *Med Biol Eng Comput* **45**, 421-435.

Huntley AC, Walter RM Jr (1990) Quantitative determination of skin thickness in diabetes mellitus: relationship to disease parameters. *J Med* **21**, 257-264.

Iwase M, Imoto H, Murata A, Nakamura U, Nohara S, Uchizono Y, Iino K, Iida M (2007) Altered postural regulation of foot skin oxygenation and blood flow in patients with type 2 diabetes mellitus. *Exp Clin Endocrinol Diabetes* **115**, 444-447.

Jaap AJ, Hammersley MS, Shore AC, Tooke JE (1994) Reduced microvascular hyeraemia in subjects at risk of developing type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* **37**, 214-216.

Jaap AJ, Shore AC, Gartside IB, Gamble J, Tooke JE (1993) Increased microvascular fluid permeability in young type 1 (insulin-dependent) diabetic patients. *Diabetologia* **36**, 648-652.

Jaap AJ, Shore AC, Stockman AJ, Tooke JE (1996) Skin capillary density in subjects with impaired glucose tolerance and patients with type 2 diabetes. *Diabet Med* **13**, 160-164.

Jaap AJ, Shore AC, Tooke JE (1997) Relationship of insulin resistance to microvascular dysfunction in subjects with fasting hyperglycaemia. *Diabetologia* **40**, 238-243.

Jacobs MJ, Ubbink DT, Kitslaar PJ, Tordoir JH, Slaaf DW, Reneman RS (1992) Assessment of the microcirculation provides additional information in critical limb ischaemia. *Eur J Vasc Surg* **6**, 135-141.

Jaffer U, Aslam M, Standfield N (2008) Impaired hyperaemic and rhythmic vasomotor response in type 1 diabetes mellitus patients: a predictor of early peripheral vascular disease. *Eur J Vasc Endovasc Surg* **35**, 603-606.

Jeffcoate WJ, Harding KG (2003) Diabetic foot ulcers. *Lancet* **361**, 1545-1551.

Johnson JM, Brengelmann GL, Hales JR, Vanhoutte PM, Wenger CB (1986) Regulation of the cutaneous circulation. *Fed Proc* **45**, 2841-2850.

Johnstone MT, Creager SJ, Scales KM, Cusco JA, Lee BK, Creager MA (1993) Impaired endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. *Circulation* **88**, 2510-2516.

Jörneskog G, Brismar K, Fagrell B (1995a) Skin capillary circulation is more impaired in the toes of diabetic than non-diabetic patients with peripheral vascular disease. *Diabet Med* **12**, 36-41.

Jörneskog G, Brismar K, Fagrell B (1995b) Skin capillary circulation severely impaired in toes of patients with IDDM, with and without late diabetic complications. *Diabetologia* **38**, 474-480.

Jörneskog G, Brismar K, Fagrell B (1998) Pronounced skin capillary ischemia in the feet of diabetic patients with bad metabolic control. *Diabetologia* **41**, 410-415.

Jörneskog G, Fagrell B (1996) Discrepancy in skin capillary circulation between fingers and toes in patients with type 1 diabetes. *Int J Microcirc Clin Exp* **16**, 313-319.

Karthikesalingam A, Holt PJ, Moxey P, Jones KG, Thompson MM, Hinchliffe RJ (2010) A systematic review of scoring systems for diabetic foot ulcers. *Diabet Med* **27**, 544-549.

Kasalová Z, Prázný M, Skrha J (2006) Relationship between peripheral diabetic neuropathy and microvascular reactivity in patients with type 1 and type 2 diabetes mellitus -- neuropathy and microcirculation in diabetes. *Exp Clin Endocrinol Diabetes* **114**, 52-57.

Kawalec JS, Pfennigwerth TC, Hetherington VJ, Logan JS, Penfield VK, Flauto JA, Shearer PM (2004). A review of lasers in healing diabetic ulcers. The Foot. 14: 68-71.

Kellogg DL Jr (2006) In vivo mechanisms of cutaneous vasodilation and vasoconstriction in humans during thermoregulatory challenges. *J Appl Physiol* **100**, 1709-1718.

Kennedy L, Baynes JW (1984) Non-enzymatic glycosylation and the chronic complications of diabetes: an overview. *Diabetologia* **26**, 93-98.

Kennedy WR, Wendelschafer-Crabb G, Johnson T (1996) Quantitation of epidermal nerves in diabetic neuropathy. *Neurology* **47**, 1042-1048.

Khan F, Elhadd TA, Greene SA, Belch JJ (2000) Impaired skin microvascular function in children, adolescents, and young adults with type 1 diabetes. *Diabetes Care* **23**, 215-220.

Khodabandehlou T, Zhao H, Vimeux M, Le Dévéhat C (1997) The autoregulation of the skin microcirculation in healthy subjects and diabetic patients with and without vascular complications. *Clin Hemorheol Microcirc* **17**, 357-362.

Kiernan JA (1972) The involvement of mast cells in vasodilatation due to axon reflexes in injured skin. *Q J Exp Physiol Cogn Med Sci* **57**, 311-317.

Klaesner JW, Hastings MK, Zou D, Lewis C, Mueller MJ (2002) Plantar tissue stiffness in patients with diabetes mellitus and peripheral neuropathy. *Arch Phys Med Rehabil* **83**, 1796-1801.

Koïtka A, Abraham P, Bouhanick B, Sigaudo-Roussel D, Demiot C, Saumet JL (2004) Impaired pressure-induced vasodilation at the foot in young adults with type 1 diabetes. *Diabetes* **53**, 721-725.

Krishnan STM, Rayman G (2006) Microcirculation and Diabetic Foot. In: Boulton AJM, Peter R. Cavanagh PR, Rayman G. Foot in Diabetes, The (Fourth Edition). John Wiley & Sons, Ltd., pp41-50.

Ksander GA, Vistnes LM, Rose EH (1977) Excisional wound biomechanics, skin tension lines, and elastic contraction. *Plast Reconstr Surg* **59**, 398-406.

Kubo K, Morimoto M, Komuro T, Tsunoda N, Kanehisa H, Fukunaga T (2007) Age-related differences in the properties of the plantar flexor muscles and tendons. *Med Sci Sports Exerc* **39**, 541-547.

Lavery LA, Armstrong DG, Harkless LB (1996) Classification of diabetic foot wounds. *J Foot Ankle Surg* **35**, 528-531.

Ledoux WR, Blevins JJ (2007) The compressive material properties of the plantar soft tissue. *J Biomech* **40**, 2975-2981.

Lee AR (2005) Enhancing dermal matrix regeneration and biomechanical properties of 2nd degree-burn wounds by EGF-impregnated collagen sponge dressing. *Arch Pharm Res* **28**, 1311-1316.

Lee AR, Moon HK (2003) Effect of topically applied silver sulfadiazine on fibroblast cell proliferation and biomechanical properties of the wound. *Arch Pharm Res* **26**, 855-860.

Lefrandt JD, Bosma E, Oomen PH, Hoeven JH, Roon AM, Smit AJ, Hoogenberg K (2003) Sympathetic mediated vasomotion and skin capillary permeability in diabetic patients with peripheral neuropathy. *Diabetologia* **46**, 40-47.

Levy DM, Karanth SS, Springall DR, Polak JM (1989) Depletion of cutaneous nerves and neuropeptides in diabetes mellitus: an immunocytochemical study. *Diabetologia* **32**, 427-433.

Lindberger M, Schröder HD, Schultzberg M, Kristensson K, Persson A, Ostman J, Link H (1989) Nerve fibre studies in skin biopsies in peripheral neuropathies. I. Immunohistochemical analysis of neuropeptides in diabetes mellitus. *J Neurol Sci* **93**, 289-296.

Lioupis C (2005) The role of distal arterial reconstruction in patients with diabetic foot ischemia. *Int J Low Extrem Wounds* **4**, 45-49.

Lippens S, Denecker G, Ovaere P, Vandenabeele P, Declercq W (2005) Death penalty for keratinocytes: apoptosis versus cornification. *Cell Death Differ* **12**, 1497-1508.

Lupi O, Semenovitch I, Treu C, Bouskela E (2008) Orthogonal polarization technique in the assessment of human skin microcirculation. *Int J Dermatol* **47**, 425-431.

Malik RA, Newrick PG, Sharma AK, Jennings A, Ah-See AK, Mayhew TM, Jakubowski J, Boulton AJ, Ward JD (1989) Microangiopathy in human diabetic neuropathy: relationship between capillary abnormalities and the severity of neuropathy. *Diabetologia* **32**, 92-102.

Malik RA, Veves A, Masson EA, Sharma AK, Ah-See AK, Schady W, Lye RH, Boulton AJ (1992) Endoneurial capillary abnormalities in mild human diabetic neuropathy. *J Neurol Neurosurg Psychiatry* **55**, 557-561.

Mathura KR, Vollebregt KC, Boer K, De Graaff JC, Ubbink DT, Ince C (2001) Comparison of OPS imaging and conventional capillary microscopy to study the human microcirculation. *J Appl Physiol* **91**, 74-78.

Maty SC, Fried LP, Volpato S, Williamson J, Brancati FL, Blaum CS (2004) Patterns of disability related to diabetes mellitus in older women. *J Gerontol A Biol Sci Med Sci* **59**, 148-153.

Mayfield JA, Reiber GE, Sanders LJ, Janisse D, Pogach LM, American Diabetes Association (2004) Preventive foot care in diabetes. *Diabetes Care* **27**, S63-64.

McCauley RL, Li YY, Chopra V, Herndon DN, Robson MC (1994) Cytoprotection of human dermal fibroblasts against silver sulfadiazine using recombinant growth factors. *J Surg Res* **56**, 378-384.

McCauley RL, Li YY, Poole B, Evans MJ, Robson MC, Heggers JP, Herndon DN (1992) Differential inhibition of human basal keratinocyte growth to silver sulfadiazine and mafenide acetate. *J Surg Res* **52**, 276-285.

McGuire MF (1980) Studies of the excisional wound: I. Biomechanical effects of undermining and wound orientation on closing tension and work. *Plast Reconstr Surg* **66**, 419-427.

Meyer MF, Schatz H (1998) Influence of metabolic control and duration of disease on microvascular dysfunction in diabetes assessed by laser Doppler anemometry. *Exp Clin Endocrinol Diabetes* **106**, 395-403.

Meyer W, Neurand K, Radke B (1982) Collagen fibre arrangement in the skin of the pig. *J Anat* **134**, 139-148.

Mofid Y, Ossant F, Imberdis C, Josse G, Patat F (2006) In-vivo imaging of skin under stress: Potential of high-frequency (20 MHz) static 2-D elastography. *IEEE Trans Ultrason Ferroelectr Freq Control* **53**, 925-35.

Morris SJ, Shore AC, Tooke JE (1995) Responses of the skin microcirculation to acetylcholine and sodium nitroprusside in patients with NIDDM. *Diabetologia* **38**, 1337-1344.

Mortazavi S, Rahmani M, Rahnama A, Saeed-Pour A, Nouri E, Hosseini N, Aghaiee M (2009) The Stimulatory Effects of Topical Application of Radioactive Lantern Mantle Powder on Wound Healing. *Dose Response* **7**, 149-159.

Mueller MJ, Zou D, Lott DJ (2005) "Pressure gradient" as an indicator of plantar skin injury. *Diabetes Care* **28**, 2908-2912.

Murray H J, Young M J, Hollis S, Boulton A J. The association between callus formation, high pressures and neuropathy in diabetic foot ulceration. Diabet Med 1996: 13: 979-982.

Nabuurs-Franssen MH, Houben AJ, Tooke JE, Schaper NC (2002) The effect of polyneuropathy on foot microcirculation in Type II diabetes. *Diabetologia* **45**, 1164-1171.

Netten PM, Wollersheim T, Thien T, Lutterman JA (1996) Skin microcirculation of the foot in diabetic neuropathy. *Clin Sci* **91**, 559-565.

Noyes FR (1977) Functional properties of knee ligaments and alterations induced by immobilization: a correlative biomechanical and histological study in primates. *Clin Orthop Relat Res* **123**, 210-242.

Ng GY (2010) Comparing therapeutic ultrasound with microamperage stimulation therapy for improving the strength of Achilles tendon repair. *Connect Tissue Res* **52**, 178-82.

Ng GY, Chung PY, Wang JS, Cheung RT (2011) Enforced bipedal downhill running induces Achilles tendinosis in rats. *Connect Tissue Res.* **52**, 466-471.

Ng GY, Ng CO, See EK (2004) Comparison of therapeutic ultrasound and exercises for augmenting tendon healing in rats. *Ultrasound Med Biol* **30**, 1539-1543.

Nikkels-Tassoudji N, Henry F, Letawe C, Pierard-Franchimont C, Lefebvre P, Pierard GE (1996) Mechanical properties of the diabetic waxy skin. *Dermatology* **192,** 19-22.

Paron NG, Lambert PW (2000) Cutaneous manifestations of diabetes mellitus. *Prim Care* **27**, 371-383.

Parry DA, Barnes GR, Craig AS (1978) A comparison of the size distribution of collagen fibrils in connective tissues as a function of age and a possible relation between fibril size distribution and mechanical properties. *Proc R Soc Lond B Biol Sci* **203**, 305-321.

Parving HK, Viberti GC, Keen H, Christiansen JS, Lassen NA (1983) Hemodynamic factors in the genesis of diabetic microangiopathy. *Metabolism* **32**, 943-949..

Pascual Huerta J, Alarcón García JM (2007) Effect of gender, age and anthropometric variables on plantar fascia thickness at different locations in asymptomatic subjects. *Eur J Radiol* **62**, 449-453.

Pasquali-Ronchetti I, Baccarani-Contri M (1997) Elastic fiber during development and aging. *Microsc Res Tech* **38**, 428-435.

Pataky Z, Assal JP, Conne P, Vuagnat H, Golay A (2005) Plantar pressure distribution in Type 2 diabetic patients without peripheral neuropathy and peripheral vascular disease. *Diabet Med* **22**, 762-767.

Pathak AP, Silver-Thorn MB, Thierfelder CA, Prieto TE (1998) A rate-controlled indentor for in vivo analysis of residual limb tissues. *IEEE Trans Rehabil Eng* **6**, 12-20.

Payne C, Turner D, Miller K. Determinants of plantar pressures in the diabetic foot. J Diabetes Complications 2002: 16: 277-283.

Perrry D, Bharara M, Armstrong DG, Mills J (2012) Intraoperative fluorescence vascular angiography: during tibial bypass. *J Diabetes Sci Technol* **6**, 204-8.

Perry LC, Connors AW, Matrisian LM, Nanney LB, Charles PD, Reyes DP, Kerr LD, Fisher J (1993) Role of transforming growth factor-beta and epidermal growth factor in the wound-healing process: an in vivo biomechanical evaluation. *Wound Repair Regen* **1**, 41-46.

Pham HT, Economides PA, Veves A (1998) The role of endothelial function on the foot. Microcirculation and wound healing in patients with diabetes. *Clin Podiatr Med Surg* **15**, 85-93.

Picart C, Carpentier PH, Brasseur S, Galliard H, Piau JM (1998) Systemic sclerosis: blood rheometry and laser Doppler imaging of digital cutaneous microcirculation during local cold exposure. *Clin Hemorheol Microcirc* **18**, 47-58.

Pickett BP, Burgess LP, Livermore GH, Tzikas TL, Vossoughi J (1996) Wound healing. Tensile strength vs healing time for wounds closed under tension. *Arch Otolaryngol Head Neck Surg* **122**, 565-568.

Pittman J (2007) Effect of aging on wound healing: current concepts. *J Wound Ostomy Continence Nurs* **34**, 412-415.

Pollard T (2007) A new research focus for skin breakdown. *J Wound Care* **16**, 281.

Prakash S, Paul WE, Robbins PW (2007) Fibrosin, a novel fibrogenic cytokine, modulates expression of myofibroblasts. *Exp Mol Pathol* **82**, 42-48.

Raskin P, Pietri AO, Unger R, Shannon WA Jr (1983) The effect of diabetic control on the width of skeletal muscle capillary basement membrane in patients with type 1 diabetes mellitus. *N Engl J Med* **309**, 1546-1550.

Rayman G, Hassan A, Tooke JE (1986a) Blood flow in the skin of the foot related to posture in diabetes mellitus. *Br Med J* **292**, 87-90.

Rayman G, Malik RA, Sharma AK, Day JL (1995) Microvascular response to tissue injury and capillary ultrastructure in the foot skin of type I diabetic patients. *Clin Sci (Lond)* **89**, 467-474.

Rayman G, Williams SA, Spencer PD, Smaje LH, Wise PH, Tooke JE (1986b) Impaired microvascular hyperaemic response to minor skin trauma in type 1 diabetes. *Br Med J (Clin Res Ed)* **292**, 1295-1298.

Reddy GK, Stehno-Bittel L, Enwemeka CS (2001). Laser photostimultation accelerates wound healing in diabetic rats. Wound Repair and Regeneration. 9: 248-255.

Reiber GE, Lipsky BA, Gibbons GW (1998) The burden of diabetic foot ulcers. *Am J Surg* **176**, 5S-10S.

Reiber GE, Vileikyte L, Boyko EJ, del Aguila M, Smith DG, Lavery LA, Boulton AJ (1999) Causal pathways for incident lower-extremity ulcers in patients with diabetes from two settings. *Diabetes Care* **22**, 157-162.

Reihsner R, Melling M, Pfeiler W, Menzel EJ (2000) Alterations of biochemical and two-dimensional biomechanical properties of human skin in diabetes mellitus as compared to effects of in vitro non-enzymatic glycation. *Clin Biomech (Bristol, Avon)*; **15**: 379-386.

Rendell M, Bamisedun O (1992) Diabetic cutaneous microangiopathy. *Am J Med* **93**, 611-618.

Rendell M, Bergman T, O' Donnell G, Drobny E, Borgos J, Bonner RF (1989) Microvascular blood flow, volume, and velocity measured by laser Doppler techniques in IDDM. *Diabetes* **38**, 819-824.

Robertson DD, Mueller MJ, Smith KE, Commean PK, Pilgram T, Johnson JE (2002) Structural changes in the forefoot of individuals with diabetes and a prior plantar ulcer. *J Bone Joint Surg Am* **84-A**, 1395-1404.

Rossi M, Carpi A (2004) Skin microcirculation in peripheral arterial obliterative disease. *Biomed Pharmacother* **58**, 427-431.

Said G (2007) Diabetic neuropathy--a review. *Nat Clin Pract Neurol* **3**, 331-340.

Sandeman D, Shearman C (1999) Clinical aspects of lower limb ulceration. *In: Mani R, Falanga V, Shearman C, Sandeman D, editors. Chronic wound healing--clinical measurement and basic science. London: W.B. Saunders*, pp4-25.

Sandeman DD, Shore AC, Tooke JE (1992) Relation of skin capillary pressure in patients with insulin-dependent diabetes mellitus to complications and metabolic control. *N Engl J Med* **327**, 760-764.

Schultz-Ehrenburg U, Blazek V (2001) Value of quantitative photoplethysmography for functional vascular diagnostics. Current status and prospects. *Skin Pharmacol Appl Skin Physiol* **14**, 316-323.

Seirafi H, Farsinejad K, Firooz A, Davoudi SM, Robati RM, Hoseini MS, Ehsani AH, Sadr B (2009) Biophysical characteristics of skin in diabetes: a controlled study. *J Eur Acad Dermatol Venereol* **23**, 146-149.

Shakespeare W (2005) Natural course of wound repair versus impaired healing in chronic skin ulcers. In: Avi Shai and Howard Maibach. *Wound healing and ulcers of the skin. Diagnosis and therapy — The practical approach.* Springer Berlin Heidelberg, 7-17.

Shore AC (2000) Capillaroscopy and the measurement of capillary pressure. *Br J Clin Pharmacol* **50**, 501-513.

Shore AC, Jaap AJ, Tooke JE (1994) Capillary pressure in patients with NIDDM. *Diabetes* **43**, 1198-1202.

Shore AC, Price KJ, Sandeman DD, Green EM, Tripp JH, Tooke JE (1991) Impaired microvascular hyperaemic response in children with diabetes mellitus. *Diabet Med* **8**, 619-623.

Silver FH, Freeman JW, DeVore D (2001) Visoelastic properties of human skin and processed dermis. *Skin Res Technol* **7**, 18-23.

Silver FH, Kato YP, Ohno M, Wasserman AJ (1992) Analysis of mammalian connective tissue: relationship between hierarchical structures and mechanical properties. *J Long Term Eff Med Implants* **2**, 165-198.

Silver IA (1977) Local factors in tissue oxygenation. *J Clin Pathol Suppl (R Coll Pathol)* **11**, 7-13.

Singh N, Armstrong DG, Lipsky BA (2005) Preventing foot ulcers in patients with diabetes. *JAMA* **293**, 217-228.

Singh TP, Groehn H, Kazmers A (2003) Vascular function and carotid intimal-medial thickness in children with insulin-dependent diabetes mellitus. *J Am Coll Cardiol* **41**, 661-665.

Singleton JR, Smith AG, Russell JW, Feldman EL (2003) Microvascular complications of impaired glucose tolerance. *Diabetes* **52**, 2867-2873.

Smith D, Cullen MJ, Nolan JJ (2004) The cost of managing diabetic foot ulceration in an Irish hospital. *Ir J Med Sci* **173**: 89-92.

Sternberg M, Cohen-Forterre L, Peyroux J (1985) Connective tissue in diabetes mellitus: biochemical alterations of the intercellular matrix with special reference to proteoglycans, collagens and basement membrane. *Diabete Metab* **11**, 27-50.

Stevens MJ, Goss DE, Foster AV, Pitel D, Edmonds ME, Watkins PJ (1993) Abnormal digital pressure measurements in diabetic neuropathic foot ulceration. *Diabet Med* **10**, 909-915.

Swain ID, Grant LJ (1989) Methods of measuring skin blood flow. *Phys Med Biol* **34**, 151-175.

Tesfaye S, Malik R, Ward JD (1994) Vascular factors in diabetic neuropathy. *Diabetologia* **37**, 847-854.

Thomas DE, Elliott EJ, Naughton GA (2006) Exercise for type 2 diabetes mellitus. *Cochrane Database Syst Rev* **19**: CD002968.

Thomas VJ, Patil KM, Radhakrishnan S, Narayanamurthy VB, Parivalavan R (2003) The role of skin hardness, thickness, and sensory loss on standing foot power in the development of plantar ulcers in patients with diabetes mellitus--a preliminary study. *Int J Low Extrem Wounds* **2**, 132-139.

Tilton RG, Faller AM, Burkhardt JK, Hoffman PL, Kilo C, Williamson JR, (1985) Pericyte degeneration and acellular capillaries are increased in the foot of human diabetic patients. *Diabetologia* **28**, 895-900.

Tooke JE (1995) Microvascular function in human diabetes. A physiological perspective. *Diabetes* **44**, 721-726.

Tooke Je (1999) A Pathophysiological Framework for the Pathogenesis of Diabetic Microangiopathy, Oxford University Press, New York.

Tooke JE, Morris SJ, Shore AC (1996) Microvascular functional abnormalities in diabetes: the role of the endothelium. *Diabetes Res Clin Pract* **31 Suppl**, S127-132.

Truong AB, Khavari PA (2007) Control of keratinocyte proliferation and differentiation by p63. *Cell Cycle* **6**, 295-299.

Uccioli L, Mancini L, Giordano A, Solini A, Magnani P, Manto A, Cotroneo P, Greco AV, Ghirlanda G (1992) Lower limb arterio-venous shunts, autonomic neuropathy and diabetic foot. *Diabetes Res Clin Pract* **16**, 123-130.

Uccioli L, Monticone G, Durola L, Russo F, Mormile F, Mennuni G, Menzinger G (1994) Autonomic neuropathy influences great toe blood pressure. *Diabetes Care* **17**, 284-287.

Umapathi T, Tan WL, Loke SC, Soon PC, Tavintharan S, Chan YH (2007) Intraepidermal nerve fiber density as a marker of early diabetic neuropathy. *Muscle Nerve* **35**: 591-598.

Urbancic-Rovan V, Bernjak A, Stefanovska A, Azman-Juvan K, Kocijancic A (2006) Macro- and microcirculation in the lower extremities--possible relationship. *Diabetes Res Clin Pract* **73**, 166-173.

Urbancic-Rovan V, Stefanovska A, Bernjak A, Azman-Juvan K, Kocijancic A (2004) Skin blood flow in the upper and lower extremities of diabetic patients with and without autonomic neuropathy. *J Vasc Res* **41**, 535-45.

Vane JR, Anggård EE, Botting RM (1990) Regulatory functions of the vascular endothelium. *N Engl J Med* **323**, 27-36.

Veves A, Akbari CM, Primavera J, Donaghue VM, Zacharoulis D, Chrzan JS, DeGirolami U, LoGerfo FW, Freeman R (1998) Endothelial dysfunction and the expression of endothelial nitric oxide synthetase in diabetic neuropathy, vascular disease, and foot ulceration. *Diabetes* **47**, 457-463.

Veves A, Backonja M, Malik RA (2008) Painful diabetic neuropathy: epidemiology, natural history, early diagnosis, and treatment options. *Pain Med* **9**, 660-674.

Vinik AI, Erbas T, Park TS, Pierce KK, Stansberry KB (2001) Methods for Evaluation of Peripheral Neurovascular Dysfunction. *Diabetes Technol Ther* **3**, 29-50.

Vinik AI, Park TS, Stansberry KB, Pittenger GL (2000) Diabetic neuropathies. *Diabetologia* **43**, 957-973.

Vinik AI, Maser RE, Mitchell BD, Freeman R (2003) Diabetic autonomic neuropathy. *Diabetes Care* **26**, 1553-1579.

Vogt M and Ermert H (2005) Development and evaluation of a high-frequency ultrasound-based system for in vivo strain imaging of the skin. IEEE Trans Ultrason Ferroelectr Freq Control **52**, 375-85.

Wagh AA, Roan E, Chapman KE, Desai LP, Rendon DA, Eckstein EC, Waters CM (2008) Localized elasticity measured in epithelial cells migrating at a wound edge using atomic force microscopy. *Am J Physiol Lung Cell Mol Physiol* **295**, L54-60.

Wagner FW Jr (1981) The dysvascular foot: a system for diagnosis and treatment. *Foot Ankle Int* **2**, 64-122.

Walk D (2009) Role of skin biopsy in the diagnosis of peripheral neuropathic pain. *Curr Pain Headache Rep* **13**, 191-196.

Waller JM, Maibach HI (2006) Age and skin structure and function, a quantitative approach (II): protein, glycosaminoglycan, water, and lipid content and structure. *Skin Res Technol* **12**, 145-154.

Walmsley D, Wales JK, Wiles PG (1989) Reduced hyperaemia following skin trauma: evidence for an impaired microvascular response to injury in the diabetic foot. *Diabetologia* **32**, 736-739.

Ward JD (1997) Upright posture and the microvasculature in human diabetic neuropathy: a hypothesis. *Diabetes* **46**, S94-97.

White WL, Brody GS, Glaser AA, Marangoni RD, Beckwith TG, Must JS, Lehman JA Jr (1971) Tensiometric studies of unwounded and wounded skin: results using a standardized testing method. *Ann Surg* **173**, 19-25.

Whitney KA (2003) Foot deformities, biomechanical and pathomechanical changes associated with aging including orthotic considerations, Part II. *Clin Podiatr Med Surg* **20**, 511-526.

Wiernsperger NF (2001) In defense of microvascular constriction in diabetes. *Clin Hemorheol Microcirc* **25**, 55-62.

Wild S, Roglic G, Green A, Sicree R, King H (2004) Global prevalence of diabetes. Estimates for the year 2000 and projections for 2030. *Diabetes Care* **27**, 1047-1053.

Williams DF, Harrison ID (1977) The variation of mechanical properties in different areas of a healing wound. *J Biomech* **10**, 633-642.

Williams SB, Cusco JA, Roddy MA, Johnstone MT, Creager MA (1996) Impaired nitric oxide-mediated vasodilation in patients with non-insulin-dependent diabetes mellitus. *J Am Coll Cardio* **27**, 567-574.

Wilson SB, Jennings PE, Belch JJ (1992) Detection of microvascular impairment in type I diabetics by laser Doppler flowmetry. *Clin Physiol* **12**, 195-208.

Wright CI, Kroner CI, Draijer R (2006) Non-invasive methods and stimuli for evaluating the skin's microcirculation. *J Pharmacol Toxicol Methods* **54**, 1-25.

Yasuda H, Dyck PJ (1987) Abnormalities of endoneurial microvessels and sural nerve pathology in diabetic neuropathy. *Neurology* **37**, 20-28.

Yeung CK, Guo X, Ng YF (2006).Pulsed ultrasound treatment accelerates the repair of Achilles tendon rupture in rats. J Orthop Res; 24(2):193-201.

Yosipovitch G, Schneiderman J, van Dyk DJ, Chetrit A, Milo G, Boner G (1996) Impairment of the postural venoarteriolar response in young type 1 diabetic patients. A study by laser doppler flowmetry. *Angiology* **47**, 687-691.

Yvonne-Tee GB, Rasool AH, Halim AS, Rahman AR (2006) Noninvasive assessment of cutaneous vascular function in vivo using capillaroscopy, plethysmography and laser-Doppler instruments: its strengths and weaknesses. *Clin Hemorheol Microcirc* **34**, 457-473.

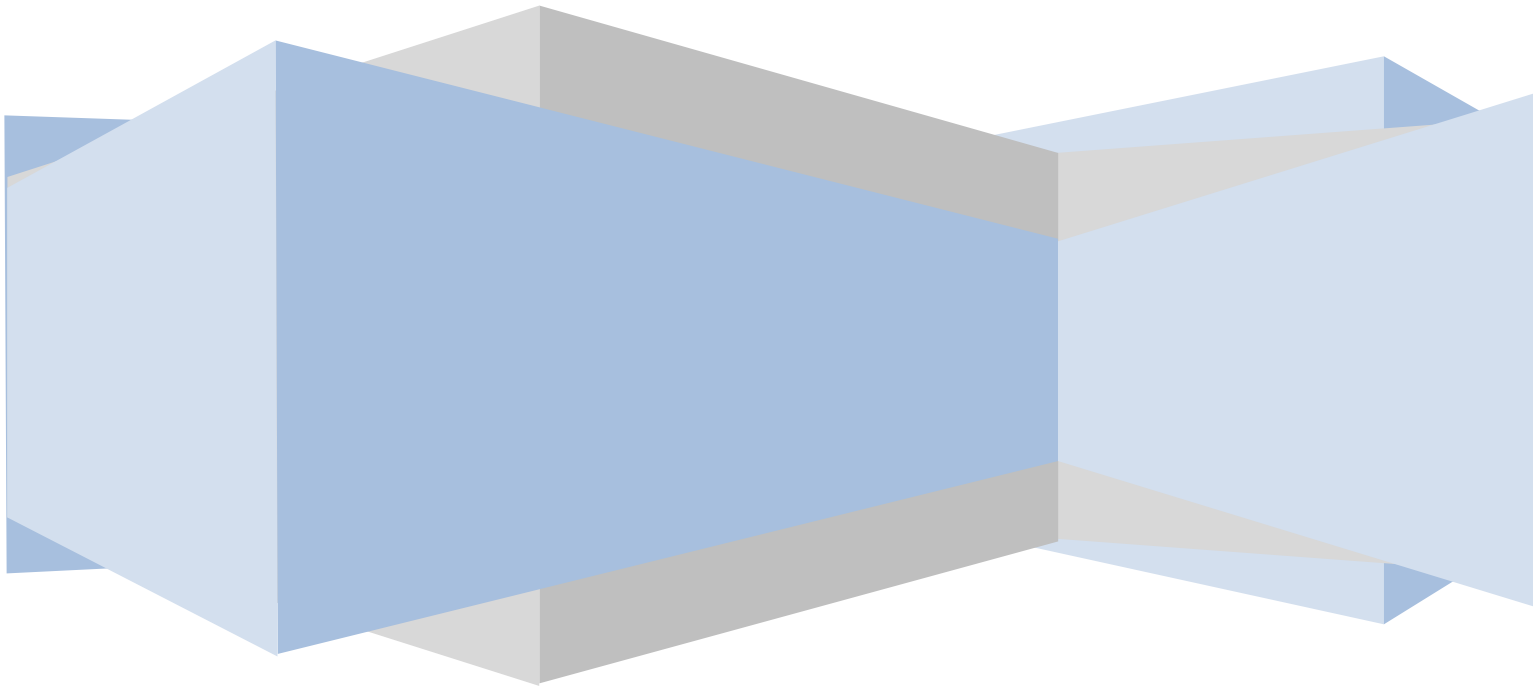
Zheng YP, Choi YK, Wong K, Chan S, Mak AF (2000) Biomechanical assessment of plantar foot tissue in diabetic patients using an ultrasound indentation system. *Ultrasound Med Biol* **26**, 451-456.

Zheng YP, Mak AF (1996) An ultrasound indentation system for biomechanical properties assessment of soft tissues in-vivo. *IEEE Trans Biomed Eng* **43**, 912-918.

Zheng Y, Mak AF (1999) Effective elastic properties for lower limb soft tissues from manual indentation experiment. *IEEE Trans Rehabil Eng* **7**, 257-267.

Zimny S, Dessel F, Ehren M, Pfohl M, Schatz H (2001) Early detection of microcirculatory impairment in diabetic patients with foot at risk. *Diabetes Care* **24**, 1810-1814.

APPENDICES



APPENDIX I

The Hong Kong Polytechnic University
Department of Rehabilitation Sciences

Research Project Informed Consent Form

Project Title:	Assessment of biomechanical properties of plantar tissue and diabetic foot ulcers
Chief Investigator:	Miss Clare Chao, PhD candidate, Department of Rehabilitation Science, The Hong Kong Polytechnic University
Co-investgator:	Dr. Gladys Cheing, PhD, Department of Rehabilitation Sciences, The Hong Kong Polytechnic University

Project information:
The purposes of this study are (i) to identify the factors that may lead to diabetic ulcer in Type 2 diabetic persons and (ii) to develop an innovative quantitative assessment for diabetic ulcer.

For the first part of the study, 4 groups of subjects will be compared: (a) Type 2 diabetic peripheral neuropathic subjects, (b) Type 2 diabetic neuroischaemic subjects, (c) Type 2 diabetic neuropathic subjects with history or present foot ulceration, and (d) non-diabetic control subjects. Comparison will be made for (i) plantar tissue thickness and elasticity (ii) skin microcirculation and morphology and (iii) foot skin temperature. High frequency ultrasound System, tissue ultrasound palpation system, laser Doppler flowmetry and skin thermometer sensor will be used for the assessment.

For the second part of this study, a novel non-contact air-jet optical indentation system will be developed for assessing the biomechanical properties of soft tissue. The reliability and validity of the machine will be tested on assessing the biomechanical properties of diabetic foot ulcers.

Subjects will be invited to go to the Hong Kong Polytechnic University for the measurements. All the measurements methods are absolutely safe with no known side-effects. Subjects will not be exposed to any painful stimulation during assessment. Subjects are under no obligation to take part in this study and free to withdraw from the study at any time during the sessions. Subject's personal information and data acquired from this study is confidential and will not be disclosed to people who are not related to this study.

If you have any complaint about the conduct of this research study, please do not hesitate to contact Mrs. Michelle Leung, Secretary of Departmental Research Committee of The Hong Kong Polytechnic University at tel. no. 2766 5397.

If you would like more information about this study, please contact Dr. Gladys Cheing at tel. no. 2766 6738. Thank you for your interest in participating in this study.

Consent:

I, _____, have been explained the details of this study. I voluntarily consent to participate in this study. I understand that I can withdraw from this study at any time without giving reasons, and my clinical management inside the hospital will not be affected for my withdrawal. I am aware of any potential risk in joining this study. I also understand that my personal information will not be disclosed to people who are not related to this study and my name or photograph will not appear on any publications resulted from this study.

I can contact the chief investigator, Dr Gladys Cheing at telephone 2766 6738 for any questions about this study. If I have complaints related to the investigator(s), I can contact Mrs Michelle Leung, secretary of Departmental Research Committee, at 2766 5397. I know I will be given a signed copy of this consent form.

Signature (subject):_____ Date: _____

Signature (witness):_____ Date: _____

Signature (researcher):_____ Date: _____

APPENDIX II

香港理工大學康復治療科學系科研同意書

科研題目： 糖尿病患者足底部軟組織生物力學特性檢測與足部潰瘍之相關研究

科研人員： 趙月蘭小姐，香港理工大學康復治療科學系博士學生
鄭荔英博士，香港理工大學康復治療科學系助理教授

科研內容：

本研究之目的包括: (一)驗明糖尿病患者足部潰瘍之成因、(二) 用定量檢測技術來驗查開放式糖尿病足部潰瘍傷口的軟組織變化。

此科研的第一部份研究對象包括: (一) 2 型糖尿病周邊神經病變患者、(二) 2 型糖尿病神經缺血性患者、(三) 過去或現在患有 2 型糖尿病周邊神經性足潰瘍患者、(四)非糖尿病健康人士。此研究將採用高頻超聲波、超聲波軟組織觸診檢測器、激光多普勒儀、皮膚溫度儀等技術來測試 2 型糖尿病患者及健康人士之皮膚組織形態變化、厚度與硬度變化、足部血液微循環、皮膚溫度等變化。

此研究的第二部份是研發使用噴氣式光學印壓方法檢測技術來測試開放傷口的軟組織變化，與及鑑定本治療測試的噴氣式光學印壓方法檢測系統的可靠度及準確度。

研究對象將被邀請到香港理工大學進行所有測試。

對研究對象和社會的益處：

此研究為糖尿病人患足部潰瘍危險因素和病發機理提供更有效的檢測及理解，研究結果將對今後治療治療糖尿病足提供寶貴資料。

潛在危險性：

此研究的所有測試都十分安全，沒有潛在危險性，測試過程中不會引起疼痛。參與者有權在任何時候、無任何原因放棄參與此次研究，不會導致任何懲罰或不公平對待。參與者的資料將不會洩露給與此研究無關的人員。

研究對象如對此項研究有疑問，可在辦工時間內致電鄭荔英博士(2766 6738)查詢。若研究對象對此研究有任何投訴，可聯繫梁女士（部門科研委員會秘書），電話：2766 5397。多謝閣下的積極參與。

同意書：

本人_____已瞭解此次研究的具體情況。本人願意參加此次研究，本人有權在任何時候、無任何原因放棄參與此次研究，而此舉將不會影響本人在院內所接受的治療與管理。本人明白參加此研究課題的潛在危險性以及本人的資料將不會洩露給與此研究無關的人員，我的名字或相片不會出現在任何出版物上。

若本人對此研究有何疑問，可致電研究負責人鄭荔英博士 (電話：2766 6738)查詢。若本人對此研究人員有任何投訴，可以聯繫梁女士（部門科研委員會秘書），電話：2766 5397。本人亦明白，參與此研究課題需要本人簽署一份同意書。

參與者簽名：_____ 日期：_____

見證人簽名：_____ 日期：_____

研究員簽名：_____ 日期：_____

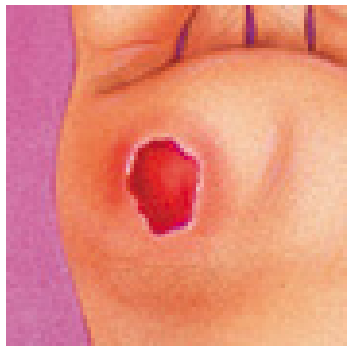
檢查須知

參加者年齡必須為四十至八十歲，並自願參加。

測試沒有潛在危險性，測試過程中不會引起疼痛。參與者有權在任何時候、無需任何原因放棄參與此次研究，而此舉不會影響其在醫院管理局或本院所接受的醫治。參與者的資料將不會洩露給與此研究無關的人員。參加者的名字不會出現在任何出版物上。

此項研究所使用的檢查方法，已通過香港理工大學及伊利沙伯醫院的安全評審。

是次檢查的費用全免。參加者的個人資料將受到保密。



閣下如有任何查詢
可在辦公時間內致電
伊利沙伯醫院物理治療師
趙姑娘
電話：2958 2602

如需預約檢查
請聯絡香港理工大學
康復治療科學系
物理治療部王姑娘
(研究助理)

電話：6404 1788

檢查地點
紅磡香港理工大學

伊利沙伯醫院
QUEEN ELIZABETH HOSPITAL

THE HONG KONG POLYTECHNIC UNIVERSITY
香港理工大學

糖尿病足

糖尿病

糖尿病患者無法製造或有效地使用體內的胰島素，以致體內血糖過高。暫時還未有根治糖尿病的方法，主要依靠多做運動及飲食控制。

常見糖尿病併發症所影響之器官或部位包括：

- 眼部
- 腦部
- 心臟
- 腎臟
- 足部

患者如能及早診斷，可有助減低糖尿病引起的併發症。

糖尿病足常見的問題

- 周邊神經病變，包括:足部感覺障礙、運動神經障礙、自律神經障礙
- 周邊動脈阻塞疾病
- 足部血液微循環改變
- 足部潰瘍，嚴重時，患者或須要接受截趾或截肢手術

足部感覺障礙是糖尿病足最常見的慢性併發症之一，患者足部感到麻木感、灼熱感、刺痛或鈍痛或感覺異常。他們常而對外物刺激反應降低，於夜間、冬天、下雨、喝酒會加重症狀。

預防勝於治療

根據保守的估計，1/10 的糖尿病患者曾因感覺障礙而尋求醫療幫助，末梢神經感覺異常，可以造成嚴重足部病變例如潰瘍，甚至須要接受截肢手術。另外，感覺障礙可增加跌倒受傷的風險。

免費檢查

參加者必需前往香港理工大學，由物理治療師提供檢查。此檢查將採用高頻超聲波、超聲波軟組織觸診檢測器、激光多普勒儀、錄影式微血管鏡檢測儀、皮膚溫度儀、噴氣式光標印壓檢測器等技術來測試第二型型糖尿病患者及健康人士之皮膚組織形態變化、厚度與硬度變化、足部血液微循環、皮膚溫度等變化、糖尿病足部潰瘍傷口的軟組織變化。

檢查範圍

- 足部觸感覺
- 足部皮膚組織厚度與硬度變化
- 足部血液微循環
- 足部潰瘍傷口的軟組織變化

所有檢查費用全免，參加者可即時獲知檢查結果。

香港特別行政區政府
衞生署
香港灣仔皇后大道東 213 號
胡忠大廈 17 及 21 樓

THE GOVERNMENT OF THE HONG KONG
SPECIAL ADMINISTRATIVE REGION
DEPARTMENT OF HEALTH,
WU CHUNG HOUSE, 17TH & 21ST FLOORS,
213 QUEEN'S ROAD EAST, WAN CHAI,
HONG KONG.

本署檔號 OUR REF.: (11-4) in DH/HA&P/8/2/4 Pt.4
來函檔號 YOUR REF.:
電　　話 TEL.: 2961 8645
圖文傳真 FAX.: 2127 7329

18 February 2011

CHAO Yuet Lan
Department of Rehabilitation Sciences
The Hong Kong Polytechnic University

Dear Sir/Madam,

Animals (Control of Experiments) Ordinance
Chapter 340

I refer to your application we received on 6 January 2011 and forward herewith the following licence(s) issued under the above Ordinance:-

Form 2 : Licence to Conduct Experiments

Your attention is drawn to regulations 4 and 5 of the Animals (Control of Experiments) Regulations as excerpted below:-

> "4. Records
> Every licensee shall keep up-to-date a book in the form set out as Form 6 in the Schedule in which he shall record the particulars therein indicated of all experiments performed by him.
>
> 5. Returns
> Every licensee shall render to the Director of Health on or before the 1st day of January each year a return in the form set out as Form 7 in the Schedule of all experiments performed by him during the preceding twelve months."

Copies of Form 6 and Form 7 are enclosed for your convenience. Failure to comply with either regulation 4 or regulation 5 is an offence, each offence punishable by a fine of HK$500 and to imprisonment for 3 months. Conviction of an offence against either regulation 4 or regulation 5 or failure to comply with either regulation may result in your licence being cancelled.

/P.2.....

Please also be reminded that if you wish to continue your experiments after the specified periods as stated on the above licence / endorsements / teaching permit, you should renew them at least two months before the end-dates. On the other hand, if you have completed or stopped your experiments before the specified periods, you should inform us immediately.

Yours faithfully,

(Dr Yonnie LAM)
for Director of Health

* *Remarks:-*

A "Code of Practice – Care and Use of Animals for Experimental Purposes" was prepared by the Agriculture, Fisheries and Conservation Department on the advice of the Animal Welfare Advisory Group.

Please visit the Agriculture, Fisheries and Conservation Department's website at <http://www.afcd.gov.hk/english/publications/publications_qua/files/code.pdf> for details of the Code of Practice.

Encl.

Form 2

Licence to Conduct Experiments

Name : CHAO Yuet Lan [Ref No.: (11-4) in DH/HA&P/8/2/4 Pt.4]
Address : Department of Rehabilitation Sciences, The Hong Kong Polytechnic University

By virtue of section 7 of the Animals (Control of Experiments) Ordinance, Chapter 340, the above-named is hereby licensed to conduct the type of experiment(s), at the place(s) and upon the conditions, hereinafter mentioned.

Type of experiment(s)

Rats will be used in the following experiments:

1. Under anaesthesia, full thickness skin wounds will be created on the posterior aspect of hind limb of the animals by biopsy punch. The wound will then be dressed with antiseptics and covered by gauze to prevent infection. Elasticity of wound bed soft tissues will be measured non-invasively using the optical coherence tomography (OCT)-based air jet indentation device. The limb of the animals will be fixed by a splint during measurement to avoid movement. At different time points, the animals will be sacrificed by carbon dioxide euthanasia. Specimens will be harvested from the wound bed for analyses.
2. Streptozotocin will be injected intraperitoneally into the animals to induce diabetes. Blood samples will be collected for glucose measurement. Diabetic rats will be divided into groups. Skin wounds will be created and managed as above. The animals will be treated with active pulsed electromagnetic field energy (PEMF) or monochromatic infrared energy (MIRE) at a distance of a few centimetres from the wound surface. No intervention will be given to control animals. At different time points, the animals will be sacrificed by carbon dioxide euthanasia. Wound closure will be evaluated and biopsies will be obtained for analyses.

In the above experiments, the conditions of the animals will be monitored.

Place(s) where experiment(s) may be conducted

(a) Orthopaedic Rehabilitation Research and Microscopy Laboratory (ST422), The Hong Kong Polytechnic University
(b) Centralised Animal Facilities (Y1423), The Hong Kong Polytechnic University

---

Conditions

1. Such experiment(s) may only be conducted for the following purposes-

   (a) To determine whether the OCT-based air jet indentation system can be used as a quantitative measurement to monitor phases of wound healing.
   (b) To examine the effects and the underlying mechanisms of PEMF and MIRE on promoting diabetic wound healing.

2. This licence is valid from 18 February 2011 to 17 February 2013

Dated 18 February 2011

DEPARTMENT OF HEALTH 衞生署 HONG KONG

Licensing Authority

PROCEEDINGS

of the

Eighth International Conference on the Ultrasonic Measurement and Imaging of Tissue Elasticity©

Vlissingen, Zeeland, The Netherlands
September 14 – 17, 2009

100 ***IN VIVO* MONITORING OF DIABETIC FOOT ULCER HEALING USING OCT AIR-JET INDENTATION.**

Clare Yuet–Lan Chao[1,4], Yong–Ping Zheng[2,3], Yan–Ping Huang[2], Gladys Lai–Ying Cheing[4].*
[1]Physiotherapy Department, Queen Elizabeth Hospital, Hong Kong, CHINA; [2]Health Technology and Informatics Department, [3]Research Institute of Innovative Products and Technologies, [4]Rehabilitation Sciences Department, The Hong Kong Polytechnic University, Hong Kong SAR, CHINA.

Background: Chronic wounds such as diabetic foot ulcers and pressure ulcers are growing challenges for the health care system. In UK, an estimated population of 500,000 people is diagnosed with a chronic wound each year, and the total cost of the health service is estimated to be 3% of the total health care budget [1]. The evaluation of wound status and monitoring of wound healing have become a priority from both research and clinical perspectives. Accurate evaluation of the physiologic status of a chronic wound provides important information to guide appropriate treatment decisions and monitoring treatment effectiveness. So far, there is a lack of accurate quantitative methods to assess wound tissue properties. The status of a wound should not be judged by its appearance only. A critical outcome of the wound repairing process is the restoration of the mechanical properties of tissue strength [2].

Aims: Firstly, to measure the mechanical properties of diabetic ulcer tissues by a newly developed non–contact optical coherence tomography (OCT)–based air–jet indentation system [3]. Secondly, to examine the test/retest reliability of the system for measuring the mechanical properties of diabetic ulcer tissues.

Methods: Figure 1 shows the experimental setup. Five male subjects with diabetic foot ulcers were recruited. Their mean age was 57±9 yrs. The mean duration of diabetes was 14.0±4.7 yr. A total of 7 wounds from the subjects was examined. All ulcers were located below the ankle, and the mean duration of the ulceration was 6.0±8.2 months. A total of 23 measuring sites at both the central wound bed (n=13) and peri–ulcer areas (n=10) were evaluated with the OCT–based air–jet indentation system. Four cycles of loading and unloading, with approximately 36 s duration at an indentation rate of 0.03 to 0.06 mm/s, were carried out for each indentation trial. Test/retest reliability was performed at all measuring points.

Results: Our results indicated that the peri–ulcer area tended to be stiffer than the central wound bed area (stiffness coefficient: 0.47±0.18 vs. 0.40±0.29 N/mm, respectively), but not significantly (p=0.47). Figure 2 shows a typical indentation curve along with time obtained on one subject. A high value of test/retest reliability was demonstrated (ICC: 0.986; Pearson's correlation: r=0.972, p<0.001).

Conclusions: Our preliminary findings demonstrated that the peri–ulcer showed a higher stiffness than the ulcerated tissues, but further study with more subjects is needed to confirm these findings. We found that the novel air–jet system is a reliable tool for characterizing the biomechanical properties of tissues around the wound in a non–contact way, which will allow better monitoring of wound tissues during the healing process *in vivo*. However, small sample size is a limitation of the study.

Acknowledgements: This project is partially supported by the HKSAR Research Grant Council (PolyU5318/05E, PolyU 5126/07E) and the Hong Kong Polytechnic University (J–BB69).

References:

[1] Pollard T. A new research focus for skin breakdown. J Wound Care 16: 281, 2007.
[2] Gamelli RL, He LK. Incisional wound healing. Model and analysis of wound breaking strength. Methods Mol Med 78:37–54, 2003.
[3] Huang, et al. An optical coherence tomography (OCT)–based air jet indentation system for measuring the mechanical properties of soft tissues. Meas Sci Technol 20: 15805, 2009.

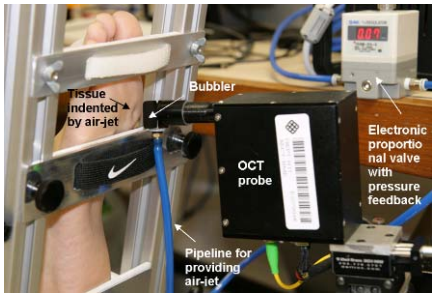


Figure 1: The experimental setup for the plantar tissue assessment using the OCT air–jet indentation system.

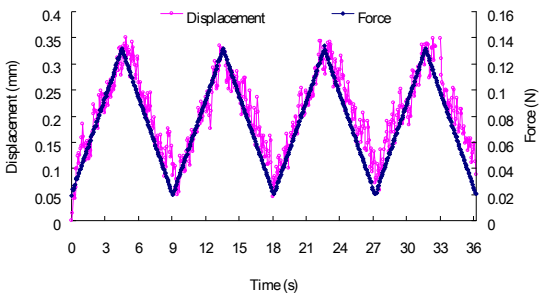


Figure 2: A typical indentation curve obtained on one subject at the central wound bed area.

* *indicates Presenter*

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(a) Service Priority
(b) Original Research in Health Care
(c) Work Place Harmony and Job Satisfaction
(d) Miscellaneous

Key-note Speakers:

Dr. Thomas TSANG, JP,
Controller, CHP

Prof. Andrew CHAN, SBS, JP
Director, EMBA Programme, CUHK

Kowloon Central Cluster
九龍中聯網

In vivo monitoring of diabetic foot ulcer healing using OCT air-jet indentation.

Chao CYL[1,4], Zheng YP[2,3], Huang YP[2], Cheing GLY[4]

[1]Physiotherapy Department, Queen Elizabeth Hospital; [2]Health Technology and Informatics Department, [3]Research Institute of Innovative Products and Technologies, [4]Department of Rehabilitation Sciences, The Hong Kong Polytechnic University

Introduction

Chronic wounds such as diabetic foot ulcers and pressure ulcers are growing challenges for the health care system. In UK, an estimated population of 500,000 people is diagnosed with a chronic wound each year, and the total cost of the health service is estimated to be 3% of the total health care budget.[1] The evaluation of wound status and monitoring of wound healing have become a priority from both research and clinical perspectives. Accurate evaluation of the physiologic status of a chronic wound provides important information to guide appropriate treatment decisions and monitoring treatment effectiveness. So far, there is a lack of accurate quantitative methods to assess wound tissue properties. The status of a wound should not be judged by its appearance only. A critical outcome of the wound repairing process is the restoration of the mechanical properties of tissue strength.[2]

Objectives

Firstly, to measure the mechanical properties of diabetic ulcer tissues by a newly developed non-contact optical coherence tomography (OCT)-based air-jet indentation system.[3] Secondly, to examine the test/retest reliability of the system for measuring the mechanical properties of diabetic ulcer tissues.

Methodology

Figure 1 shows the experimental setup. Five male subjects with diabetic foot ulcers were recruited. Their mean age was 57±9 years. The mean duration of diabetes was 14.0±4.7 years. A total of 7 wounds from the subjects were examined. All ulcers were located below the ankle, and the mean duration of the ulceration was 6.0±8.2 months. A total of 23 measuring sites at both the central wound bed (n=13) and peri-ulcer areas (n=10) were evaluated with the OCT-based air-jet indentation system. Four cycles of loading and unloading, with approximately 36 s duration at an indentation rate of 0.03 to 0.06 mm/s, were carried out for each indentation trial. Test/retest reliability was performed at all measuring points.

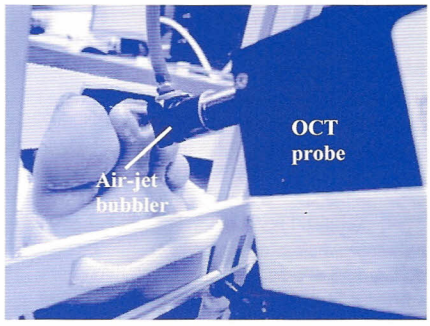

Figure 1: The experimental setup for the wound tissue assessment using the OCT air-jet indentation system.

Results

Our results indicated that the peri-ulcer area tended to be stiffer than the central wound bed area (stiffness coefficient: 0.47±0.18 vs. 0.40±0.29 N/mm, respectively), but not significant (p=0.47). Figure 2 shows a typical indentation curve along with time obtained on one subject. A high value of test/retest reliability was demonstrated (ICC: 0.986; Pearson's correlation: r=0.972, p<0.001).

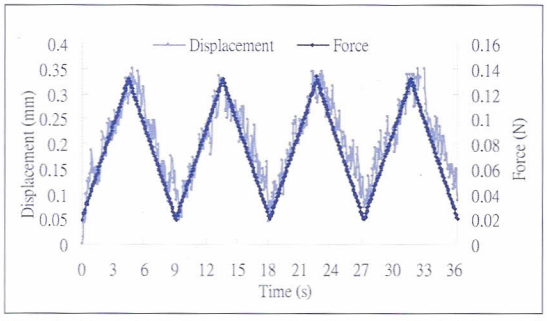

Figure 2: A typical indentation curve obtained on one subject at the central wound bed area.

Conclusion

Our preliminary findings demonstrated that the peri-ulcer showed a higher degree of stiffness than the ulcerated tissues, but further study with more subjects is needed to confirm these findings. We found that the novel air-jet system is a reliable tool for characterizing the biomechanical properties of tissues around the wound in a non-contact way, which will allow better monitoring of wound tissues during the healing process *in vivo*. However, small sample size is a limitation of the study.

References

1. Pollard T. A new research focus for skin breakdown. *J Wound Care* 2007;16: 281.
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In vivo Monitoring of Diabetic Foot Ulcer Healing Using OCT Air-jet Indentation

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[1]Physiotherapy Department, Queen Elizabeth Hospital; [2]Health Technology and Informatics Department,
[3]Research Institute of Innovative Products and Technologies, [4]Department of Rehabilitation Sciences,
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Introduction

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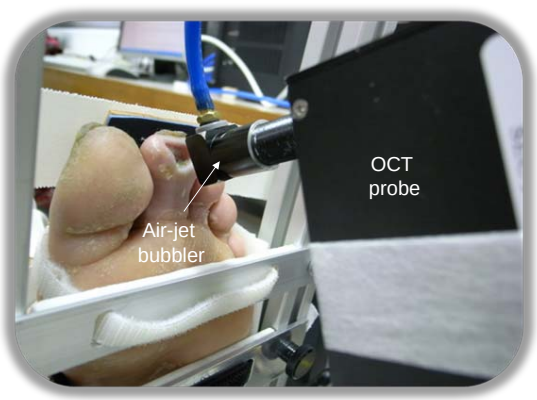

Figure 1. The experimental setup for the wound tissue assessment using the OCT air-jet indentation system.

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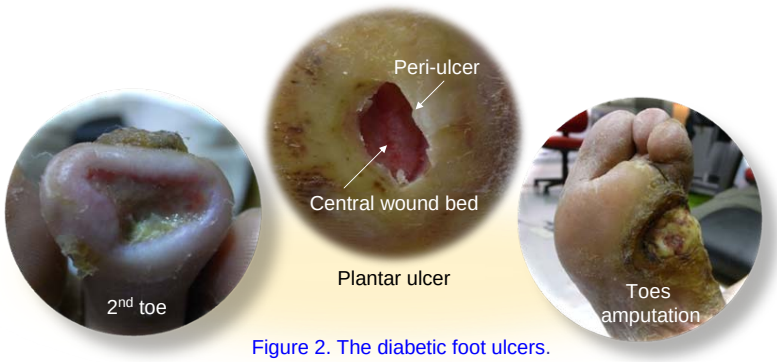

Figure 2. The diabetic foot ulcers.

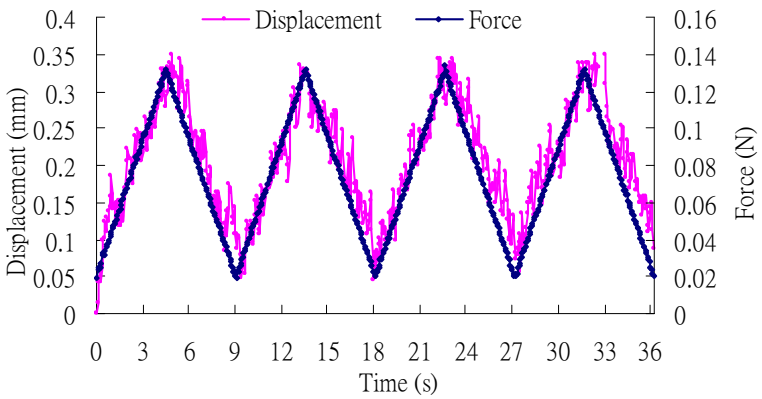

Figure 3. A typical indentation curve obtained on one subject at the central wound bed area.

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Our preliminary findings demonstrated that the peri–ulcer showed a higher degree of stiffness than the ulcerated tissues, but further study with more subjects is needed to confirm these findings. We found that the novel air–jet system is a reliable tool for characterizing the biomechanical properties of tissues around the wound in a non–contact way, which will allow better monitoring of wound tissues during the healing process *in vivo*. However, small sample size is a limitation of the study.

Key References

1. Pollard T. A new research focus for skin breakdown. *J Wound Care* 2007;16: 281.
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Physiotherapy Volume 97 Supplement S1

Wednesday 22 June 2011 12:00
RAI: Exhibit Halls 2 & 3

THE EPIDERMAL THICKNESS AND BIOMECHANICAL PROPERTIES OF PLANTAR TISSUES IN DIABETIC FOOT

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[1]The Hong Kong Polytechnic University, Department of Rehabilitation Science, Hong Kong SAR, China, [2]Queen Elizabeth Hospital, Physiotherapy Department, Hong Kong SAR, China, [3]The Hong Kong Polytechnic University, Department of Health Technology and Informatics, Hong Kong SAR, China

Purpose: The present study aimed to examine changes in the thickness of the epidermis and in the biomechanical properties of plantar soft tissue in people with type 2 diabetes mellitus with or without neuropathy and ulceration.
Relevance: Diabetic foot problem is a common complication for people with diabetes but it is unclear whether it initially develops from the surface of the skin, or underneath plantar tissues.
Participants: Seventy-two people with diabetes, including 22 people with neuropathies, 16 with foot ulcerations, 34 pure diabetics without complications; and 40 healthy controls participated in the study.
Methods: The thickness of the epidermal layer of the plantar skin was examined using high-frequency ultrasonography; the thickness and stiffness of the entire plantar soft tissue were measured by using the Tissue Ultrasound Palpation System at the big toe, the first, third, and fifth metatarsal heads, and the heel pad.
Analysis: A one-way analysis of variance (ANOVA) was used for all testing variables between the study groups.
Results: As compared with the control group, the average epidermal thickness of the plantar skin was reduced by 15% in people with diabetic foot ulceration and 9% in people with neuropathy, but had increased by 6% in pure diabetics. There was an 8% increase in the entire thickness of the plantar soft tissue in the three diabetic groups at all testing sites (all $p<0.05$), with the exception of the tissue at the first metatarsal head. The stiffness of the plantar soft tissue increased in all diabetic groups at all testing sites as compared with that in the control group (all $p<0.05$).
Conclusions: The epidermal plantar skin of people with diabetes becomes thinner and the plantar soft tissues stiffen, particularly if they also suffer from neuropathy or ulceration, which increases the risk of tissue breakdown and the formation of ulcers.
Implications: The present study demonstrated a decrease in the epidermal thickness of the plantar skin and reduced flexibility in the entire plantar soft tissues in people with diabetic neuropathy and ulceration. This implies that changes associated with diabetes in the biomechanical properties of plantar skin and soft tissues may be important factors leading to foot ulceration in people with diabetes. Therefore, the importance of regular examinations of the sole of the foot and the wearing of proper shoes should be impressed upon people with diabetes in order to prevent them from developing foot complications.
Key-words: 1. Epidermal thickness 2. plantar soft tissues 3. diabetic foot
Funding acknowledgements: The project was supported by the General Research Fund provided by the Research Grants Council of the Hong Kong SAR Government (PolyU 5126/07E).
Ethics approval: Ethical approval was obtained from the Hong Kong Polytechnic University and Queen Elizabeth Hospital.

Session name:
CHRONIC DISEASE MANAGEMENT 3

Programme track/theme:
Professional Practice - CHRONIC DISEASE MANAGEMENT

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Epidermal Thickness and Biomechanical Properties of Plantar Tissues in Diabetic Foot

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[1]Department of Rehabilitation Sciences, The Hong Kong Polytechnic University; [2]Physiotherapy Department, Queen Elizabeth Hospital;
[3]Health Technology and Informatics Department, The Hong Kong Polytechnic University, Hong Kong SAR, China

Introduction

Diabetic foot is a common complication for people with diabetes but it is unclear whether the change is initiated from the skin surface, or underneath plantar tissues.

Purpose

To examine the changes in epidermal thickness and biomechanical properties of plantar soft tissue in people with type 2 diabetes mellitus with or without neuropathy and ulceration.

Participants

72 people with diabetes, including:

(1) 22 people with neuropathies;

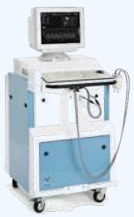
(2) 16 with foot ulcerations;

(3) 34 pure diabetics without complications;

and 40 healthy control subjects

Methods

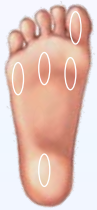
- Thickness of the **epidermal layer of the plantar skin** was examined using high frequency ultrasonography



- Thickness and stiffness of the **entire plantar soft tissue** were measured by using the Tissue Ultrasound Palpation System

Methods

5 Measuring points in the feet:



(i) pulp of big toe

(ii) 1st metatarsal head (MTH)

(iii) 3rd MTH

(iv) 5th MTH

(v) the heel pad

Data Analysis

A one-way analysis of variance (ANOVA) was used for all testing variables between the study groups.

Results

As compared with the control group, the **epidermal thickness** of the plantar skin was:

- ⬇ 15% in people with diabetic foot ulceration
- ⬇ 9% in people with neuropathy
- ⬆ 6% in pure diabetics (Fig 1 & 2)

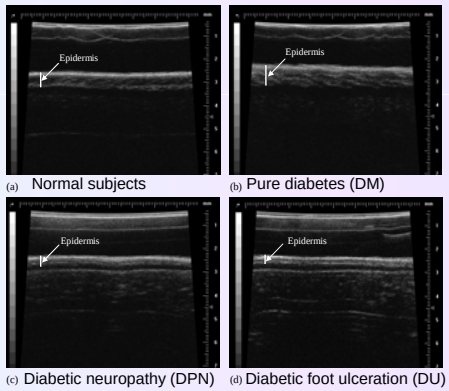


Fig 1. Ultrasonic images of the plantar skin

Results

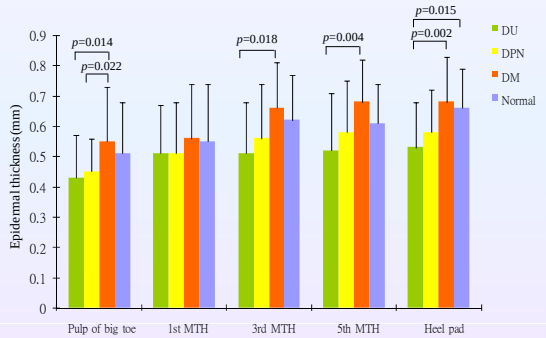


Fig 2. Epidermal thickness of plantar skin at different sites of the foot

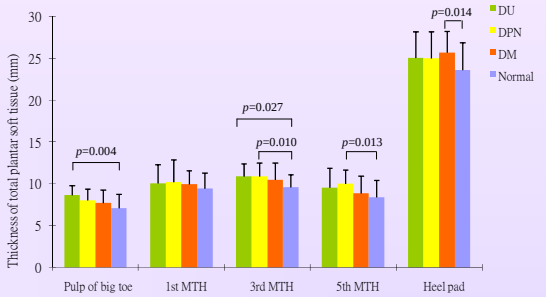


Fig 3. Total thickness of plantar soft tissues at different regions of the foot

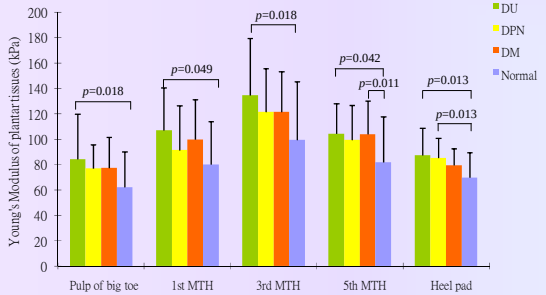


Fig 4. Stiffness of plantar soft tissues at different region of the foot

Results

- There was an 8% increase in the entire thickness of the plantar soft tissue in the three diabetic groups at all measuring points (all $p<0.05$) (Fig 3), with the exception of the tissue at the 1st metatarsal head.
- The stiffness of the plantar soft tissue increased in all diabetic groups at all measuring points as compared with that in the control group (all $p<0.05$) (Fig 4).

Discussion & Conclusion

The epidermal plantar skin of people with diabetes becomes thinner and the plantar soft tissues stiffen, particularly if they also suffer from neuropathy or ulceration, which increases the risk of tissue breakdown and the formation of ulcers.

Recommendations

- To prevent foot complication, regular examination of the sole of the foot in people with diabetes and proper shoes wear should be reinforced.
- Regular exercise may control blood sugar level and delay the onset of neuropathy.

Acknowledgements

Ethical approval was obtained from the Hong Kong Polytechnic University and Queen Elizabeth Hospital, Hong Kong.

The project was supported by the General Research Fund provided by the Research Grants Council of the Hong Kong SAR Government (PolyU 5126/07E).

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The Association Between Skin Blood Flow and Oedema on Epidermal Thickness in the Diabetic Foot

Gladys Lai-Ying Cheing[1]; Clare Yuet-Lan Chao[1,2]; Yong-Ping Zheng[3]

[1]Department of Rehabilitation Sciences, The Hong Kong Polytechnic University, Hong Kong

[2]Physiotherapy Department, Queen Elizabeth Hospital, Hong Kong

[3]Department of Health Technology and Informatics, The Hong Kong Polytechnic University, Hong Kong

Background

Skin blood flow plays an important role in maintaining the health of the skin. The development of interstitial oedema may impede oxygen diffusion to the skin. The aim of this study was to evaluate the association of skin blood flow and oedema and epidermal thickness in the feet of people with and without diabetic neuropathy as compared to a healthy control group.

Methods

Eighty-seven subjects, including 19 people with diabetic neuropathy and foot ulceration (DU group), 35 people with diabetes but without neuropathy (DM group), and 33 non-diabetic healthy controls, participated in the study. High frequency ultrasonography was used to measure the epidermal thickness and oedema in papillary skin at the big toe as reflected by the thickness of the subepidermal low echogenic band (SLEB). The capillary nutritive blood flow was measured by the use of video capillaroscopy and skin blood flux was monitored by laser-Doppler flowmetry.

Results

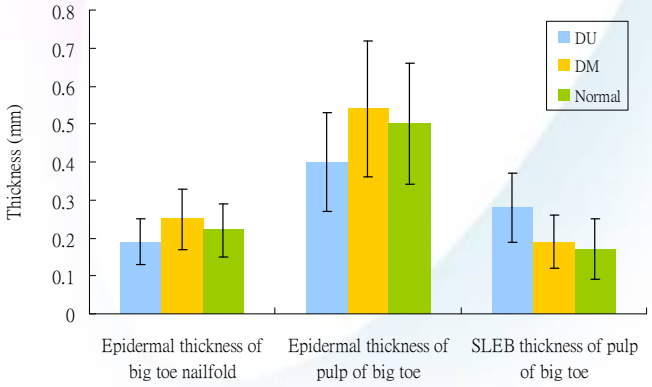

Figure 1. The epidermal and subepidermal low echogenic band thickness at different regions of the big toe in people with or without diabetes.

As compared with the healthy controls:
Epidermal thickness: ⇧ 7.2% in DM group
⇩ 16.5% in DU group (all p<0.05, Figure 1)
SLEB thickness: ⇧64.7% in DU group
⇧11.8% in DM group (p<0.001, Figure 1)

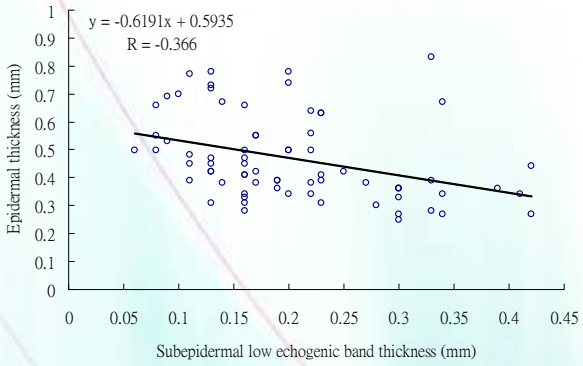

Figure 2. The correlation curve between epidermal and subepidermal low echogenic band thickness at the pulp of big toe.

A significant fair negative correlation (p=0.002, r=-0.366) was demonstrated between the SLEB and epidermal thickness at the pulp of the big toe (Figure 2).

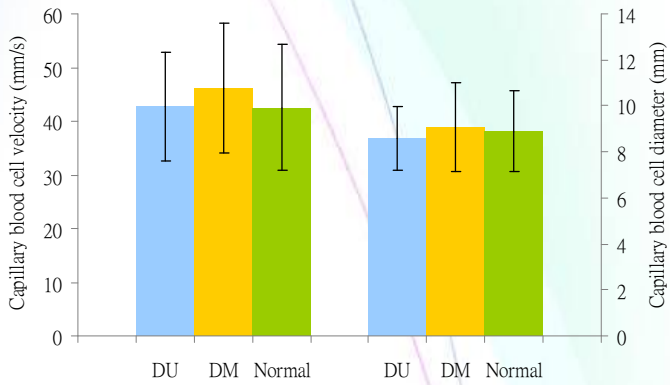

Figure 3. The capillary blood cell velocity and diameter at nailfold of big toe.

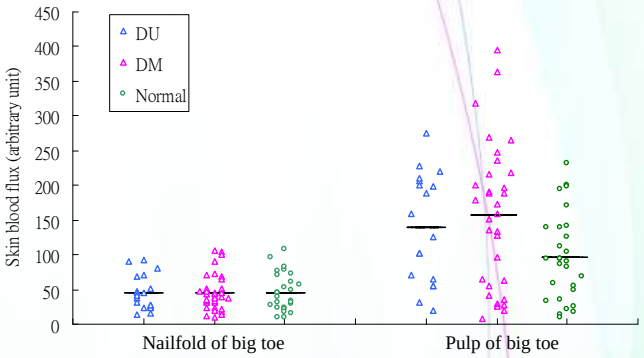

Figure 4. The resting skin blood flux at nailfold and pulp of big toe as measured by laser Doppler flowmetry. The horizontal bar represents the averaged mean value.

Skin blood flux was shown to be higher in the diabetic groups than in the controls (all p<0.05), but no significant difference was found in the resting nutritive capillary blood flow (p>0.05) (Figure 3 & 4).

Conclusions

An increase in subepidermal oedema was demonstrated in people with diabetic neuropathy and ulceration, which may partly contribute to reduced epidermal thickness at the pulp of the big toe. This may subsequently lead to the breaking down of skin in the diabetic foot.

Acknowledgement

This project is supported by the General Research Fund provided by the Research Grants Council of the Hong Kong SAR Government (PolyU5128/08E, PolyU 5600/11M).

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In Vivo and *In Vitro* Approaches to Studying the Biomechanical Properties of Healing Wounds in Rat

Thomas KW Ng[1]; Clare YL Chao[1,2]; Gabriel YF Ng[1]; Kwok-Kuen Cheung[1]; Li-Ke Wang[3]; Yong-Ping Zheng[3]; Gladys LY Cheing[1]

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Background

Cutaneous wounds can result from a break in the integrity of the skin due to punctures, abrasions, incisions, lacerations, burns, or ulcers. The main function of skin is to act as the body's biological barrier, shielding it from mechanical trauma. The biomechanical properties of skin are important to its normal functions, by allowing repeated reversible extensions and compressions during activities of daily living. The restoration of both the structural and functional properties of wounded tissue back to its pre-wounding state are good indicators of effective wound healing

Objectives

To examine the biomechanical properties of wound tissue in normal skin and in healing wounds of rat by using *in vivo* and *in vitro* approaches.

Methods

Thirty male Sprague-Dawley rats, each with a 6 mm full-thickness circular punch biopsied wound at both posterior hind limbs were used. The mechanical stiffness at both the central and margins of the wound was measured repeatedly in five rats over the same wound sites to monitor the longitudinal changes over time of before wounding, and on Days 0, 3, 7, 10, 14, and 21 after wounding *in vivo* by using an optical coherence tomography (OCT)-based air-jet indentation system (Fig 1). Five rats were euthanized at each time point, and the biomechanical properties of the wound tissues were assessed *in vitro* using a tensiometer (Fig 2).

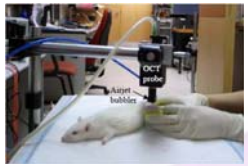

Fig 1. The *in vivo* OCT-based air-jet indentation system

Fig 2. The *in vitro* material testing system (tensiometer).

Results

1. The wound size decreased significantly in the initial few days, and closing was almost complete by Day 10.

Fig 3. The photos of the healing skin wound.

2. At the central wound bed region, the stiffness measured by the air-jet system was 16.9±2.2 N/m at the baseline pre-wounding stage. The stiffness increased significantly from Day 0 (19.8±5.3 N/m, 17.2%), peaked at Day 7 (52.1±20.6 N/m, 208.3%), then decreased progressively until Day 21 (23.7±3.2 N/m, 40.2%) (Fig 4).

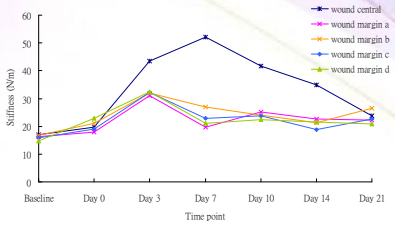

Fig 4. The changes in the stiffness of the healing wound tissues monitored longitudinally over a 3-week period as measured by the air-jet indentation system *in vivo*.

3. Various biomechanical parameters of the skin wound samples measured by the tensiometer are shown in Fig 6. It showed a marked reduction upon wounding, but a gradual increase with time (all $p<0.05$). On Day 21, the ultimate tensile strength of the skin wound tissue approached 50% of the normal skin; while the tissue recovered its stiffness at a faster rate, reaching 97% of its pre-wounded state (Fig 5).

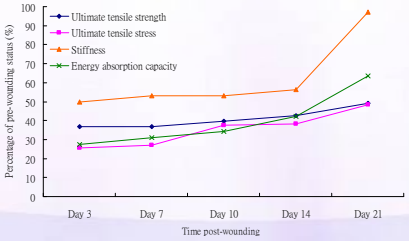

Fig 5.. Comparisons on the rate of recovery across time on variousvbiomechanical parameters obtained in the *in vitro* test with reference to the value ofpre-wounding status.

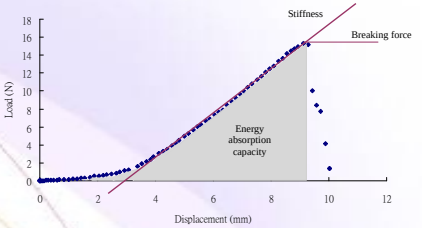

Fig 6. The load deformation curve of the healing rat skin wound as measured by using the material testing system. The slope of the steepest part represents the stiffness. and the shaped area under the curve till break point represents the energy absorption capacity..

Conclusions

Our results suggest that the stiffness of wound tissues can be an indicator for stimulating wound healing and contraction. It took less time for healing wound tissues to recover the mechanical stiffness than the maximal strength in rat skin. In conclusion, measurements made by the air-jet-indentation system and by material testing system involve different principles, but both systems can reflect the biomechanical properties of wound tissue.

Acknowledgements

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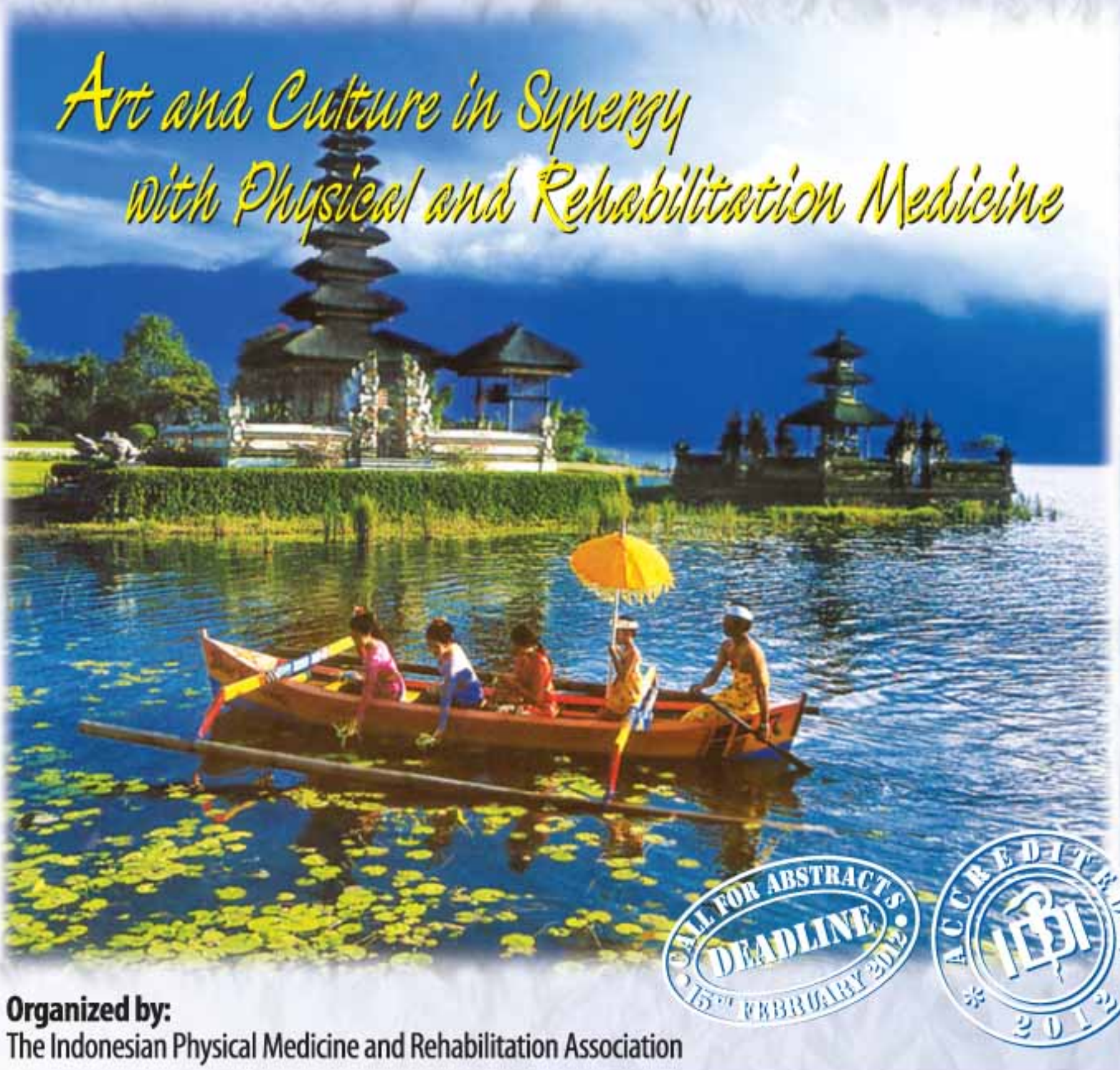

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FINAL ANNOUNCEMENT

Subepidermal Oedema In Diabetic Foot

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Background

Skin blood flow plays an important role in maintaining the health of the skin. The development of interstitial oedema may impede oxygen diffusion to the skin. The aim of this study was to evaluate the association of skin blood flow and oedema and epidermal thickness in the feet of people with and without diabetic neuropathy, as compared to a healthy control group.

Methods

Eighty-seven subjects, including 19 people with diabetic neuropathy and foot ulceration (DU group), 35 people with diabetes but without neuropathy (DM group), and 33 non-diabetic healthy controls, participated in the study. High frequency ultrasonography was used to measure the epidermal thickness and oedema in papillary skin at the big toe as reflected by the thickness of the subepidermal low echogenic band (SLEB). The capillary nutritive blood flow was measured by the use of video capillaroscopy and skin blood flux was monitored by laser-Doppler flowmetry.

Results

1.As compared with the healthy controls:
Epidermal thickness: ⇧ 7.2% in the DM group, ⇩16.5% in DU group (all $p<0.05$, Figure 1)
SLEB thickness: ⇧64.7% in the DU group, ⇧11.8% in DM group ($p<0.001$, Figure 1)

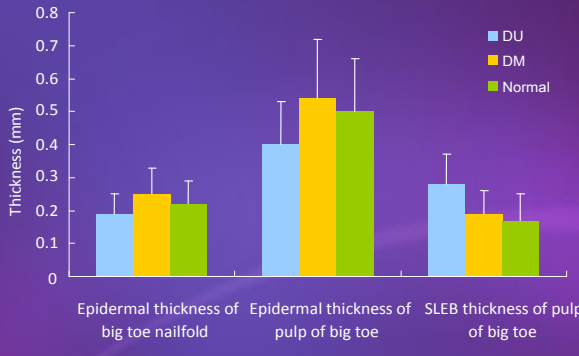

Figure 1. The epidermal and subepidermal low echogenic band thickness at different regions of the big toe in people with or without diabetes.

2. A significant fair negative correlation ($p=0.002$, $r=-0.366$) was demonstrated between the SLEB and epidermal thickness at the pulp of the big toe (Figure 2).

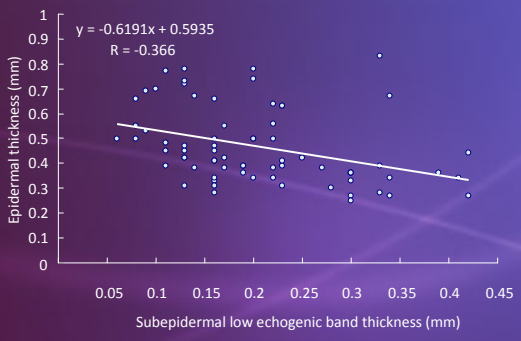

Figure 2. The correlation curve between epidermal and subepidermal low echogenic band thickness at the pulp of big toe.

3. Skin blood flux was shown to be higher in the diabetic groups than in the controls (all $p<0.05$), but no significant difference was found in the resting nutritive capillary blood flow ($p>0.05$) (Figure 3 & 4).

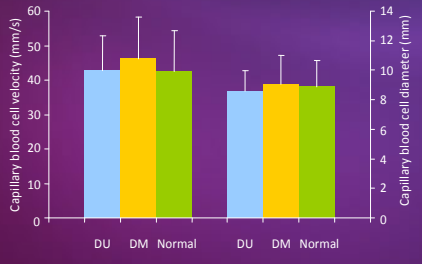

Figure 3. The capillary blood cell velocity and diameter at the nailfold of the big toe.

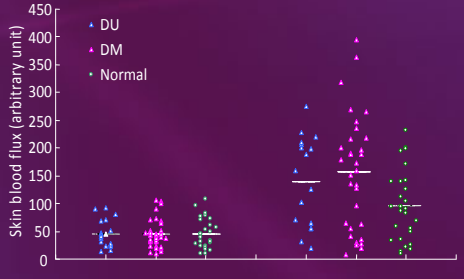

Figure 4. The resting skin blood flux at nailfold and pulp of big toe as measured by laser Doppler flowmetry. The horizontal bars represent the averaged mean value.

Conclusions

An increase in subepidermal oedema was demonstrated in people with diabetic neuropathy and ulceration, which may partly contribute to reduced epidermal thickness at the pulp of the big toe. This may subsequently lead to the breaking down of skin in the diabetic foot.

Acknowledgement

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REVIEW ARTICLE

Microvascular dysfunction in diabetic foot disease and ulceration

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Summary

Diabetic foot disease and ulceration is a major complication that may lead to the amputation of the lower limbs. Microangiopathy may play a significant role in the pathogenesis of tissue breakdown in the diabetic foot. However, the precise mechanisms of this process remain unclear and poorly understood. Microvasculature in the skin is comprised of nutritive capillaries and thermoregulatory arteriovenous shunt flow. It is regulated through the complex interaction of neurogenic and neurovascular control. The interplay among endothelial dysfunction, impaired nerve axon reflex activities, and microvascular regulation in the diabetic patient results in the poor healing of wounds. Skin microvasculature undergoes both morphologic changes as well as functional deficits when parts of the body come under stress or injury. Two important theories that have been put forward to explain the abnormalities that have been observed are the haemodynamic hypothesis and capillary steal syndrome. With advances in medical technology, microvasculature can now be measured quantitatively. This article reviews the development of microvascular dysfunction in the diabetic foot and discusses how it may relate to the pathogenesis of diabetic foot problems and ulceration. Common methods for measuring skin microcirculation are also discussed. Copyright © 2009 John Wiley & Sons, Ltd.

Keywords microcirculation; foot ulceration; foot disease; skin; diabetes

Introduction

Microvascular dysfunction is an integral component of the pathological complications that occur in the diabetic foot. However, the aetiology of microvascular abnormalities in the pathogenesis of diabetic foot ulceration has not yet been established. The development in diabetics of foot ulceration in the presence of a strong peripheral pulse has led to the hypothesis that microvascular dysfunction plays a significant role in the breaking down of tissues in the diabetic foot. The amputation of a lower limb then becomes a possibility which, should it occur, would cause great distress to a patient and impose a significant burden on health care resources. Therefore, much more research on diabetic foot problems or ulceration is needed.

Types of diabetic foot ulceration and their causal pathway

Diabetic foot ulceration can be classified as vascular (10%), neuropathic (40%), or neuro-ischaemic (40%) in origin [1,2]. Vascular ulcer usually refers to the absence of foot pulses, mainly at the posterior tibial and dorsalis

pedis areas, coupled with an abnormal ankle brachial index of less than 0.9. Neuro-ischaemic ulcer is a further impairment of foot sensation to vascular ulcer [3]. Pure neuropathic ulcer is characterized by no clinical evidence of macrovascular disease, along with the loss of the protective sensation associated with diabetic sensory neuropathy [4]. The most common causal pathway to diabetic foot ulceration can be identified as a combination of the loss of sensation, abnormal foot structure and stress, unperceived trauma, and poor management of associated foot injury [5]. Large vessel diseases have been considered the major cause of ischaemic foot ulceration, and early revascularization in diabetics with peripheral arterial disease has been found to reduce the risk of associated ulceration and subsequent amputation [6]. While occlusive small vessel diseases have been refuted to exist in the diabetic foot [7], it has been postulated that the alteration of diabetic foot microcirculation is an important factor in the poor healing of wounds associated with chronic diabetic foot ulcerations [8]. A thorough understanding of the specific microvascular impairments in diabetes is therefore helpful in consolidating the concepts involved in the pathogenesis of diabetic foot disease.

Microvascular abnormalities in diabetes

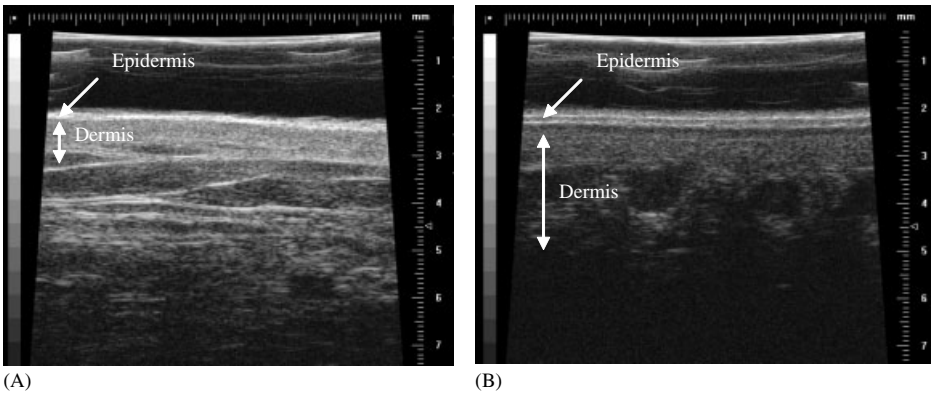
Microvasculature abnormalities specific to diabetics include micro-angiopathic complications of the retina, kidneys, skin, and peripheral nervous systems. What these tissues have in common is the insulin-dependent intracellular accumulation of glucose. Hyperglycemia is the unique central causative factor in vascular abnormalities induced by diabetes, including impaired vascular permeability, vascular tone, and the auto-regulation of blood flow [8]. These changes are accomplished indirectly by the alteration of multiple metabolic pathways. Chronic hyperglycemia leads to structural and functional changes in the nerve microvasculature in people with diabetic peripheral neuropathy, which in turn cause reduced endoneurial perfusion and hypoxia [9–12]. A reduction in the supply of oxygen to nerves and tissues causes a disturbance in the metabolism of cells, which significantly impedes the viability of tissues and the wound healing process.

Anatomy of skin morphology and microcirculation

The morphological structure of the skin is composed of two layers: an outer epidermis and an inner dermis (Figure 1). The epidermis contains keratin and has no blood supply, with nutrition provided by the papillary layer of the dermis. The dermis consists of papillary and reticular layers of collagen and elastic fibers. It consists of a microvascular network that provides tissues with nutrients and eliminates waste products [13]. Skin microcirculation is composed of nutritive capillary blood flow and thermoregulatory arteriovenous shunt flow. It is organized into two horizontal plexuses: an upper subpapillary plexus and a lower cutaneous plexus (Figure 2). The whole vascular network of the skin varies considerably from one area to another. In a normal foot, it has been estimated that 80% to 90% of total skin blood flow (SBF) passes through the arteriovenous shunts circulation while the remaining 10% to 20% passes through the more distal nutritive capillary bed [14]. These nutritional capillaries are organized into functional units, with each dermal papilla supplied by one to three capillary loops [15]. As the exchange of nutrients and metabolites between blood and tissues occurs at the capillary level, the integrity of the capillary circulation has an impact on the health of the entire skin.

Neurogenic and neurovascular regulation of SBF

SBF is under the regulation of several humoral and neural factors including: (1) central neural reflex control from



(A) (B)

Figure 1. Ultrasonic images of human skin showing different skin thicknesses at different regions of the foot. Images are taken by high frequency ultrasound with central frequency at 55MHz (Visualsonic Inc, Vevo 708, Toronto, Canada). (A) Dorsum of the foot (non-glabrous skin). (B) Plantar skin at metatarsal head (glabrous skin)

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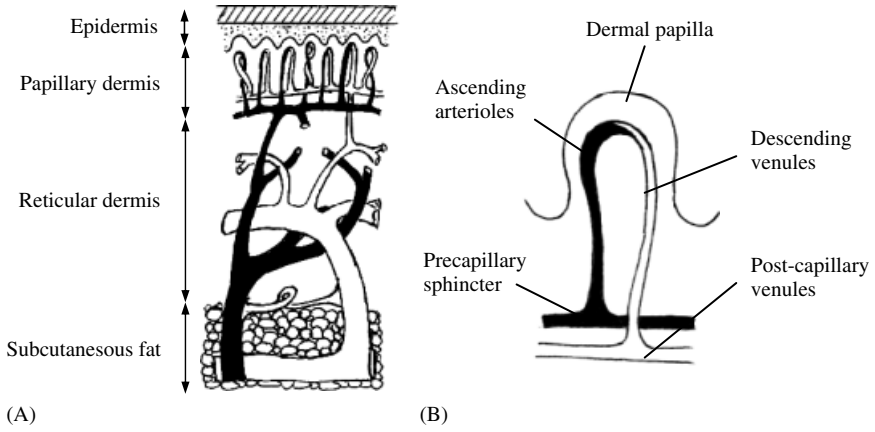

Figure 2. Schematic representation of the microvasculature in human skin. (A) Upper nutritive capillaries loops and a lower thermoregulatory arteriovenous shunt circulation in the dermal layer of the skin (B) A single capillary loop inside a dermal papilla

Table 1. The sympathetic vasodilatory and vasoconstrictor systems in the regulation of skin blood flow

Thermoregulatory systems	Neurotransmitters	Function	Skin innervation
Vasodilatory system Sympathetic cholinergic nerves	Nitric oxide Acetylcholine Vasoactive Intestinal peptide	Dissipate body heat	Non-glabrous skin
Vasoconstrictor system Sympathetic adrenergic nerves	Norepinephrine Noradrenaline Neuropeptide Y	Conserve body heat	Glabrous skin & non-glabrous skin

the long, descending autonomic fibers, (2) short reflex arcs though the spinal cord, and (3) local reflexes within the skin [16,17] and the integrity of the endothelium.

Central neural reflex control via sympathetic nerve fibers

Human cutaneous microcirculation is controlled by both sympathetic adrenergic vasoconstrictor nerves and sympathetic vasodilator nerves [18,19]. SBF is controlled by the opening and closing of arteriovenous anatomoses and precapillary arterioles. Arteriovenous anatomoses are thick-walled and low in resistance, allowing blood to flow at a high rate directly from the arterioles to the venules. In glabrous (hairless) skin, there are numerous arteriovenous anatomoses that are richly innervated by sympathetic vasoconstrictor nerves, while in non-glabrous (hairy) skin there are very few or even no arteriovenous anatomoses that are innervated by both sympathetic vasodilator and vasoconstrictor nerves (Table 1) [20]. Only the vasoconstrictor system is tonically active in a thermoneutral environment. Because of the sympathetic tone of the vasculature, the arteriovenous shunts are maintained in a constricted state under normothermic conditions. The loss of this tone due to sympathetic neuropathy may result in the opening of the shunt and cause a deviation of blood flow from the skin [21].

In diabetes, the role of the sympathetic nervous system in the development of diabetes-associated microcirculatory alternations remains unclear. A decrease in sympathetic tone [22,23], an increase in capillary flow and pressure [14,24,25], and an increase in capillary permeability [26,27] have been reported in people in different stages of diabetes. Cacciatori *et al*. [28] found an almost complete abolition of peripheral sympathetic activity in type 2 diabetic patients with foot ulceration.

Nerve axon reflex mediated vasodilation

Nerve axon reflex mediated vasodilation is another important mechanism for the regulation of microcirculation. This mechanism is thought to be neurally mediated by nociceptive C-fibers [29] (Figure 3). C-nociceptive nerve fibers can be stimulated by heat or other noxious stimuli to initiate orthodromic conduction to the spinal cord and antidromic conduction to other axon branches. This in turn leads to the release of local vasodilatory substances such as neuropeptides substance P, bradykinin, adenosine analog ATP, and calcitonin gene-related peptide (CGRP) from the terminals in the skin and tissues that they innervate. Such neuropeptides may either act directly on vascular smooth muscles or indirectly through secondary pathways that include the mast cell release of histamine and sweat gland secretion of bradykinin and vasoactive intestinal polypeptide, to cause vasodilation to the immediate area of the receptive field of particular sensory neurons [21,30]. This axon reflex is also known as the Lewis triple flare response when induced by the antidromic stimulation of sensory nerves. This accounts for 75% to 90% of the dilatory capacity in mostly non-glabrous skin. In healthy subjects, it was found that the

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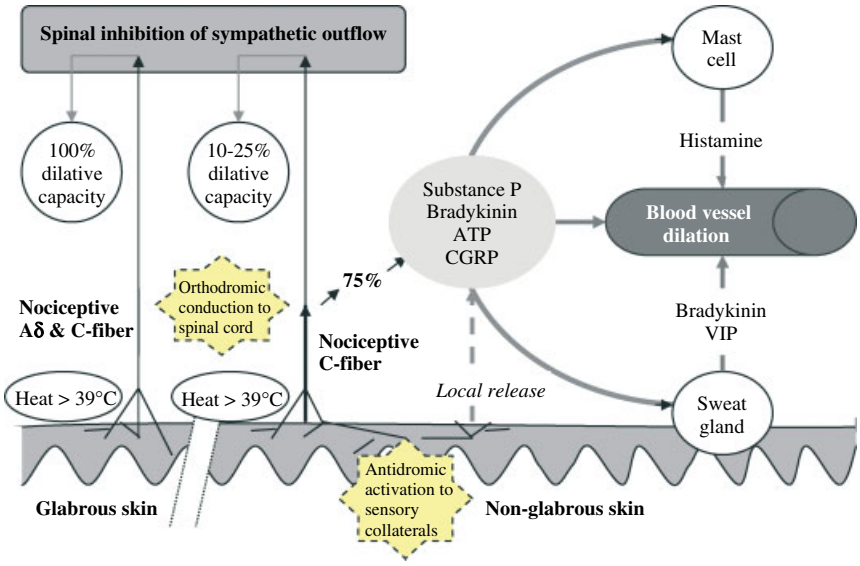

Figure 3. The nerve axon reflex mediated vasodilation in glabrous and non-glabrous skin

nerve axon reflex-related vasodilation accounts for one-third of the total endothelium-dependent vasodilation at both the forearm and foot levels [31]. However, the presence of diabetic neuropathy results in a reduction in the percentage contribution of this neurovascular vasodilation to the total response [31]. A reduction in sensory neurons for substance P and calcitonin gene-related peptide has been shown in diabetic patients [32,33]. The nerve axon reflex-related vasodilation has also been reported to be impaired in patients with diabetic peripheral neuropathy [34,35] or even to be virtually absent in severe cases [36,37].

Local sympathetic veno-arteriolar axon reflex

The sympathetically mediated vasoconstrictor reflex is also activated under gravitational stress (Figure 4). On standing, venous and arteriolar pressures increase due to the height of the column of blood between the heart and foot, resulting in an increase in capillary pressure in the foot. This could rapidly result in interstitial oedema. The increase in venous pressure also increases precapillary resistance and activates receptors in the small vein due to the force of the stretching on blood vessel walls [38]. Oedema is prevented by a local vasoconstrictor reflex mediated by a local sympathetic axon reflex and a myogenic response [38–40]. The nerve impulse in the vein is transmitted antidromically along the postganglionic sympathetic sudomotor axon to the branch point, and then orthodromically to the arteriolar, causing vasoconstriction in the precapillary sphincters. The degree of vasoconstriction is directly proportional to the change in the height of the column of blood between the heart and the limb.

Several investigators have shown that this veno-arteriolar reflex (VAR) is reduced in subjects with diabetic

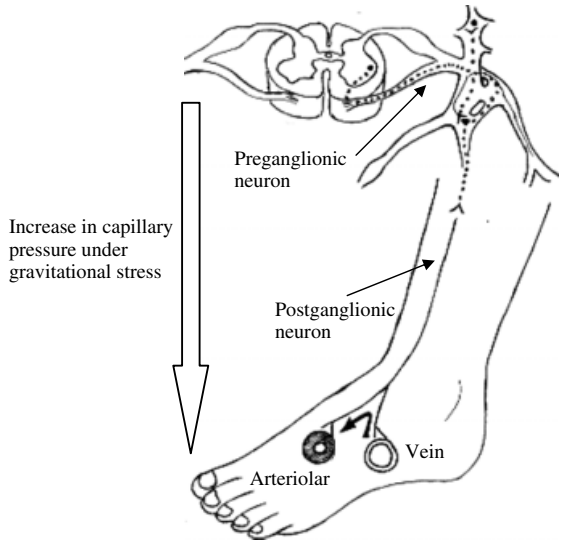

Figure 4. The sympathetic venoarteriolar axon reflex: pressure receptor in the vein is activated during leg dependency, and the nerve impulse is transmitted antidromically along the postganglionic sympathetic axon to the branch point, and then orthodromically to the arteriolar, causing vasoconstriction

autonomic neuropathy, causing oedema and orthostatic hypotension [39,41–45]. Thus, the loss of the postural regulation of blood flow and raised capillary pressure may have important consequences for the diabetic foot, due to an increase in fluid filtration and oedema formation. These abnormalities may initiate microvascular damage and contribute to the development of foot complications. The impaired veno-arteriolar reflex is found in both type 1 [45] and type 2 diabetes [28,44,46,47].

Role of endothelium in the regulation of SBF

Endothelium is a flat monolayer of cells that covers the vascular lumen throughout the body. It is well

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known that vascular endothelium plays an important role in controlling microvascular tone by synthesizing and releasing several vasodilator substances such as nitric oxide, prostacyclin, and endothelium-derived hyperpolarizing factor, and vasoconstrictor substances such as prostaglandins, angiotensin II, and endothelin [15,48]. Therefore, an intact functional endothelium is a prerequisite for the regulation of microcirculatory blood flow. As endothelial function cannot be separated from vascular smooth muscle cell function, evaluations of endothelial function usually include an assessment of both endothelial-dependent vasodilation (vascular endothelial function) and endothelial-independent vasodilation (vascular smooth muscle function). Table 2 summarizes some common endothelial-dependent and endothelial-independent vasodilators/vasoconstrictors.

Endothelial dysfunction has been demonstrated in both type 1 and type 2 diabetes mellitus [36,37,49–55]. A defect in the synthesis and function of nitric oxide has also been observed in the early course of diabetes [36,54,56]. It has been reported that changes in the endothelial function precede the development of diabetes and are present in the prediabetic stage [51]. Although it is well established that endothelium-dependent vasodilation is impaired in both micro- and macrocirculation for patients with type 1 diabetes [51,57,58], whether endothelium-independent vasodilation is also impaired in type 1 diabetic patients is a controversial issue. Singh *et al*. [55] and Koïtka *et al*. [59] found impaired endothelial-dependent vasodilation but not endothelial-independent vasodilation in those with type 1 diabetes without secondary complications. The conflicting results that have been observed may be due to differences in the methodological design of those studies and to their small sample sizes.

Structural microvascular changes in diabetes

Various structural abnormalities can be detected in the microvasculature as diabetes progresses. The most obvious structural changes are the thickening of the capillary basement membrane, diminished capillary size, and pericyte degeneration [12,60–64]. Increased hydrostatic pressure and shear forces in the microcirculation are the initial steps in the development of thickness in the capillary basement membrane. The postural regulation of blood flow is impaired in diabetes. In particular, the capability of effective precapillary vasoconstriction on standing is reduced. This exposes the capillary bed to a high hydrostatic load, producing oedema and thickening of the capillary basement membrane [43]. These stresses are thought to evoke an inflammatory response in the microvascular endothelium, with a subsequent release of extravascular matrix proteins. Over time, this process results in thickening of the basement membrane with arteriolar hyalinosis [65]. In the diabetic foot, thickening of basement membrane is also found in skeletal muscle capillaries [66]. Thickening of the basement membrane may impair normal transport across capillary walls. Furthermore, the elastic properties of the capillary walls are also reduced, limiting the capacity for vasodilation and hence impairing the normal hyperemic response to injury [67,68]. The secretory functions of the endothelium are lost in diabetic patients as the disease advances. This, together with a sclerosed basement membrane, physically limits vasodilation, resulting in a failure to meet the metabolic demands of the tissue when the body parts come under stress or injury [69].

Table 2. Examples of endothelium-dependent and endothelial-independent vasodilators/vasoconstrictors

Types of vasodilatory system	Neurotransmitters
Endothelium-dependent vasodilation	Acetylcholine (Ach) Eugenol Histamine Bradykinin
Endothelium-independent vasodilation	Sodium nitroprusside (SNP) Propofol

Functional microvascular changes in patients with diabetes

Besides structural changes, there are also functional changes in microcirculation in the skin of those with diabetes. These include altered microvascular blood flow, vascular resistance, tissue PO_2, and characteristics of vascular permeability. Functional ischaemia is expressed as an impaired ability in the microcirculation function of diabetic patients to vasodilate in response to stress or injury [36,37]. Much work has been done in the past decade to investigate why the microvasculature of diabetic patients fails to respond appropriately to stress and injury. The proposed mechanism is that blood is shunted away from the nutritional capillaries via subpapillary arteriovenous shunts, which give a much lower resistance compared to capillaries. The subpapillary arteriovenous shunts are innervated by sympathetic nerves; therefore, the presence of diabetic autonomic neuropathy with sympathetic denervation may lead to the opening of these shunts and result in a mal-distribution of blood flow between the nutritional capillaries and subpapillary vessels. This leads to a reduction in the flow of blood to the capillaries and to the development of diabetic peripheral neuropathy and other diabetic foot complications [10,12,36,70].

A number of different functional disturbances are found in the microvasculature of diabetic subjects, including impaired reactive hyperemia, thermal hyperaemia, and occlusive hyperaemia [37,42,62,71–81]. In the case of injury or infection, it is important for the hyperaemia response to increase the local blood flow sufficiently to cope with the increase in metabolic waste products. Interruptions of this increased blood flow response coupled

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with an increase in metabolic demands could create a pathological environment within the microvasculature, especially when the skin is either abnormally stressed or injured. For diabetic people, this leads to in an increased risk of developing foot ulcerations and of a delay in the healing of ulcers that have already developed.

Haemodynamic hypothesis

The haemodynamic hypothesis is a possible explanation for microvascular dysfunction in the neuropathic foot (Table 3). This hypothesis was first introduced by Parving *et al*. [68], who suggested that blood flow dysregulation is mediated by hyperglycemia in the early stage of diabetes. This process is known to stimulate the polyl pathway, which ultimately limits the production of nitric oxide. The result is an increase in microvascular flow and capillary pressure [25], which subsequently induces an endothelial injury response. In a later stage, microvascular sclerosis does occur as a result of the thickening of the capillary basement membrane due to long-term structural adaptation and remodeling [82,83]. The elasticity of the vessel walls is reduced, which physically limits vasodilation. This leads to a loss of reactive hyperemia and impaired autoregulation when the patient is faced with the demands of changes in the environment. An increase in vascular endothelium permeability is also found, which leads to oedema and to a reduction in the supply of nutrients to tissues [84].

Several studies have provided evidence supportive of the haemodynamic hypothesis and shown that capillary blood flow and pressure increase in diabetic persons [14,24,25]. However, several studies have shown contradictory results. Fagrell *et al*. [85] did not demonstrate increased capillary blood flow in diabetic patients as compared to healthy control subjects, but reported a delay in the post-occlusive hyperaemia response in the skin capillaries of the diabetic patients. Similarly, Shore *et al*. [86] did not demonstrate any difference in nailfold capillary flow pressure between those with type 2 diabetes and healthy control subjects. No correlation was found between capillary pressure and glycemic control, while a negative association between capillary pressure and the duration of diabetes was demonstrated. Therefore, the question of whether there is an increase or decrease in capillary blood flow and pressure in diabetic patients remains controversial.

Table 3. Proposed mechanisms for microvascular dysfunction in diabetic foot

Haemodynamic hypothesis	Capillary steal syndrome
• Blood flow dysregulation is mediated by hyperglycaemia • Stimulates polyl pathway and limits production of nitric oxide • Increase in microvascular flow and capillary pressure	• Sympathetic denervation in autonomic neuropathy • Loss of vasoconstriction
• Induces endothelial injury response	• Capillary steal: blood is shunt away from capillaries through arteriovenous shunt • Increase in total peripheral blood flow though arteriovenous anastomosis
• Thickening of capillary basement membrane • Microvascular sclerosis & reduced vascular elasticity which physically limit vasodilation • Loss of reactive hyperemia • Increase in vascular endothelial permeability • Oedema and reduction in nutrient supply	

Increased arteriovenous shunt flow and capillary steal syndrome

Capillary steal syndrome is another hypothesis that has been put forward to explain microvascular dysfunction in the diabetic foot (Table 3). An increase in total peripheral blood flow has been demonstrated in the diabetic neuropathic foot. This is thought to be secondary to peripheral sympathetic denervation with loss of vasoconstriction, which results in an increase in the flow of blood through the arteriovenous shunts. It has been suggested that what then develops is 'capillary steal', in which blood is shunted away from the capillaries through these vessels, resulting in reduced nutrition to the skin [87,88]. Several studies have been conducted to investigate the arteriovenous shunt flow in patients with diabetes [47,89–94]. Boulton *et al*. [89] found increased venous oxygenation in the diabetic neuropathic foot, which is consistent with arteriovenous shunting. Uccioli *et al*. [95] reported an increase in arteriovenous shunt flow in diabetic patients with neuropathy and foot ulcerations, followed by those with neuropathy alone, and then by those without neuropathy. This finding provides evidence that autonomic neuropathy exerts an influence on arteriovenous shunts, which may play a role in the pathogenesis of the diabetic foot. Houben *et al*. [92] investigated both the nutritive capillary blood flow and arteriovenous shunt flow in patients with type 1 diabetes. They found that the arteriovenous shunt flow increased, while the capillary blood flow remained unchanged. Therefore, the majority of these studies demonstrated a reduction in capillary blood flow, but their findings on arteriovenous shunt flow are controversial. Rendell *et al*. [90] found that the arteriovenous shunt flow was reduced in patients with type 1 diabetes as compared with healthy control subjects, which is contradictory to what happens in capillary steal syndrome. Jörneskog *et al*. [93] also reported that total skin microcirculation was similar in both patients with type 1 diabetes and control subjects, whereas the capillary circulation was markedly reduced in diabetic patients both with and without late complications. Further, Jörneskog *et al*. [91] found a reduction in capillary blood flow but not in arteriovenous shunt flow in diabetic patients with peripheral vascular disease, indicating that a sufficient amount of blood

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Table 4. The advantages and disadvantages of common techniques for measuring skin microcirculation

Techniques	Advantages	Disadvantages
$TcPO_2$	Cheap and easy to use Relatively free of operator bias	The area and depth of tissue are difficult to characterize Poor spatial resolution Time is required to obtain a stable measure of resting value
PPG	Relatively cheap and easy to use Experienced operators not required Has good prognostic value	Limited spatial resolution Dependent on the optical geometry of the sensor Uncertainty regarding the physiological origin (e.g., skin or muscle)
Capillaroscopy	Has good spatial resolution Can direct to a single capillary Allows for both morphological and blood flow measurement Can measure capillary diameter, length, and density in a specified area	Limited to the peripheral regions Restricted to measurements of nutritive capillaries only Requires high image quality for valid measurements Relatively complex and time consuming
LDF	Relatively cheap and easy to use Does not require specialist training Instrument is well validated	Limited spatial resolution Penetration depth is limited by its wavelength Direct contact between the probe and skin is required Restricted to single point measurement Inability to measure absolute flow values
LDI	Non-contact method Can measure skin blood perfusion in a larger area with better spatial resolution Provides color mapping on skin perfusion	Time is needed to produce the perfusion imaging
OPS imaging	Non-invasive and convenient Allows for blood flow measurement in both the skin and internal organs	It is a contact method technique, which may impede the microcirculation

$TcPO_2$, transcutaneous oxygen tension measurement; PPG, photoplethysmosgraphy; LDF, laser Doppler flowmetry; LDI, laser Doppler imaging; OPS, orthogonal polarization spectral.

reaches the area but does not enter the capillaries. Moreover, Nabuurs-Franssen *et al*. [47] demonstrated a reduction in capillary blood flow in type 2 polyneuropathy patients, with the reduction being most severe in those with a history of foot ulceration. Still a point of debate is whether the microvascular impairments observed in diabetic patients can be explained by the haemodynamic hypothesis or are a result of the capillary steal syndrome.

Clinical implications and management of diabetic foot

The microvascular complications of diabetes such as neuropathy can lead to a loss of sensation that in turn leads to the development of foot ulceration due to reduced perceptions of foot trauma. Neuropathy is the main factor associated with the impaired endothelium-dependent and endothelium-independent vasodilations, and subsequent predisposition to foot ulceration [36]. The reduction or absence of the nerve axon reflex together with the presence of neuropathy further renders the diabetic foot unable to mount a vasodilatory response when body parts come under stress or injury. It has been proven that glycemic control may prevent or partially reverse neuropathy and modulate neuropathy [96]. Therefore, in preventing microvascular disease, good control of hyperglycemia is the most effective intervention. It is recommended that people with diabetes receive an annual somatosensory examination on the level of sensation in their feet. A simple screening examination of the neurological, vascular, dermatological, and musculoskeletal systems are important to identify changes occurring in the high risk foot [97]. In managing the high risk foot, it is of the utmost importance to prevent and manage high risk conditions. Once foot ulceration develops, the main principles of good wound care should be followed, such as debridement, adequate off-loading and the prompt treatment of infections, and the use of moist wound dressings [98].

Methods for studying skin microcirculation

The concept of measuring skin microcirculation is based on quantifying the optical and thermal properties of the skin. Four measurement parameters including blood flow, blood volume, intracellular oxygenation, and cellular respiration are of particular interest in determining microvascular status [99]. Blood flow has been considered a primary indicator of haemodynamic status. The light reflected by the skin is due to the multiple scattering of photos by the various elements of the skin's structure. Therefore, there is considerable interest in determining the flows of haemoglobin distributed in different plexuses of cutaneous microcirculation [100]. Several techniques can be used in an *in vivo* study to investigate SBF. They include transcutaneous oxygen tension measurement, photoplethysmosgraphy, capillaroscopy, laser Doppler flowmetry (LDF), laser Doppler imaging, and orthogonal polarization spectral imaging (OPS). Table 4 summarizes the advantages and disadvantages of each measurement tool.

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Transcutaneous oxygen tension measurement

Transcutaneous oxygen tension measurement is a non-invasive method that quantifies the oxygen molecules that are transferred out through the skin. These oxygen molecules probably come from skin capillaries as well as the subpapillary plexus [101]. Oxygen is capable of diffusing throughout the tissues of the body and skin, although the diffusion rate is very low under normal body temperatures. The application of heat to a localized area of skin in the range of 37 °C to 45 °C can sufficiently enhance the flow of oxygen through the dermis to allow for non-invasive measurements of capillary oxygen levels [99]. This is an indirect measure of the amount of oxygen that is delivered from the blood in the total process of skin microcirculation out of the surface of the skin. However, these measurements can be inaccurate because they appear to fluctuate with skin temperature and room temperature. In principle, the measured transcutaneous oxygen tension measurement values can be compared with normal ranges to provide an indication of the nutritional status of the tissues.

Photo-plethysmography

Photo-plethysmography records the changes in optical intensity in response to changes in the volume of blood in sampled tissue [102,103]. It allows for the measurement of venous blood volume or pulsatile arterial blood volume [104]. This technique requires a light source (red or near infrared light) and a photodetector. When the light enters the skin, it is scattered, reflected, refracted, and absorbed before again reaching the photodetector [105]. The degree of absorption is dependent on the volume of blood in the tissue; tissue volume is inversely related to the recorded signal [106].

Capillaroscopy

Capillaroscopy is one of the most sensitive methods for estimating the nutritional status of skin tissues at the microscopic level *in vivo*. This technique allows for a real-time 2D visualization of the capillaries to evaluate the morphology, density, and blood cell velocity of the capillaries (videocapillaroscopy) [103,107]. The capillaries can be studied better in the nailfold than in other parts of the body [108]. In the nailfold, the capillaries may appear as lines, and the whole capillary loop can be visualized as the capillaries are lying parallel to the surface of the skin. In other regions of the skin, the capillaries may appear as black dots as they are lying perpendicular to the surface of the skin [108].

Laser Doppler flowmetry and imaging

LDF is a non-invasive continuous measurement of skin microvascular perfusion based on the effect of light on moving red blood cells (RBC) in a restricted volume of tissue [103,108]. It is the most widely used form of measuring microcirculation. LDF mainly measures the flow of blood in arterioles, venous plexuses, and arteriovenous anastomises, but only a small part is contributed by the motion of blood cells in the nutritional capillaries [109,110]. It uses a monochromatic low-powered red laser light that is transmitted to the skin through a fiberoptic cable. RBCs moving in the skin backscatter light from the laser, producing a Doppler frequency shift that is sensed by the photodetector [108]. The output from the LDF is referred to a perfusion flux instead of flow, and the response is proportional to RBC velocity and RBC concentration. The extraction of RBC velocity and volume requires mathematical models. It is assumed that the blood cell velocities are randomly distributed in direction. The microvascular perfusion flux is then expressed in terms of arbitrary units and is calculated as the product of the number of RBCs in the sampling volume times the mean velocity, which is proportional to blood flow [99,108].

Laser Doppler imaging is a non-contact device used to map the SBF perfusion [111]. This device is comprised of a helium neon laser, a scanner, an optical detector system, and a computer [111]. It is based on the same principle as LDF, but can be used for scanning tissue areas of various sizes instead of just measuring a single point as in LDF. Once the region of interest is defined, it can be used to monitor and capture blood flow images in minutes. The device can then generate a color-coded perfusion map profile of the defined region, with dark blue representing the lowest and red the highest area of blood perfusion [112].

Orthogonal polarization spectral imaging

OPS imaging is a non-invasive technique developed in the last decade to visualize the microcirculation in both the skin and internal organs [113]. It is a handheld device that uses a polarized light technique to examine the absorption of haemoglobin [113]. OPS imaging allows for the *in vivo* assessment of the microcirculation up to a depth of 3 mm in the skin [114]. The tissue being investigated is illuminated with linearly polarized light at a wavelength of 538 nm. A large part of the radiation is reflected through a polarizer oriented orthogonally to the plane of the light. Only a small amount of light is able to penetrate to deeper layers, where it undergoes multiple dispersion, depolarization, or reissues to the surface [114]. The degree of penetration of polarized light depends on the density of the examined object and the power of the polarized radiation used. As polarization is preserved in reflection, only photons scattered from relatively deep in the tissue contribute to the images. The microcirculatory parameters obtained by OPS imaging have been demonstrated to be comparable to those obtained by capillaroscopy [115].

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Conclusion

Skin microcirculation plays a prominent role in skin viability and cutaneous pathology. Disturbances in the microcirculatory function of the diabetic foot include both structural and functional abnormalities. Such disturbances, together with the neurologic and vascular complications involved in SBF regulation, further render the diabetic foot at risk of ulceration and contribute to the poor healing of wounds. Advances in medical technology allow the exact etiopathogenic mechanisms involved in skin pathology to be analyzed. An in-depth knowledge and understanding of the underlying pathology can definitely aid and accelerate the development of protective therapies to prevent diabetic foot disease and ulceration.

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Conflict of interest

No conflicts of interest exist.

References

1. Boulton A. The pathway to ulceration: aetiopathogenesis. In *The Foot in Diabetes* (2nd edn), Boulton A, Connor H, Cavanagh P (eds). Wiley: Chichester, 1994; 37–48.
2. Grunfeld C. Diabetic foot ulcers: etiology, treatment and prevention. *Adv Intern Med* 1992; **37**: 103–132.
3. Sandeman D, Shearman C. Clinical aspects of lower limb ulceration. In *Chronic Wound Healing – Clinical Measurement and Basic Science*, Mani R, Falanga V, Shearman C, Sandeman D (eds). WB Saunders: London, 1999; 4–25.
4. Brand P. The diabetic foot. In *Elenberg and Rifkin's Diabetes Mellitus: Theory and Practice* (4th edn), Rifkin H Ponte D (eds). Elsevier: New York, 1990; 829–849.
5. Brem H, Sheehan P, Rosenberg HJ, Schneider JS, Boulton AJ. Evidence-based protocol for diabetic foot ulcers. *Plast Reconstr Surg* 2006; **117**: 193S–209S.
6. Lioupis C. The role of distal arterial reconstruction in patients with diabetic foot ischemia. *Int J Low Extrem Wounds* 2005; **4**: 45–49.
7. Goldenberg S, Alex M, Joshi RA, Blumenthal HT. Nonatheromatous peripheral vascular disease of the lower extremity in diabetes mellitus. *Diabetes* 1959; **8**: 261–273.
8. Dinh T, Veves A. Microcirculation of the diabetic foot. *Curr Pharm Des* 2005; **11**: 2301–2309.
9. Giannini C, Dyck PJ. Basement membrane reduplication and pericyte degeneration precede development of diabetic polyneuropathy and are associated with its severity. *Ann Neurol* 1995; **37**: 498–504.
10. Malik RA, Newrick PG, Sharma AK, *et al*. Microangiopathy in human diabetic neuropathy: relationship between capillary abnormalities and the severity of neuropathy. *Diabetologia* 1989; **32**: 92–102.
11. Malik RA, Veves A, Masson EA, *et al*. Endoneurial capillary abnormalities in mild human diabetic neuropathy. *J Neurol Neurosurg Psychiatry* 1992; **55**: 557–561.
12. Yasuda H, Dyck PJ. Abnormalities of endoneurial microvessels and sural nerve pathology in diabetic neuropathy. *Neurology* 1987; **37**: 20–28.
13. Hagisawa S, Shimada T. Skin morphology and its mechanical properties associated with loading. In *Pressure Ulcer Research: Current and Future Perspectives*, Bader D, Bouten C, Colin D, Oomens C (eds). Springer-Verlag: Berlin, Germany, 2005; 161–185.
14. Flynn MD, Edmonds ME, Tooke JE, Watkins PJ. Direct measurement of capillary blood flow in the diabetic neuropathic foot. *Diabetologia* 1988; **31**: 652–656.
15. Fontaine J, Harkless L, Davis C, Allen M, Shireman P. Current concepts in diabetic microvascular dysfunction. *J Am Podiatr Med Assoc* 2006; **96**: 245–252.
16. Coffman JD, Cohen RA. Alpha-adrenergic and serotonergic mechanisms in the human digit. *J Cardiovasc Pharmacol* 1988; **11**: S49–S53.
17. Henriksen O. Sympathetic reflex control of blood flow in human peripheral tissues. *Acta Physiol Scand* 1991; **143**: 33–39.
18. Charkoudian N. Skin blood flow in adult human thermoregulation: how it works, when it does not, and why. *Mayo Clin Proc* 2003; **78**: 603–612.
19. Kellogg DL Jr. *In vivo* mechanisms of cutaneous vasodilation and vasoconstriction in humans during thermoregulatory challenges. *J Appl Physiol* 2006; **100**: 1709–1718.
20. Johnson JM, Brengelmann GL, Hales JR, Vanhoutte PM, Wenger CB. Regulation of the cutaneous circulation. *Fed Proc* 1986; **45**: 2841–2850.
21. Vinik AI, Erbas T, Park TS, Pierce KK, Stansberry KB. Methods for evaluation of peripheral neurovascular dysfunction. *Diabetes Technol Ther* 2001; **3**: 29–50.
22. Wiernsperger NF. In defense of microvascular constriction in diabetes. *Clin Hemorheol Microcirc* 2001; **25**: 55–62.
23. Hoffman RP, Sinkey CA, Kienzle MG, Anderson EA. Muscle sympathetic nerve activity is reduced in IDDM before overt autonomic neuropathy. *Diabetes* 1993; **42**: 375–380.
24. Netten PM, Wollersheim T, Thien T, Lutterman JA. Skin microcirculation of the foot in diabetic neuropathy. *Clin Sci* 1996; **91**: 559–565.
25. Sandeman DD, Shore AC, Tooke JE. Relation of skin capillary pressure in patients with insulin-dependent diabetes mellitus to complications and metabolic control. *N Engl J Med* 1992; **327**: 760–764.
26. Jaap AJ, Shore AC, Gartside IB, Gamble J, Tooke JE. Increased microvascular fluid permeability in young type 1 (insulin-dependent) diabetic patients. *Diabetologia* 1993; **36**: 648–652.
27. Lefrandt JD, Bosma E, Oomen PH, *et al*. Sympathetic mediated vasomotion and skin capillary permeability in diabetic patients with peripheral neuropathy. *Diabetologia* 2003; **46**: 40–47.
28. Cacciatori V, Dellera A, Bellavere F, *et al*. Comparative assessment of peripheral sympathetic function by postural vasoconstriction arteriolar reflex and sympathetic skin response in NIDDM patients. *Am J Med* 1997; **102**: 365–370.
29. Cable NT. Unlocking the secrets of skin blood flow. *J Physiol* 2006; **572**: 613.
30. Kiernan JA. The involvement of mast cells in vasodilatation due to axon reflexes in injured skin. *Q J Exp Physiol Cogn Med Sci* 1972; **57**: 311–317.
31. Hamdy O, Abou-Elenin K, LoGerfo FW, Horton ES, Veves A. Contribution of nerve-axon reflex-related vasodilation to the total skin vasodilation in diabetic patients with and without neuropathy. *Diabetes Care* 2001; **24**: 344–340.
32. Levy DM, Karanth SS, Springall DR, Polak JM. Depletion of cutaneous nerves and neuropeptides in diabetes mellitus: an immunocytochemical study. *Diabetologia* 1989; **32**: 427–433.
33. Lindberger M, Schröder HD, Schultzberg M, *et al*. Nerve fibre studies in skin biopsies in peripheral neuropathies. I. Immunohistochemical analysis of neuropeptides in diabetes mellitus. *J Neurol Sci* 1989; **93**: 289–296.
34. Uccioli L, Monticone G, Durola L, *et al*. Autonomic neuropathy influences great toe blood pressure. *Diabetes Care* 1994; **17**: 284–287.
35. Caselli A, Rich J, Hanane T, Uccioli L, Veves A. Role of C-nociceptive fibers in the nerve axon reflex-related vasodilation in diabetes. *Neurology* 2003; **60**: 297–300.
36. Veves A, Akbari CM, Primavera J, *et al*. Endothelial dysfunction and the expression of endothelial nitric oxide synthetase in diabetic neuropathy, vascular disease, and foot ulceration. *Diabetes* 1998; **47**: 457–463.
37. Arora S, Pomposelli F, LoGerfo FW, Veves A. Cutaneous microcirculation in the neuropathic diabetic foot improves

significantly but not completely after successful lower extremity revascularization. *J Vasc Surg* 2002; **35**: 501–505.
38. Rayman G, Hassan A, Tooke JE. Blood flow in the skin of the foot related to posture in diabetes mellitus. *Br Med J* 1986; **292**: 87–90.
39. Belcaro G, Nicolaides AN. The venoarteriolar response in diabetics. *Angiology* 1991; **42**: 827–835.
40. Henriksen L, Sejrsen P. Local reflex in microcirculation in human cutaneous tissue. *Acta Physiol Scand* 1976; **98**: 227–231.
41. Khodabandehlou T, Zhao H, Vimeux M, Le Dévéhat C. The autoregulation of the skin microcirculation in healthy subjects and diabetic patients with and without vascular complications. *Clin Hemorheol Microcirc* 1997; **17**: 357–362.
42. Golster H, Hyllienmark L, Ledin T, Ludvigsson J, Sjöberg F. Impaired microvascular function related to poor metabolic control in young patients with diabetes. *Clin Physiol Funct Imaging* 2005; **25**: 100–105.
43. Belcaro G, Nicolaides AN, Volteas N, Leon M. Skin flow the venoarteriolar response and capillary filtration in diabetics. A 3-year follow-up. *Angiology* 1992; **43**: 490–495.
44. Iwase M, Imoto H, Murata A, *et al*. Altered postural regulation of foot skin oxygenation and blood flow in patients with type 2 diabetes mellitus. *Exp Clin Endocrinol Diabetes* 2007; **115**: 444–447.
45. Yosipovitch G, Schneiderman J, van Dyk DJ, Chetrit A, Milo G, Boner G. Impairment of the postural venoarteriolar response in young type 1 diabetic patients. A study by laser doppler flowmetry. *Angiology* 1996; **47**: 687–691.
46. Zimny S, Dessel F, Ehren M, Pfohl M, Schatz H. Early detection of microcirculatory impairment in diabetic patients with foot at risk. *Diabetes Care* 2001; **24**: 1810–1814.
47. Nabuurs-Franssen MH, Houben AJ, Tooke JE, Schaper NC. The effect of polyneuropathy on foot microcirculation in Type II diabetes. *Diabetologia* 2002; **45**: 1164–1171.
48. Vane JR, Anggård EE, Botting RM. Regulatory functions of the vascular endothelium. *N Engl J Med* 1990; **323**: 27–36.
49. Williams SB, Cusco JA, Roddy MA, Johnstone MT, Creager MA. Impaired nitric oxide-mediated vasodilation in patients with non-insulin-dependent diabetes mellitus. *J Am Coll Cardiol* 1996; **27**: 567–574.
50. Johnstone MT, Creager SJ, Scales KM, Cusco JA, Lee BK, Creager MA. Impaired endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. *Circulation* 1993; **88**: 2510–2516.
51. Elhadd TA, Kennedy G, Hill A, *et al*. Abnormal markers of endothelial cell activation and oxidative stress in children, adolescents and young adults with type 1 diabetes with no clinical vascular disease. *Diabetes Metab Res Rev* 1999; **15**: 405–411.
52. Morris SJ, Shore AC, Tooke JE. Responses of the skin microcirculation to acetylcholine and sodium nitroprusside in patients with NIDDM. *Diabetologia* 1995; **38**: 1337–1344.
53. Arora S, Smakowski P, Frykberg RG, *et al*. Differences in foot and forearm skin microcirculation in diabetic patients with and without neuropathy. *Diabetes Care* 1998; **21**: 1339–1344.
54. Khan F, Elhadd TA, Greene SA, Belch JJ. Impaired skin microvascular function in children, adolescents, and young adults with type 1 diabetes. *Diabetes Care* 2000; **23**: 215–220.
55. Singh TP, Groehn H, Kazmers A. Vascular function and carotid intimal-medial thickness in children with insulin-dependent diabetes mellitus. *J Am Coll Cardiol* 2003; **41**: 661–665.
56. Vinik AI, Park TS, Stansberry KB, Pittenger GL. Diabetic neuropathies. *Diabetologia* 2000; **43**: 957–973.
57. Caballero AE, Arora S, Saouaf R, *et al*. Microvascular and macrovascular reactivity is reduced in subjects at risk for type 2 diabetes. *Diabetes* 1999; **48**: 1856–1862.
58. Jaap AJ, Shore AC, Tooke JE. Relationship of insulin resistance to microvascular dysfunction in subjects with fasting hyperglycaemia. *Diabetologia* 1997; **40**: 238–243.
59. Koıtka A, Abraham P, Bouhanick B, Sigaudo-Roussel D, Demiot C, Saumet JL. Impaired pressure-induced vasodilation at the foot in young adults with type 1 diabetes. *Diabetes* 2004; **53**: 721–725.
60. Malik RA, Newrick PG, Sharma AK, *et al*. Microangiopathy in human diabetic neuropathy: relationship between capillary abnormalities and the severity of neuropathy. *Diabetologia* 1989; **32**(2): 92–102.
61. Jaap AJ, Shore AC, Stockman AJ, Tooke JE. Skin capillary density in subjects with impaired glucose tolerance and patients with type 2 diabetes. *Diabet Med* 1996; **13**(2): 160–164.
62. Rayman G, Mailk RA, Sharma AK, Day JL. Microvascular response to tissue injury and capillary ultrastructure in the foot skin of type I diabetic patients. *Clin Sci (Lond)* 1995; **89**(5): 467–474.
63. Cameron NE, Eaton SE, Cotter MA, Tesfaye S. Vascular factors and metabolic interactions in the pathogenesis of diabetic neuropathy. *Diabetologia* 2001; **44**: 1973–1988.
64. Tesfaye S, Malik R, Ward JD. Vascular factors in diabetic neuropathy. *Diabetologia* 1994; **37**: 847–854.
65. Tilton RG, Faller AM, Burkhardt JK, *et al*. Pericyte degeneration and acellular capillaries are increased in the foot of human diabetic patients. *Diabetologia* 1985; **28**: 895–900.
66. Raskin P, Pietri AO, Unger R, Shannon WAJ. The effect of diabetic control on the width of skeletal muscle capillary basement membrane in patients with type 1 diabetes mellitus. *N Engl J Med* 1983; **309**(25): 1546–1550.
67. Tooke JE. Microvascular function in human diabetes. A physiological perspective. *Diabetes* 1995; **44**: 721–726.
68. Parving HK, Viberti GC, Keen H, Christiansen JS, Lassen NA. Hemodynamic factors in the genesis of diabetic microangiopathy. *Metabolism* 1983; **32**: 943–949.
69. Singleton JR, Smith AG, Russell JW, Feldman EL. Microvascular complications of impaired glucose tolerance. *Diabetes* 2003; **52**: 2867–2873.
70. Flynn MD, Tooke JE. Diabetic neuropathy and the microcirculation. *Diabet Med* 1995; **12**: 298–301.
71. Rayman G, Williams SA, Spencer PD, Smaje LH, Wise PH, Tooke JE. Impaired microvascular hyperaemic response to minor skin trauma in type I diabetes. *Br Med J (Clin Res Ed)* 1986; **292**: 1295–1298.
72. Walmsley D, Wales JK, Wiles PG. Reduced hyperaemia following skin trauma: evidence for an impaired microvascular response to injury in the diabetic foot. *Diabetologia* 1989; **32**: 736–739.
73. Shore AC, Price KJ, Sandeman DD, Green EM, Tripp JH, Tooke JE. Impaired microvascular hyperaemic response in children with diabetes mellitus. *Diabet Med* 1991; **8**: 619–623.
74. Wilson SB, Jennings PE, Belch JJ. Detection of microvascular impairment in type I diabetics by laser Doppler flowmetry. *Clin Physiol* 1992; **12**: 195–208.
75. Jörneskog G, Fagrell B. Discrepancy in skin capillary circulation between fingers and toes in patients with type 1 diabetes. *Int J Microcirc Clin Exp* 1996; **16**: 313–319.
76. Jörneskog G, Brismar K, Fagrell B. Pronounced skin capillary ischemia in the feet of diabetic patients with bad metabolic control. *Diabetologia* 1998; **41**: 410–415.
77. Meyer MF, Schatz H. Influence of metabolic control and duration of disease on microvascular dysfunction in diabetes assessed by laser Doppler anemometry. *Exp Clin Endocrinol Diabetes* 1998; **106**: 395–403.
78. Kasalová Z, Prázný M, Skrha J. Relationship between peripheral diabetic neuropathy and microvascular reactivity in patients with type 1 and type 2 diabetes mellitus – neuropathy and microcirculation in diabetes. *Exp Clin Endocrinol Diabetes* 2006; **114**: 52–57.
79. Rendell M, Bamisedun O. Diabetic cutaneous microangiopathy. *Am J Med* 1992; **93**: 611–618.
80. Jaap AJ, Hammersley MS, Shore AC, Tooke JE. Reduced microvascular hyeraemia in subjects at risk of developing type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 1994; **37**: 214–216.
81. Jaffer U, Aslam M, Standfield N. Impaired hyperaemic and rhythmic vasomotor response in type 1 diabetes mellitus patients: a predictor of early peripheral vascular disease. *Eur J Vasc Endovasc Surg* 2008; **35**: 603–606.
82. Chittenden SJ, Shami SK. Microvascular investigations in diabetes mellitus. *Postgrad Med J* 1993; **69**: 419–428.
83. Pham HT, Economides PA, Veves A. The role of endothelial function on the foot. Microcirculation and wound healing in patients with diabetes. *Clin Podiatr Med Surg* 1998; **15**: 85–93.
84. Deckert T, Feldt-Rasmussen B, Borch-Johnsen K, Jensen T, Kofoed-Enevoldsen A. Albuminuria reflects widespread vascular damage: the Steno hypothesis. *Diabetologia* 1989; **32**: 219–226.
85. Fagrell B, Hermansson IL, Karlander SG, Ostergren J. Vital capillary microscopy for assessment of skin viability and

microangiopathy in patients with diabetes mellitus. *Acta Med Scand Suppl* 1984; **687**: 25–28.
86. Shore AC, Jaap AJ, Tooke JE. Capillary pressure in patients with NIDDM. *Diabetes* 1994; **43**: 1198–1202.
87. Krishnan STM, Rayman G. Microcirculation and diabetic foot. In *Foot in Diabetes* (4th edn), Boulton AJM, Peter R, Cavanagh PR, Rayman G (eds). John Wiley & Sons: 2006; 41–50.
88. Uccioli L, Mancini L, Giordano A, *et al*. Lower limb arteriovenous shunts, autonomic neuropathy and diabetic foot. *Diabetes Res Clin Pract* 1992; **16**: 123–130.
89. Boulton AJ, Scarpello JH, Ward JD. Venous oxygenation in the diabetic neuropathic foot: evidence of arteriovenous shunting? *Diabetologia* 1982; **22**: 6–8.
90. Rendell M, Bergman T, O' Donnell G, Drobny E, Borgos J, Bonner RF. Microvascular blood flow, volume, and velocity measured by laser Doppler techniques in IDDM. *Diabetes* 1989; **38**: 819–824.
91. Jörneskog G, Brismar K, Fagrell B. Skin capillary circulation is more impaired in the toes of diabetic than non-diabetic patients with peripheral vascular disease. *Diabet Med* 1995; **12**: 36–41.
92. Houben AJ, Nieuwenhuijzen Kruseman AC, Bouhouch E, Slaaf DW, Schaper NC. Peripheral macro- and microcirculation in short-term insulin-dependent diabetes mellitus: the role of prostaglandins in early haemodynamic changes. *Eur J Clin Invest* 1993; **23**: 662–667.
93. Jörneskog G, Brismar K, Fagrell B. Skin capillary circulation severely impaired in toes of patients with IDDM, with and without late diabetic complications. *Diabetologia* 1995; **38**: 474–480.
94. Stevens MJ, Goss DE, Foster AV, Pitel D, Edmonds ME, Watkins PJ. Abnormal digital pressure measurements in diabetic neuropathic foot ulceration. *Diabet med* 1993; **10**: 909–915.
95. Uccioli L, Mancini L, Giordano A, *et al*. Lower limb arteriovenous shunts, autonomic neuropathy and diabetic foot. *Diabetes Res Clin Pract* 1992; **16**: 123–130.
96. Veves A, Backonja M, Malik RA. Painful diabetic neuropathy: epidemiology, natural history, early diagnosis, and treatment options. *Pain Med* 2008; **9**: 660–674.
97. Mayfield JA, Reiber GE, Sanders LJ, Janisse D, Pogach LM. American Diabetes Association. Preventive foot care in diabetes. *Diabetes Care* 2004; **27**: S63–S64.
98. Dinh TL, Veves A. Treatment of diabetic ulcers. *Dermatol Ther* 2006; **19**: 348–355.
99. Cobb J, Claremont D. Noninvasive measurement techniques for monitoring of microvascular function in the diabetic foot. *Int J Low Extrem Wounds* 2002; **1**: 161–169.
100. Berardesca E, Lévêque JL, Masson P. European Group for Efficacy Measurements on Cosmetics and Other Topical Products (EEMCO Group). EEMCO guidance for the measurement of skin microcirculation. *Skin Pharmacol Appl Skin Physiol* 2002; **15**: 442–456.
101. Fagrell B. Advances in microcirculation network evaluation: an update. *Int J Microcirc Clin Exp* 1995; **15**: 34–40.
102. Challoner A. Photoelectric plethysmography for estimating cutaneous blood flow. In *Non-Invasive Physiological Measurement*, Rolfe P (ed). London: Academic Press: 1979; 125–151.
103. Wright CI, Kroner CI, Draijer R. Non-invasive methods and stimuli for evaluating the skin's microcirculation. *J Pharmacol Toxicol Methods* 2006; **54**: 1–25.
104. Schultz-Ehrenburg U, Blazek V. Value of quantitative photoplethysmography for functional vascular diagnostics. Current status and prospects. *Skin Pharmacol Appl Skin Physiol* 2001; **14**: 316–323.
105. Swain ID, Grant LJ. Methods of measuring skin blood flow. *Phys Med Biol* 1989; **34**: 151–175.
106. Allen J, Frame JR, Murray A. Microvascular blood flow and skin temperature changes in the fingers following a deep nspiratory gasp. *Physiol Meas* 2002; **23**: 365–373.
107. Shore AC. Capillaroscopy and the measurement of capillary pressure. *Br J Clin Pharmacol* 2000; **50**: 501–513.
108. Yvonne-Tee GB, Rasool AH, Halim AS, Rahman AR. Noninvasive assessment of cutaneous vascular function *in vivo* using capillaroscopy, plethysmography and laser-Doppler instruments: its strengths and weaknesses. *Clin Hemorheol Microcirc* 2006; **34**: 457–473.
109. Bongard O, Fagrell B. Variations in laser Doppler flux and flow motion patterns in the dorsal skin of the human foot. *Microvasc Res* 1990; **39**: 212–222.
110. Fagrell B. Peripheral vascular disease. In *Laser: Doppler Flowmetry Boston*, Shepherd AP, Öberg AA (eds). Kluwer Academic Publishers: 1990; 201–214.
111. Picart C, Carpentier PH, Brasseur S, Galliard H, Piau JM. Systemic sclerosis: blood rheometry and laser Doppler imaging of digital cutaneous microcirculation during local cold exposure. *Clin Hemorheol Microcirc* 1998; **18**: 47–58.
112. Humeau A, Steenbergen W, Nilsson H, Strömberg T. Laser Doppler perfusion monitoring and imaging: novel approaches. *Med Biol Eng Comput* 2007; **45**: 421–435.
113. Groner W, Winkelman JW, Harris AG, *et al*. Orthogonal polarization spectral imaging: a new method for study of the microcirculation. *Nat Med* 1999; **5**: 1209–1212.
114. Lupi O, Semenovitch I, Treu C, Bouskela E. Orthogonal polarization technique in the assessment of human skin microcirculation. *Int J Dermatol* 2008; **47**: 425–431.
115. Mathura KR, Vollebregt KC, Boer K, De Graaff JC, Ubbink DT, Ince C. Comparison of OPS imaging and conventional capillary microscopy to study the human microcirculation. *J Appl Physiol* 2001; **91**: 74–78.

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Biomechanical properties of the forefoot plantar soft tissue as measured by an optical coherence tomography-based air-jet indentation system and tissue ultrasound palpation system

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ABSTRACT

Background: The forefoot medial plantar area withstand high plantar pressure during locomotion, and is a common site that develops foot lesion problems among elderly people. The aims of the present study were to (1) determine the correlation between the biomechanical properties of forefoot medial plantar soft tissue measured by a newly developed optical coherence tomography-based air-jet indentation system and by tissue ultrasound palpation system, and (2) to compare the biomechanical properties of plantar soft tissues of medial forefoot between a young and old adult group.
Methods: Thirty healthy subjects were classified as the young or older group. The biomechanical properties of plantar soft tissues measured at the forefoot by the air-jet indentation system and tissue ultrasound palpation system were performed, and the correlation of the findings obtained in the two systems were compared.
Findings: A strong positive correlation was obtained from the findings in the two systems ($r = 0.88$, $P < 0.001$). The forefoot plantar soft tissue of the older group was significantly stiffer at the second metatarsal head and thinner at both metatarsal heads than that of the young group (all $P < 0.05$). The stiffness coefficient at the second metatarsal head was 28% greater than that at the first metatarsal head in both study groups.
Interpretation: Older subjects showed a loss of elasticity and reduced thickness in their forefoot plantar soft tissue, with the second metatarsal head displaying stiffer and thicker plantar tissue than the first metatarsal head. The air-jet indentation system is a useful instrument for characterizing the biomechanical properties of soft tissue.

1. Introduction

The forefoot medial plantar soft tissues are the site that withstands the highest plantar pressure during weight bearing (Duckworth et al., 1985; Armstrong et al., 1998). It comprises of a complex framework of skin, fat cells, fascia layers, and muscles (Bojsen-Moller and Flagstad, 1976). Each layer of this multilayered composite of tissues has various mechanical properties and the extent to which it deforms in response to external stress may vary. However, the total thickness of the plantar soft tissue composed of various layers can work together and serves as a cushion for optimizing load-bearing during ambulation.

Tissue breakdown of the foot frequently occurs at the plantar aspect of the metatarsal head region (Reiber et al., 1998). This area is subject to micro-tears due to the compound stresses of tension, compression, and shearing force during repetitive gait cycles, which make it more susceptible to the breakdown of tissue (Reiber et al., 1998). Abnormal foot biomechanics caused by changes in the properties of tissue has been postulated as a key risk factor for the breakdown (Cevera et al., 1997; Whitney, 2003). It is apparent that foot lesions occur mostly in the older population (Whitney, 2003) or in pathological conditions such as diabetes. A substantially stiffer tissue may cause a reduction in the cushioning effect of plantar tissue, hence less shock-absorbing ability during the loading periods of ambulation. It may lead to foot complications such as metatarsalgia or development of ulcers. Nevertheless, as human tissue has the capability to adapt to mechanical loading by changing its structure and composition, an understanding of the quantitative changes in the biomechanical properties of tissue is of crucial importance for many biomedical applications. Understanding these physiological changes may assist in both the prevention and treatment of the diabetes associated foot problems such as ulceration.

Age is a key factor that may influence the biomechanical properties of the plantar soft tissue layers. Aged human skin appears loose and

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sagging, with less resilience and elasticity than young skin due to the progressive degradation of a protein polymer in the dermis (Pasquali-Ronchetti and Baccarani-Contri, 1997). Aging also causes a reduction in the cohesiveness of the dermoepidermal junction in the intact skin, thus rendering the aging person more susceptible to partial thickness skin injuries such as skin tears (Pittman, 2007). Hsu et al. (1998) found that elderly people have a thicker layer of soft tissue and decreased elasticity in the heel pad region. Kubo et al. (2007) reported that the maximum strain of the plantar flexor tendon decreased with age. Pascual Huerta and Alarcón García (2007) found that thickness of the plantar fascia did not correlate with age. Most previous studies have examined the effects of aging on the thickness of skin or its associated change in mechanical properties. Very few studies have investigated the effects of aging on changes in the biomechanical properties of the total thickness of plantar soft tissue, i.e. from the surface of the skin to the soft tissue–bone interface. Thus, it is worth understanding the changes in the biomechanical properties of the plantar soft tissue as a whole.

Several indentation methods have been developed in the past decades to characterize the mechanical properties of the thickness or stiffness of plantar soft tissue. The main difference of these methods comes from the techniques to measure the displacement of the indenter or the deformation of the tissues. Measurements with three-dimensional position sensors or different indentation speeds through a driving motor have been reported (Klaesner et al., 2002; Pathak et al., 1998). Imaging techniques such as magnetic resonance imaging system and ultrasound have also been proposed for this purpose (Hsu et al., 2000; Gefen et al., 2001). However, system portability is always a problem for *in vivo* operations with a bulk measurement device. In order to solve this problem, Zheng and Mak (1996) and Zheng et al. (2000) have developed a hand-held tissue ultrasound palpation system (TUPS) that utilizes single ultrasound transducer in tandem with a load cell to measure the stiffness of plantar soft tissues in healthy and people with diabetes. The thickness of the tissue can also be obtained with ultrasound measurement (Zheng et al., 2000; Abouaesha et al., 2001; Hsu et al., 2002). Therefore, ultrasound indentation has become one of the most popular indentation techniques in recent decades to measure the mechanical properties of plantar soft tissues with its capability of real-time imaging function.

Optical coherence tomography (OCT) is a fast developing imaging modality that provides high resolution two-dimensional imaging of internal biological structures by measuring their optical reflection (Huang et al., 1991). With this technique, Huang et al. (2009) developed a novel OCT-based air-jet indentation system for characterizing the biomechanical properties of soft tissues in a non-contact way. The concept is to utilize the optical interferometric technique for obtaining the deformation of the tested specimen during indentation. By using a pressure controlled air jet as an indenter and OCT-signals to extract the deformation induced, the biomechanical properties of soft tissue could then be objectively and quantitatively deduced. The stiffness coefficient of this air-jet indentation system has been shown to have a high correlation ($r = 0.89$, $P < 0.001$) with the corresponding Young's moduli obtained by conventional mechanical testing in a phantom model. This novel device could potentially be used for quantitative assessment of skin area that requires a non-contact application such as wound or eye tissue. The present study aimed to (1) determine the correlation between the findings obtained from this newly developed air-jet indentation system and the use of TUPS, and to (2) to examine the biomechanical properties of forefoot plantar soft tissue between a young and old adult group using both the air-jet indentation system and TUPS.

2. Methods

2.1. Subjects

Thirty healthy subjects (17 male, 13 female) without any foot lesions, orthopaedic foot problems, or diabetes participated in the present study. They were classified into the young group ($n = 19$, age: 22 to 35 years) and the older group ($n = 11$, age: 55 to 74 years). Ethical approval was obtained from a local university and written informed consent was obtained for each participant. The demographic characteristics of the subjects are shown in Table 1.

2.2. Experimental setup

An examination of the biomechanical properties of plantar soft tissue was performed at the first and second metatarsal heads (MTHs) of the right foot by both the air-jet indentation system and TUPS. All of the participants were positioned comfortably in a sitting position with the testing leg supported on a stool. The testing knee was maintained in a slightly flexed position by a small towel placed underneath the knee, and the ankle was kept in a neutral position by a tailor-made stainless steel ankle foot supporting frame. The location of the testing points of the first and second MTHs was marked by a pen to standardize the measurement site for the two measurement tools and between trials. The skin of the tested area was cleaned with alcohol to facilitate ultrasound and penetration of optical signal into the plantar soft tissues. Preconditioning of four loading–unloading trials was performed on each testing point before the actual measurements were taken for each instrument, to ensure that the soft tissue was in a steady state. Two trials of indentation measurements were conducted on each measuring point with each instrument, with a 5-minute rest that allowed for recovery of tested soft tissue between the measurements. The mean of the two measurements was computed for further data analysis. There was 10 min rest between the two measurement tools and the sequence of measurement tools was randomized.

2.3. Instrumentation

2.3.1. The OCT-based air-jet indentation system

The non-contact OCT-based air-jet indentation system comprises of two parts: the testing probe and the data collection part (Fig. 1). Details of the system design could be found in Huang et al. (2009). The probe consists of a time domain OCT system and an air-jet bubbler that contains a super luminescent diode light source (DenseLight, DL-CS3055 A, Singapore) operated at a central wavelength of 1310 nm, a nominal −3 dB spectral bandwidth of 50 nm, and a nominal output power of 5 mW. This provides an axial resolution of 18 μm and an imaging depth of approximately 2 to 3 mm in highly scattered materials. A visible red light beam was used together with the invisible infrared beam in order to guide the detection point. The fiber-based OCT probe was modified to allow for the installation of an air-jet bubbler for producing the air jet. The diameter of the orifice of the air-jet bubbler was 1 mm. The probe was then affixed on a rigid stand with an adjustable height. The fine control knobs could make fine adjustments in the anteroposterior or mediolateral direction so that the laser beam could be accurately adjusted to focus vertically at around 5 mm below the surface of the bubbler during *in vivo* measurements (Fig. 2).

With an improvement of the system compared to our prototype (Huang et al., 2009), an electronic proportional valve with pressure feedback (ITV 1030-311L-Q, SMC Corporation, Tokyo, Japan) at a measurement range of 0.5 MPa was installed before the bubbler to

Table 1
Demographic characteristics of the subjects.

	Young group ($n = 19$)	Older group ($n = 11$)	*P*-value
Age (years)	27.11 (4.18)	62.18 (5.72)	<0.001
Female:male	8:11	5:6	0.861
Weight (kg)	65.88 (13.15)	64.13 (9.07)	0.700
Height (m)	1.70 (0.08)	1.66 (0.07)	0.097
Body Mass Index (kg/m^2)	22.80 (3.80)	23.60 (2.13)	0.465

Data are in mean (SD).

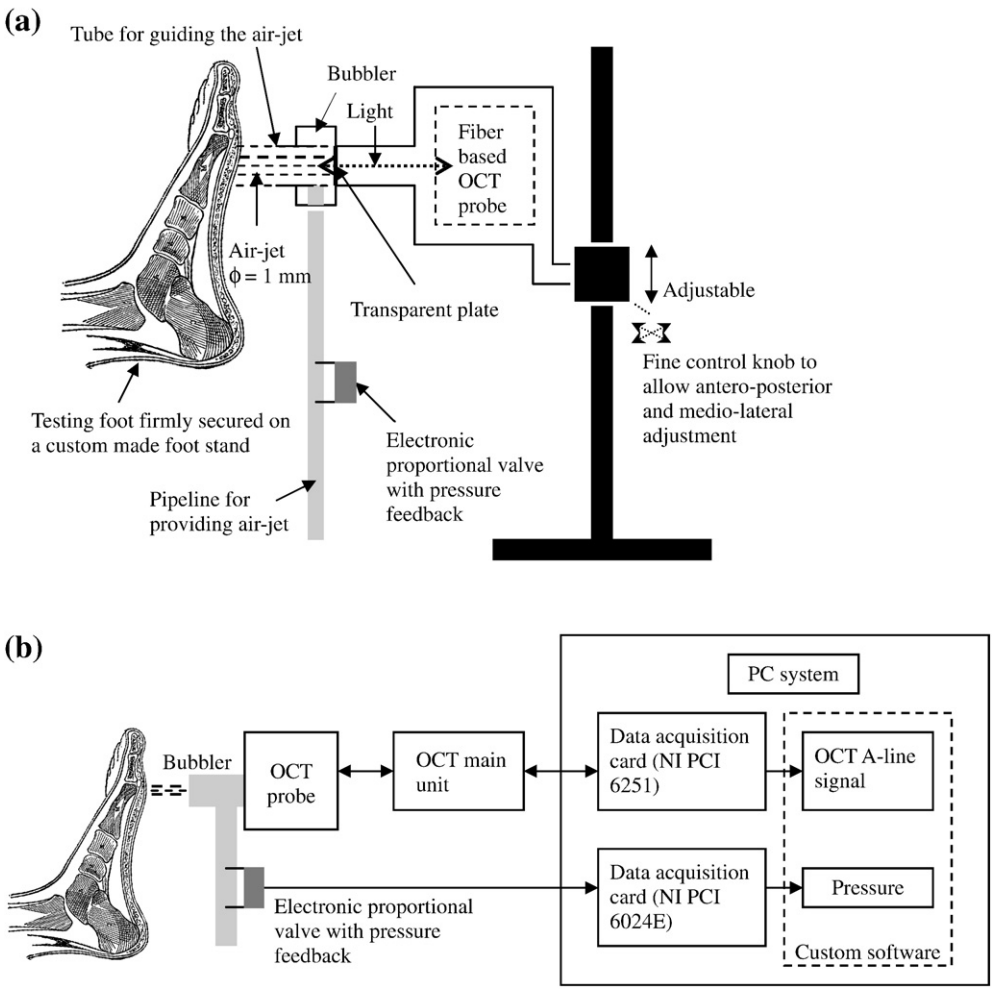

Fig. 1. Schematics of the (a) probe and (b) data collection part of the OCT-based air-jet indentation system.

continuously control and monitor the pressure of the air jet by voltage control. Calibration was performed to evaluate the relationship between the force on the tested specimen and the pressure. The relationship is expressed as $y = 0.1864x - 0.191$ with r^2 equal to 0.9997, where x is the pressure value read from the software, and y is the force (N). A transparent plate was installed at the top of the bubbler to seal the

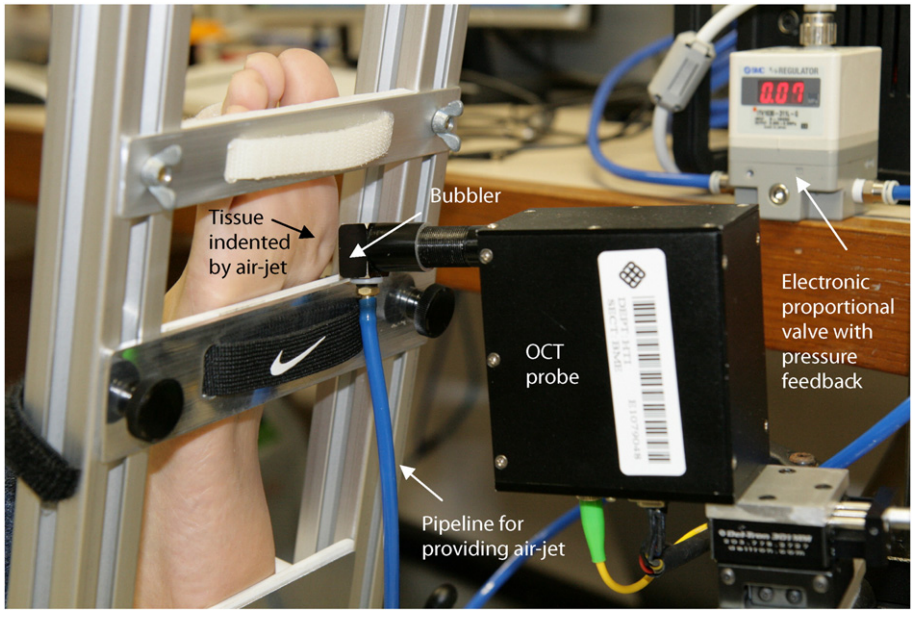

Fig. 2. A picture shows the setup of testing on plantar soft tissue *in vivo* by the air-jet indentation system.

pressurized air from the OCT components while laser beams could pass through (Fig. 1a). The data collection part of the system is shown in Fig. 1b. It involves PC software to collect the OCT and pressure signals. A second function of the software is to control the operation of the electronic valve. The deformation of the plantar soft tissue during indentation can be extracted by a cross-correlation technique to track the displacement of the skin surface as the deformation of the soft tissue.

Four cycles of loading and unloading with a total duration of approximately 36 s at an indentation rate of around 0.4 mm/s was carried out for each indentation trial loading cycle. The maximum indentation force used was 1.4 N and it gave a maximum displacement of about 1.6 mm in the softest forefoot plantar tissue being assessed. The stiffness coefficient k (N/mm) for the force/deformation ratio was then calculated from each test and it was used to represent the biomechanical properties of the soft tissue. Only the loading phase at the second to fourth cycles was utilized to calculate the stiffness coefficient.

$$k = F / d \tag{1}$$

where F is the indentation force and d is the deformation of the tissue. This was obtained by a regression of the force/deformation curve of the indentation.

2.3.2. Tissue ultrasound palpation system

A tissue ultrasound palpation system (TUPS) comprised of a pen-sized, hand-held indentation probe was used to measure the thickness and elasticity of the plantar soft tissue. It was shown to have high reliability and validity in previous studies done in the same laboratory (Zheng and Mak, 1996, 1999). A 10 MHz ultrasound transducer with a diameter of 3 mm at the tip of the probe served as both the indenter and sensor for measuring thickness and deformation of tissues. A 10 N load cell was connected in series with the ultrasound transducer to record the corresponding force during indentation. Frames of A-line signals of 4 K points sampled at a rate of 100 MHz were obtained for the extraction of initial soft tissue thickness and deformation during indentation. A PC program was used to display the real-time ultrasound reflection signals, and force response. The force response and ultrasound US A-line signal were captured during the indentation test with an approximate rate of 25 frames per second. All of the ultrasound and force data were then synchronized and recorded for subsequent offline analysis. Four to five cycles of loading and unloading, with a total duration of approximately 8 s were carried out for each indentation trial to ensure a stable manual indentation rate. The rate was estimated by monitoring the force and deformation curve shown in the computer screen. In comparison with air-jet indentation, the indentation rate used for TUPS measurement was approximately 4 times larger. According to the results of our previous study, the variation of measured modulus was negligible under these indentation rates (Zheng et al., 1999). The thickness and deformation of the plantar soft tissue layer were determined from the flight time of the ultrasound echo signal that was reflected from the soft tissue–bone interface. A constant sound speed of 1540 m/s in soft tissue was used in the current study (Goss et al., 1980). The effective Young's modulus was calculated using the following equation described by Hayes et al. (1972):

$$E = \frac{1-\nu^2}{2a\kappa(\nu, a/h)}\frac{p}{W} \tag{2}$$

where P is the applied force, w is the indentation depth, a is the radius of the indenter, ν is the Poisson's ratio, h is the tissue thickness, and κ is a scaling factor which is a function of ν and a/h.

2.4. Statistical analysis

Data analyses were conducted using SPSS (version 15.0 for Windows, company information). A two-sample t test and the Mann–Whitney U test were used to test for the demographic variables between the two study groups. The Pearson correlation coefficient was used to assess the relationship between the continuous variables of the stiffness coefficient k (N/m) as measured by the air-jet indentation system under the first and second MTHs and Young's modulus value E (kPa) by TUPS under the same sites and also the relationship between plantar tissue thickness and stiffness. The independent sample t test was used to compare the changes in stiffness coefficient, Young's modulus, and thickness between the young and older groups. The paired t test was used for within group comparison for the measurements of all these variables between the 2 measurement sites. The level of significance was set at 0.05 for all procedures.

3. Results

3.1. The correlation of the findings obtained between the air-jet indentation system and the TUPS

The indentation curve and a typical load-deformation record during the loading and unloading cycle as measured by the air-jet indentation system are shown in Fig. 3a and b respectively. The final calculation of the parameter in the loading phases at the second to fourth cycles is shown in Fig. 3c. The Pearson's correlation revealed a strong positive correlation ($r = 0.88$, $P < 0.001$) between the stiffness coefficient as measured by the air-jet indentation system and the effective Young's modulus as measured by TUPS (Fig. 4).

3.2. Comparison of the biomechanical properties of forefoot plantar soft tissue between the young and older groups

Table 2 compares the thickness and stiffness of the forefoot plantar soft tissue between the young and older groups at the two testing sites. In the older group, the average stiffness coefficient at the second MTH was 18% greater than that of the young subjects ($P < 0.05$). However, no significant difference ($P > 0.05$) in the stiffness coefficient was found between the two groups at the first MTH. The stiffness of the plantar soft tissue was site specific, and the mean stiffness coefficient measured at the second MTH was 28% greater than that in the first MTH ($P < 0.001$). Similar results were found for the stiffness measured from the TUPS. For the tissue thickness measurement, the plantar soft tissue measured in the older group was significantly thinner than that in the young group (both $P < 0.001$). The plantar soft tissue at the second MTH tended to be thicker than the first MTH but did not reach statistical significance ($P = 0.121$). No significant correlation was found between the thickness and Young's moduli of the plantar soft tissue ($P > 0.05$).

4. Discussion

The present study demonstrated a strong positive correlation of the findings obtained between the air-jet indentation system and TUPS. Therefore, we have demonstrated that the air-jet indentation system is capable of characterizing the biomechanical properties of plantar soft tissue *in vivo* and in a non-contact way. Older subjects showed a loss of elasticity and reduced thickness in their forefoot plantar soft tissue, with the second MTH displaying stiffer and thicker plantar tissue than the first MTH.

4.1. The OCT-based air-jet indentation system

The present study used a newly developed non-contact OCT-based air-jet indentation system to characterize the biomechanical properties of soft tissue. The system is based on the concept of an optical

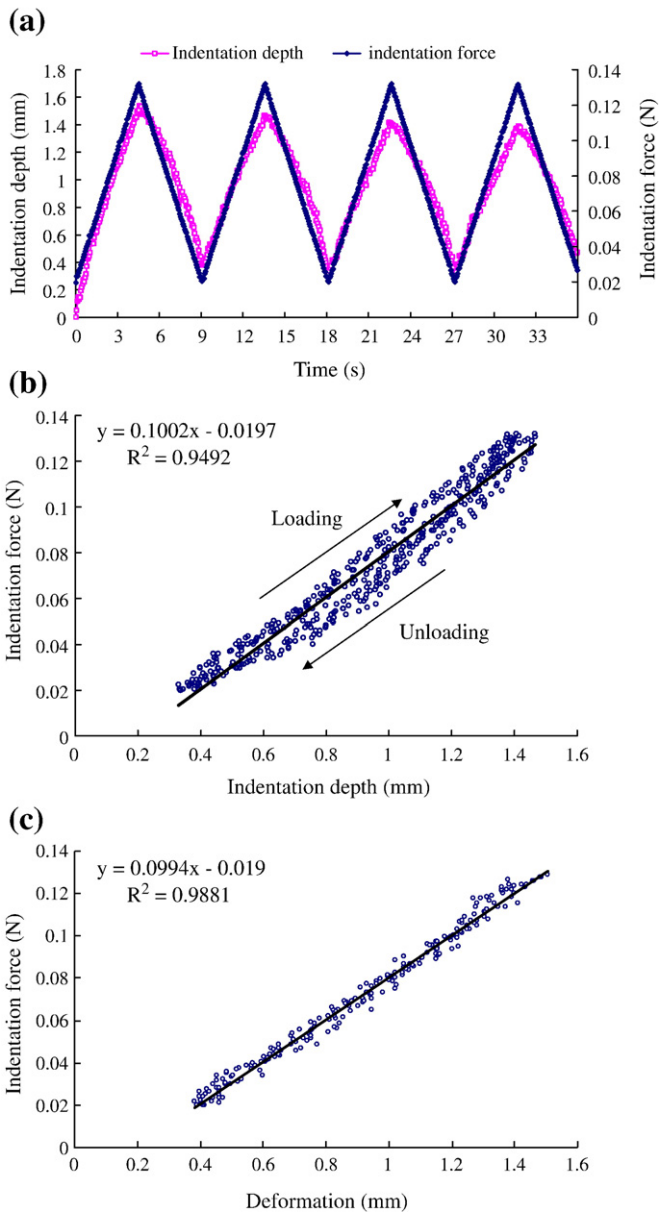

Fig. 3. (a) A typical indentation curve obtained on one subject with four loading and unloading cycles by the air-jet indentation system. (b) A typical load-indentation curve obtained from a subject and the corresponding regression curve by the air-jet indentation system at the second to fourth indentation cycles. (c) The selected data points used for estimation of stiffness coefficient k (N/mm) of plantar soft tissues. The data in the loading phases at the second to fourth cycles were selected. The relationship between the force measured within the air jet and the relative deformation of the plantar tissues is shown in the corresponding regression curve.

interferometric technique for extracting the deformation induced and pressure controlled air jet as an indenter to stimulate loading and unloading (Huang et al., 2009). The force–deformation curves can then be derived from the indentation test and the stiffness coefficient obtained represents the biomechanical properties of the whole tissue layer under the skin to be tested because the deformation was the change of all the soft tissue covering the bone. The system demonstrated a strong correlation (Pearson's coefficient: $r = 0.88$, $P < 0.001$) in evaluating the biomechanical properties of plantar soft tissue with the reference of the TUPS measurement. To date, no non-contact quantitative measurement tool is available to determinate the biomechanical properties of living tissues. The present air-jet indentation system incorporated with the fast-scanning OCT signal enables tissue deformations of level of microns to be detected. Hence, this system is

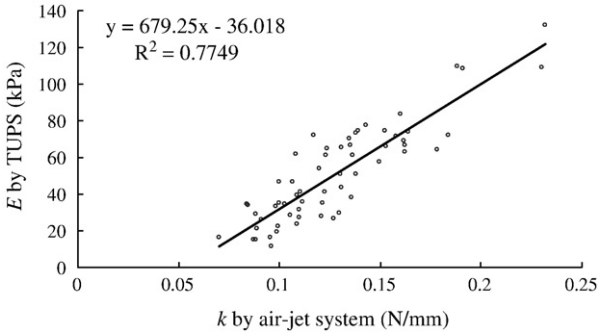

Fig. 4. The correlation between the Young's moduli of the plantar tissue mechanical properties determined using the tissue ultrasound palpation system and the stiffness coefficient measured by the air-jet indentation system.

of potential use for evaluating the biomechanical properties of fine delicate living tissue that requires non-contact applications, such as wound tissues. As one of the critical outcomes of the wound repairing process is the restoration of the mechanical properties of tissue strength, the stiffness coefficient provided by the air-jet indentation system could be used for monitoring the physiological state of the healing process of a wound. It provides quantitative measurements that are useful information for selecting appropriate treatment or monitoring treatment effectiveness. However, the system is unable to measure the tissue thickness larger than 3 mm, which is a limitation of the unit.

4.2. Effects of aging on the biomechanical properties of plantar soft tissue

The present study assessed the biomechanical properties of plantar soft tissue at the first and second MTHs because these withstand the greatest plantar pressure during ambulation and are therefore the most vulnerable sites. They are also the most common sites for diabetics to develop foot ulcerations (Armstrong et al., 1998; Gefen, 2003). The present study examined the stiffness coefficient using an air-jet indentation system that represents the mechanical properties of the whole layer of plantar tissue. A higher stiffness coefficient value implies that greater force or stress is needed to deform the soft tissue. Our results did not demonstrate any significant difference in the biomechanical properties at the plantar first MTH soft tissue between the young and older subjects. It has been reported that the intrinsic extensibility of the skin begins to decline at the age of 70 (Escoffier et al., 1989), while the skeletal mass decreases by 6% per decade after age 30 due to changes in the chemical composition of the muscle fibers (Whitney, 2003). The mean age of the older group in the present study was 62.18 (SD 5.72) years, which may be not old enough to demonstrate the change in tissue extensibility at the first MTH. As for the second MTH, our findings demonstrated that the stiffness of plantar soft tissue in the second MTH was significantly greater than ($P < 0.05$) that in the first MTH in both study groups, and the stiffness of plantar soft tissue at the second MTH in the older subjects was 18% greater than in the young subjects.

Our findings are in good agreement with those reported in the literature. By using a rigid indentation device, Klaesner et al. (2002) demonstrated that the mean stiffness coefficient value at the third MTH (0.79 (SD 0.17) N/mm) was greater than that in the first MTH (0.72 (SD 0.32) N/mm), versus 0.69 (SD 0.28) N/mm recorded in the fifth MTH among normal healthy subjects. Hsu et al. (2005) demonstrated that most of the metatarsus in the older subjects had a significantly greater elastic modulus and energy dissipation ratio than those of the younger persons. Mueller et al. (2005) demonstrated that the peak pressure gradient was substantially higher in the forefoot than in the rearfoot. Our present findings demonstrated that older subjects had stiffer plantar soft tissue, and that the change

Table 2
The stiffness coefficient measured by the air-jet system and the effective Young's modulus and thickness measured by the TUPS.

	Young group ($n=19$)	Older group ($n=11$)	Mean differences	95% CI	*P*-value
First metatarsal head					
Stiffness coefficient k (N/mm)	0.10 (0.02)	0.12 (0.02)	−0.01 (0.01)	−0.02 to 0.00	0.100
Young's modulus E (kPa)	32.91 (14.41)	36.73 (19.26)	−3.82 (6.17)	−16.47 to 8.84	0.541
Soft tissue thickness (mm)	12.70 (2.14)	9.92 (0.87)	2.77 (0.56)	1.63 to 3.92	<0.001
Second metatarsal head					
Stiffness coefficient k (N/mm)	0.14 (0.02)	0.17 (0.04)	−0.03 (0.14)	−.06 to −.00	0.046
Young's modulus E (kPa)	58.30 (15.88)	83.24 (28.77)	−24.97 (9.40)	−45.20 to −4.74	0.019
Soft tissue thickness (mm)	13.21 (1.80)	10.63 (1.78)	2.58 (0.67)	1.20 to 3.96	<0.001

Data are in mean (SD).
Abbreviation: CI, confidence interval.

seems to be more obvious at the second MTH than in the first MTH. Gefen (2003) used a finite element model to simulate the change of stress concentration in the forefoot with diabetes causing the stiffening of the soft tissue. They found with the increase of the tissue stiffness, the stress concentration in the first and second metatarsal heads might increase significantly. This may make the foot less capable of accommodating pressures and stresses on the plantar foot, and hence diminish its ability to effectively distribute foot–ground contact loads during weight-bearing situations. As demonstrated in the present study, the older group has stiffer forefoot medial plantar soft tissue, and this may lead to further reduction on the shock absorption capability of plantar tissue. This may explain why older people have a higher incidence of foot problems such as symptomatic metatarsalgia.

The thickness of the plantar soft tissues affects the amount of cushioning available for an even distribution of pressure at the foot. We found that the thickness of the plantar soft tissue in the older group measured at both the first and second MTH was lower than that of the young group at the same sites. This reduced thickness in the soft tissue might be due to a reduction in the thickness of the skin as well as to a loss of underlining subcutaneous fat. It was suggested that aged skin has abnormally deposited on the elastic material and has decreased lipid content (Waller and Maibach, 2006). Thinning of the skin as well as a loss of subcutaneous fat under the metatarsal heads region has also been reported in the aged population (Whitney, 2003). Cavanagh (1999) demonstrated that the tissue underneath the second MTH undergoes a maximum compression of 45.7% during the late stages of ground contact. It has been suggested that the thickness of the plantar soft tissues at the MTH is important for reducing pressure (Cevera et al., 1997). Abouaesha et al. (2001) reported a strong inverse relationship between plantar tissue thickness and peak plantar pressure. The reduced thickness of the plantar tissue in the aged population increases the peak plantar pressure and reduces the degree of cushioning in the foot, making the skin of the foot more susceptible to breaking down.

5. Conclusion

The present study demonstrated that the older population experiences stiffening of the forefoot plantar soft tissue, especially in the second MTH. This causes the foot to become less elastic and less able to distribute foot–ground contact force and pressure through deformation in these areas. This, together with a reduction in the thickness of the tissue diminishes the cushioning property of the plantar soft tissue during situations of standing or walking. The newly developed non-contact OCT-based air-jet indentation system is a useful instrument for characterizing the biomechanical properties of soft tissue and could potentially be used in providing quantitative examinations of fine delicate tissue such as wound areas.

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References

Abouaesha, F., van Schie, C.H., Griffths, G.D., Young, R.J., Boulton, A.J., 2001. Plantar tissue thickness is related to peak plantar pressure in the high-risk diabetic foot. Diab. Care 24, 1270–1274.
Armstrong, D.G., Lavery, L.A., Bushman, T.R., 1998. Peak foot pressures influence the healing time of diabetic foot ulcers treated with total contact casts. J. Rehabil. Res. Dev. 35, 1–5.
Bojsen-Moller, F., Flagstad, K.E., 1976. Plantar aponeurosis and internal architecture of the ball of the foot. J. Anat. 121, 599–611.
Cavanagh, P.R., 1999. Plantar soft tissue thickness during ground contact in walking. J. Biomech. 32, 623–628.
Cevera, J.J., Bolton, L.L., Kerstein, M.D., 1997. Options for diabetic patients with chronic heel ulcers. J. Diab. Complications 11, 358–366.
Duckworth, T., Boulton, A.J., Betts, R.P., Franks, C.I., Ward, J.D., 1985. Plantar pressure measurements and the prevention of ulceration in the diabetic foot. J. Bone Joint Surg. Br. 67, 79–85.
Escoffier, C., de Rigal, J., Rochefort, A., Vasselet, R., Lévêque, J.L., Agache, P.G., 1989. Age-related mechanical properties of human skin: an in vivo study. J. Investig. Dermatol. 93, 353–357.
Gefen, A., 2003. Plantar soft tissue loading under the medial metatarsals in the standing diabetic foot. Med. Eng. Phys. 25, 491–499.
Gefen, A., Megido-Ravid, M., Azariah, M., Itzchak, Y., Arcan, M., 2001. Integration of plantar soft tissue stiffness measurements in routine MRI of the diabetic foot. Clin. Biomech. 16, 921–925.
Goss, S.A., Johnston, R.L., Dunn, F., 1980. Compilation of empirical ultrasonic properties of mammalian tissues. II. J. Acoust. Soc. Am. 68, 93–108.
Hayes, W.C., Keer, L.M., Herrmann, G., Mockros, L.F., 1972. A mathematical analysis for indentation tests of articular cartilage. J. Biomech. 5, 541–551.
Hsu, T.C., Wang, C.L., Tsai, W.C., Kuo, J.K., Tang, F.T., 1998. Comparison of the mechanical properties of the heel pad between young and elderly adults. Arch. Phys. Med. Rehabil. 79, 1101–1104.
Hsu, T.C., Wang, C.L., Shau, Y.W., Tang, F.T., Li, K.L., Chen, C.Y., 2000. Altered heel-pad mechanical properties in patients with Type 2 diabetes mellitus. Diabet. Med. 17, 854–859.
Hsu, T.C., Lee, Y.S., Shau, Y.W., 2002. Biomechanics of the heel pad for type 2 diabetic patients. Clin. Biomech. 17, 291–296.
Hsu, C.C., Tsai, W.C., Chen, C.P., Shau, Y.W., Wang, C.L., Chen, M.J., et al., 2005. Effects of aging on the plantar soft tissue properties under the metatarsal heads at different impact velocities. Ultrasound Med. Biol. 31, 1423–1429.
Huang, D., Swanson, E.A., Lin, C.P., Schuman, J.S., Stinson, W.G., Chang, W., et al., 1991. Optical coherence tomography. Science 254 (5035), 1178–1181.
Huang, Y.P., Zheng, Y.P., Wang, S.Z., Chen, Z.P., Huang, Q.H., He, Y.H., 2009. An optical coherence tomography (OCT)-based air jet indentation system for measuring the mechanical properties of soft tissues. Meas. Sci. Technol. 20, 015805.
Klaesner, J.W., Hastings, M.K., Zou, D., Lewis, C., Mueller, M.J., 2002. Plantar tissue stiffness in patients with diabetes mellitus and peripheral neuropathy. Arch. Phys. Med. Rehabil. 83, 1796–1801.
Kubo, K., Morimoto, M., Komuro, T., Tsunoda, N., Kanehisa, H., Fukunaga, T., 2007. Age-related differences in the properties of the plantar flexor muscles and tendons. Med. Sci. Sports Exerc. 39, 541–547.
Mueller, M.J., Zou, D., Lott, D.J., 2005. "Pressure gradient" as an indicator of plantar skin injury. Diab. Care 28, 2908–2912.
Pascual Huerta, J., Alarcón García, J.M., 2007. Effect of gender, age and anthropometric variables on plantar fascia thickness at different locations in asymptomatic subjects. Eur. J. Radiol. 62, 449–453.

Pasquali-Ronchetti, I., Baccarani-Contri, M., 1997. Elastic fiber during development and aging. Microsc. Res. Tech. 38, 428–435.
Pathak, A.P., Silver-Thorn, M.B., Thierfelder, C.A., Prieto, T.E., 1998. A rate-controlled indentor for in vivo analysis of residual limb tissues. IEEE Trans. Rehabil. Eng. 6, 12–20.
Pittman, J., 2007. Effect of aging on wound healing: current concepts. J. Wound Ostomy Continence Nurs. 34, 412–415.
Reiber, G.E., Lipsky, B.A., Gibbons, G.W., 1998. The burden of diabetic foot ulcers. Am. J. Surg. 176, 5S–10S.
Waller, J.M., Maibach, H.I., 2006. Age and skin structure and function, a quantitative approach (II): protein, glycosaminoglycan, water, and lipid content and structure. Skin Res. Technol. 12, 145–154.
Whitney, K.A., 2003. Foot deformities, biomechanical and pathomechanical changes associated with aging including orthotic considerations, part II. Clin. Podiatr. Med. Surg. 20, 511–526.
Zheng, Y.P., Mak, A.F., 1996. An ultrasound indentation system for biomechanical properties assessment of soft tissues in-vivo. IEEE Trans. Biomed. Eng. 43, 912–918.
Zheng, Y., Mak, A.F., 1999. Effective elastic properties for lower limb soft tissues from manual indentation experiment. IEEE Trans. Rehabil. Eng. 7, 257–267.
Zheng, Y.P., Mak, A.F.T., Lue, B.K., 1999. Objective assessment of limb tissue elasticity: development of a manual indentation procedure. J. Rehabil. Res. Dev. 36, 71–85.
Zheng, Y.P., Choi, Y.K., Wong, K., Chan, S., Mak, A.F., 2000. Biomechanical assessment of plantar foot tissue in diabetic patients using an ultrasound indentation system. Ultrasound Med. Biol. 26, 451–456.

A novel noncontact method to assess the biomechanical properties of wound tissue

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ABSTRACT

A novel noncontact optical coherence tomography based air-jet indentation system was developed for characterizing the biomechanical properties of soft tissue in a noncontact way. This study aimed to measure the stiffness of diabetic foot ulcer tissues by using this air-jet indentation system, and examining the test/retest reliability. Eight subjects with diabetes (seven males, one female), with a total of 10 foot ulcers between them, participated in the study. A total of 20 measuring sites located at the central wound bed ($n=10$) or peri-ulcer areas ($n=10$), respectively, were evaluated with the air-jet indentation system. Four cycles of loading and unloading, each with a duration of approximately 36 seconds at an indentation rate of 0.08 mm/s, were carried out for each indentation trial. The test/retest reliability was examined at all measuring points. The average stiffness coefficient of the peri-ulcer area (mean $\pm$ SD: 0.47 ± 0.15 N/mm) was significantly larger than that of the central wound bed area (mean $\pm$ SD: 0.35 ± 0.23 N/mm; $p=0.042$). A high value for test/retest reliability was shown (intraclass correlation coefficient: 0.986; Pearson's correlation: $r=0.972$, $p < 0.001$). Our preliminary findings showed that the peri-ulcer area had greater stiffness than the central wound bed. This greater magnitude of hardness and inelasticity at the peri-ulcer region may scatter part of the contractile forces for wound contraction during the healing process. We found the novel air-jet system to be a reliable tool for characterizing the stiffness of soft tissues around the wound in a noncontact way.

Chronic wounds such as diabetic foot ulcers, pressure ulcers, and venous ulcers are growing challenges for the health care system. An accurate evaluation of the physiological status of a chronic wound provides important information to guide appropriate treatment decisions and monitor the effectiveness of the treatment. Thus far, there is a lack of accurate quantitative methods to assess the properties of wound tissue. The restoration of the mechanical properties of tissue strength is a potential quantitative outcome of the process of repairing the wound.[1] In order to prevent contaminating the wound, it is of the utmost importance to be able to assess the mechanical properties of wound tissues quantitatively in a noncontact way.

Wound healing and its biomechanical properties

Wound healing is a natural dynamic process of tissue repair. It involves the reproduction of cells, as well as the recovery of a damaged extracellular matrix that generates resurfacing, reconstitution, and restoration of the mechanical strength of injured tissue.[2] Under normal circumstances, wound repair exhibits the three overlapping phases of inflammation, proliferation, and maturation.[3] There are changes in biomechanical properties of wound bed soft tissue over the wound healing phases, which is partly determined by the thickness and the distribution of the extracellular matrix.[4] Toward the final phase of wound closure, the process is both accompanied by and followed by changes in physical and mechanical properties of the wound and peri-ulcer tissues during the biological transformation. The physical attributes of the collagen fibers such as the diameter of a fiber, its alignment, and the cross-linking of fibers in a healing wound are thought to be essential factors in characterizing the biomechanical properties of a wound.[5] It is well known that maturation of the collagen matrix contributes to the mechanical strength of dermal tissue and the fibroblast is the primarily cell type that provides traction force on extracellular matrix fibrils and hence brings collagen fibrils closer to each other to enhance wound contraction.[4] The myofibroblast is a key cell for the remodeling of connective tissue that takes place during wound healing and fibrosis development.[6] It is also the main contractile unit for the development of the mechanical force found in wound tissues.[7] Mechanical stresses at the wound site are thought to guide collagen fibrillogenesis and any altered tension during wound closure will affect the extent of scarring.[8] Moreover, mechanical tension is also considered to control contractile activity of the granulation tissues and myofibroblast differentiation.[9] Doillon et al.[10] showed a direct relationship between collagen fiber diameters and wound tensile strength. Doillon et al.[11] demonstrated that collagen fiber diameter increases with time post-wounding and is related to tensile strength. As wound healing progresses, the orientation of collagen fibers takes a more uniform pattern and subsequently affects its mechanical strength.[8] Impaired wound healing due to a decrease in the fibroblast replication may disturb the regeneration of a new dermal

matrix, subsequently resulting in a decrease in the mechanical strength of the wound.[12] It has been suggested that a wound becomes stiffer, stronger, and more extensible throughout a normal wound healing process, any decrease in mechanical strength may lead to a reduction in the resilience, toughness, and maximum extension of the tissue.[4] Therefore, an effective wound management program should promote the optimal mechanical strength of the wound tissue at the various stages of healing.[4]

Evaluation of the biomechanical properties of the wound bed

Theoretically, the ideal in wound healing is to regenerate tissues in such a way that the structural and functional properties of wounded tissue can be restored to the levels that existed before the wounding.[13] Very few studies so far have examined the biomechanical properties of the wound bed. The majority of these were in vitro studies of animal tissues that examined the biomechanical characteristics of excisional wounds during the early phases of healing using such material testing systems as tensiometers.[10,11,14–18] This method involves measuring excised strips with given dimensions for uni-axial tensile tests. Standard definitions for axial stress, axial strain, and the modulus of elasticity are usually employed. One of the disadvantages of a tensiometer is that it is invasive and disruptive in nature, in that the tissues being examined must be excised for testing. The biomechanical property measured in the sample tissue can be different from a living in vivo wound. With regard to this, compressive indentometry devices have been used to measure the biomechanical behavior of living wound tissue in vivo.[19] Perry et al.[20] used the Dimensional Analysis System to perform a biomechanical analysis of linear incisional wounds in rats in vivo. In addition, Gingrass et al.[21] described a method for the in vivo biomechanical characterization of linear incision wounds in rats using a vacuum pressure chamber for displacement induction and video camera to capture the displacement induced aided by observing the infrared light-reflecting targets displacement that were placed on either side of the linear incision wound. However, this contact assessment method may potentially contaminate or damage the fragile wound and induce pain during the in vivo measurements. Indeed, the majority of previous studies had focused primarily on measuring the biomechanical properties of incisional wounds in vitro, little is known about the mechanical properties of an open wound or ulcer in human beings. An optical-coherence-tomography (OCT) based air-jet indentation system has recently been developed. It can be used for the in vivo characterization of the biomechanical properties of wound tissue in a noncontact way. This system is composed of an OCT component to capture the displacement of soft tissue, as well as its stiffness under indentation.[22] The concept of the system is to utilize the optical interferometric technique for obtaining the deformation of the tested tissue during indentation. By using a pressure controlled air jet as an indenter and OCT-signals to extract the deformation that is induced, it would then be possible to objectively and quantitatively deduce the biomechanical properties of soft tissue. The instrument has been proven to be a useful tool for characterizing the biomechanical properties of soft tissue in vivo in humans.[23] As it is noncontact in nature, this device can potentially be used to characterize the biomechanical properties in any type of wound. It would be particularly useful for chronic wounds such as diabetic ulcers, pressure ulcers, and venous ulcers, making it possible to objectively monitor the wound healing process over time.

The present study aimed to characterize and compare the biomechanical properties of diabetic foot ulcers in the central wound bed and the peri-ulcer area using the newly developed noncontact OCT-based air-jet indentation system. The test/retest reliability of the system for measuring the biomechanical properties of diabetic foot ulcer tissues was also examined. The present equipment and testing procedures allow for the noninvasive in vivo biomechanical testing of wound tissues, which shed light on the biomechanical properties of the wound bed during the healing process.

MATERIALS AND METHODS

Subjects

Eight diabetic subjects (seven males, one female) with a total of 10 foot ulcers were evaluated. Their mean age was 59.9 ± 9.5 (mean $\pm$ SD) years, and the mean duration of their diabetes was 13.1 ± 6.3 (mean $\pm$ SD) years. All of the ulcers were located below the ankle, and the mean duration of the ulcerations was 6.1 ± 7.2 (mean $\pm$ SD) months. The wounds were graded using the Wagner Grading System. None of the subjects suffered from wound infections. Ethical approval for the study was obtained from the Hong Kong Polytechnic University and Queen Elizabeth Hospital, Hong Kong. Written informed consent was obtained from each participant. The demographic characteristics of the subjects are shown in Table 1.

Experimental procedures

All of the participants were positioned comfortably in a sitting position with the ulcerated foot supported on a stool. The knee of the tested leg was maintained in a slightly flexed position by a small towel placed underneath the knee, and the ankle was kept in a neutral position by a tailor-made stainless-steel ankle/foot supporting frame. An examination of the biomechanical properties of diabetic foot ulcer tissue was performed at the central wound bed ($n=10$) and peri-ulcer area ($n=10$) by the air-jet indentation system. Therefore, a total of 20 measuring sites from 10 wounds were evaluated. Preconditioning of four loading–unloading cycles was performed on each testing point before the actual measurements were taken. This was to ensure that the biomechanical properties of the soft tissue were maintained in a steady state. Two trials of indentation measurements were conducted on each measuring point, with a 5-minute rest period that allowed the tested soft tissue to recover between the measurements. The mean of the two trials was computed for further data analysis. The test/retest reliability was examined by the same assessor at all measuring points, 1 hour apart.

Table 1. Subject characteristics

Subjects	Gender	Age	Ulcer location	Ulcer duration (months)	Wound area (cm^2)	Wound grading
1	M	63	Forefoot	24	25.00	2
2	M	48	Big toe	12	0.25	1
3	M	49	Plantar foot	1	1.00	1
4	M	53	Plantar foot	2	1.50	1
			Lateral malleolus	2	4.60	2
5	M	59	Second toe	1	0.64	2
			Lateral heel	6	1.60	1
6	M	68	Heel cord	8	0.48	1
7	F	64	Metatarsal head	3	0.50	1
8	M	75	Lateral heel	2	0.96	1

Each wound was graded by Wagner Grading system.

Instrumentation

The OCT-based air-jet indentation system

The noncontact OCT-based air-jet indentation system is comprised of two parts: the testing probe and the data collection segment (Figure 1). Details of the design of the system can be found in a previously published paper by the same laboratory.[22] The probe consists of a time domain OCT system and an air-jet bubbler that contains a super luminescent diode light source (Dense Light, DL-CS3055 A, Singapore) operated at a central wavelength of 1310 nm, a nominal −3 dB spectral bandwidth of 50 nm, and a nominal output power of 5 mW. This provides an axial resolution of 18 μm and an imaging depth of approximately 2–3 mm in highly scattered materials. A visible red light beam was used together with the invisible infrared beam in order to guide the detection point. The fiber-based OCT probe was modified to allow for the installation of an air-jet bubbler for producing the air jet. The diameter of the orifice of the air-jet bubbler was 1 mm. The probe was then affixed to a rigid stand, the height of which was adjustable. The fine control knobs could make precise adjustments in the anteroposterior or mediolateral direction so that the laser beam could be accurately adjusted to focus vertically at around 5 mm below the surface of the bubbler during in vivo measurements.

In an improvement of the system as compared with our prototype,[22] an electronic proportional valve with pressure feedback (ITV 1030-311L-Q, SMC Corporation, Tokyo, Japan) at a measurement range of 0.5 MPa was installed before the bubbler to continuously control and monitor the pressure of the air jet by voltage control. Calibration was performed to evaluate the relationship between the force applied on the tested specimen and the pressure measured in the air pipe. The calibration was performed by placing a load cell at the location of the specimens or tissues and measuring the relationship between air

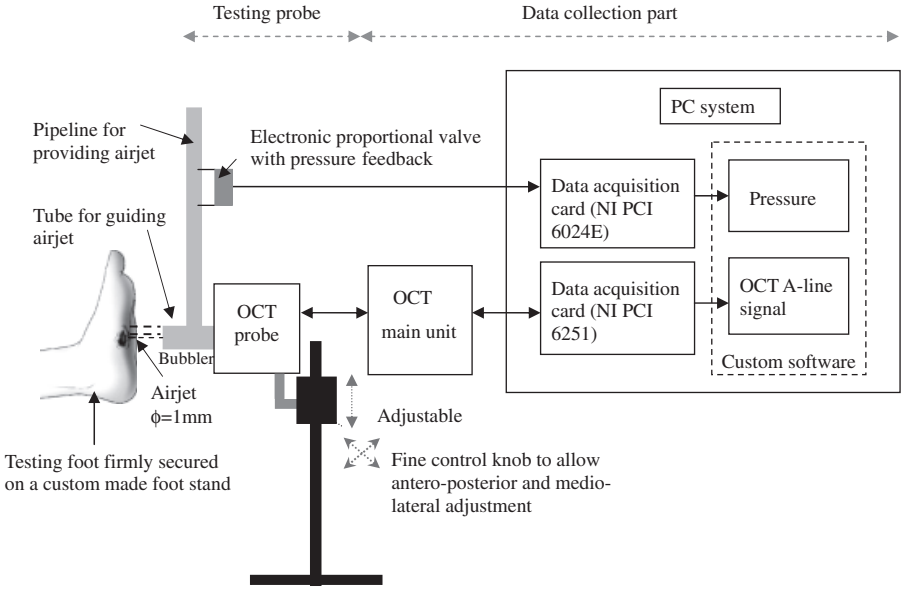

Figure 1. Schematics representation of the optical coherence tomography-based air-jet indentation system.

pressure and force sensed by the load cell. The relationship was expressed as $y=0.1864x-0.191$ with r^2 equal to 0.9997, where x was the pressure value inside the air pipe and y was the force (N) applied on the tissue surface. A transparent plate was installed at the top of the bubbler to seal the pressurized air from the OCT components while laser beams could pass through. The data collection part of the system involved the use of personal computer software to collect the OCT and pressure signals. The second function of the software was to control the operation of the electronic valve. The deformation of the wound tissue during indentation was extracted by a cross-correlation technique to track the displacement of the wound surface as the deformation of the soft tissue.

Four cycles of loading and unloading with a total duration of approximately 36 seconds at an indentation rate of around 0.08 mm/s was carried out for each indentation trial loading cycle. The maximum indentation force used was 0.14 N and it gave a maximum displacement of about 0.35 mm. The stiffness coefficient k (N/mm), that is the slope of the force–deformation relationship, was then calculated from each test (Figure 2C) and it was used to represent the biomechanical properties of the wound tissue. Only the loading phase at the second to fourth cycle was utilized to calculate the stiffness coefficient.

$$k = F/d \quad (1)$$

where F was the indentation force and d was the deformation of the tissue. This was obtained by a regression of the force/deformation curve of the indentation.

Statistical analysis

Data analyses were conducted using SPSS (version 15.0 for Windows). A paired sample t-test was used to test for the mean difference of the continuous variables of the stiffness coefficient k (N/m) between the central wound bed and peri-ulcer tissues. The Pearson correlation coefficient and intra-class coefficient (ICC) were used to assess the test/retest reliability of the air-jet indentation system. The level of significance was set at 0.05 for all analyses.

RESULTS

The indentation curve and a typical load–deformation relationship recorded during the loading and unloading cycle by using the air-jet indentation system are shown in Figure 2A and B, respectively. The final calculations of the parameter in the loading phases at the second to fourth cycles are shown in Figure 2C. The average stiffness coefficient of the peri-ulcer area (mean ± SD: 0.47 ± 0.15) was significantly greater than that of the central wound bed area (mean ± SD: 0.35 ± 0.23 N/mm; $p=0.042$). A high value for test/retest reliability was demonstrated (ICC: 0.986; Pearson's correlation: $r=0.972$, $p < 0.001$) (Figure 3).

DISCUSSION

In the present study, a novel noncontact OCT-based air-jet indentation system was presented for characterizing the biomechanical properties of wound tissues. To date, no noncontact quantitative measurement tool is available to determine the biomechanical properties of living tissues. The present air-jet indentation system incorporated with the fast-scanning OCT signal enables tissue deformations at the level of microns to be detected. The system is based on the concept of an optical interferometric technique for extracting the deformation-induced and pressure-controlled air jet as an indenter to stimulate loading and unloading.[22] The force–deformation relationship can then be derived from the indentation test (Figure 2B), and the stiffness coefficient obtained represents the biomechanical properties of the whole tissue layer under the wound

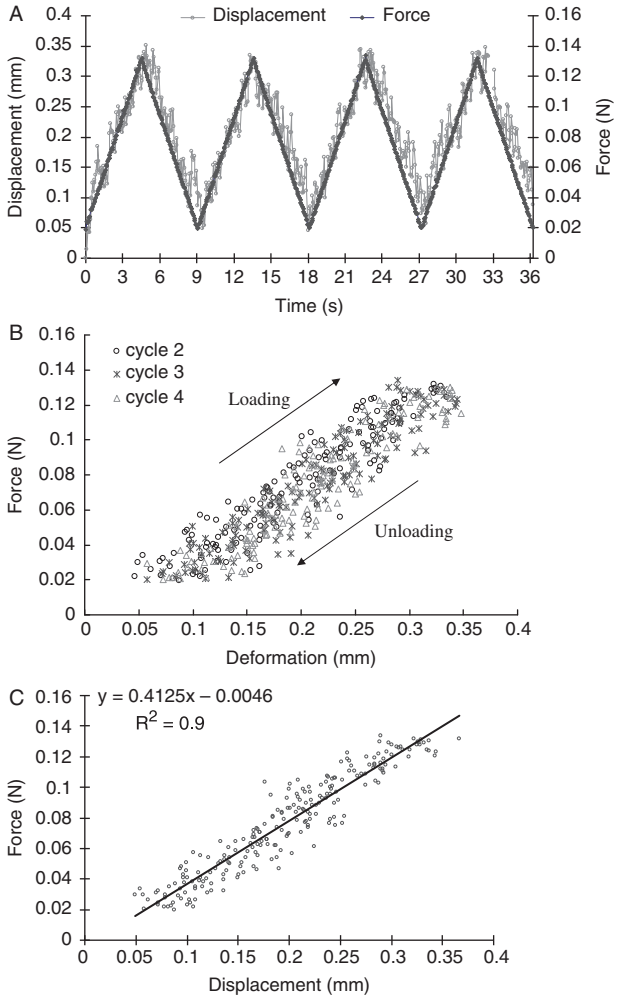

Figure 2. (A) A typical indentation curve obtained on one subject on the central wound bed tissue with four loading and unloading cycles by the air-jet indentation system. (B) A scatter plot of the force-deformation relationship obtained from a subject by the air-jet indentation system at the 2nd–4th indentation cycles. (C) The selected data points used for estimation of stiffness coefficient k (N/mm) of the wound tissues. The data in the loading phases at the 2nd–4th cycle were selected. The relationship between the force measured within the air-jet and the deformation of the wound tissues is shown in the corresponding regression curve.

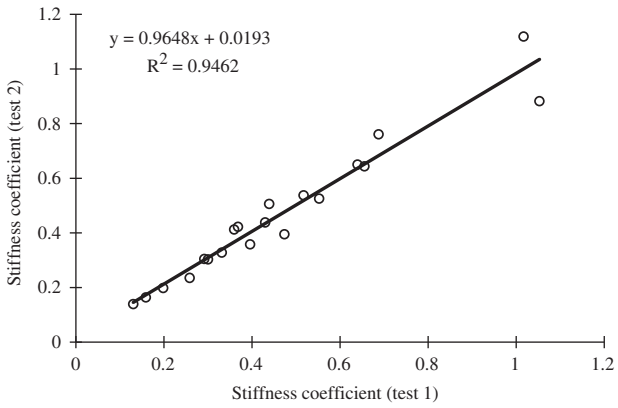

Figure 3. The correlation curve between the stiffness coefficients obtained by test 1 and test 2 on the same measuring point of the wound or peri-wound tissues.

surface being tested. The stiffness coefficient of this air-jet indentation system has been shown to have a high correlation with the corresponding Young's moduli obtained by conventional mechanical testing in a phantom model[22] as well as by a tissue ultrasound palpation system in human plantar soft tissues.[23] Our findings further demonstrate the strong test/retest reliability of this system for characterizing the biomechanical properties of diabetic foot ulcer tissues in vivo. However, the system is unable to measure the tissue thickness, which is a limitation of the system. Apart from diabetic foot ulcers, this reliable measurement tool could be used in measuring the biomechanical properties of various types of wounds, whether acute or chronic in nature. Better monitoring of the healing process for wound tissues will help to shed light on the pathogenesis of a nonhealing wound under certain pathological conditions such as pressure ulcers or diabetic foot ulcers.

The biomechanical properties of connective tissue depend on the extent to which collagen fibers have been laid down, as these are important for the functioning of the tissues. It is thought that such information will provide insights into the degree to which the collagen fibers are cross-linked and the extent of the degradation of the elastic fiber network.[24] In this regard, the mechanical properties of a connective tissue can be interpreted as a reflection of the organization of the tissue.[16] However, as there have been very few studies on the biomechanical properties of wound tissues, we postulate that the elastic modulus of wound tissue may differ from those of the surrounding healthy tissues; and the elastic modulus may change over various phases of the wound healing process. Thus, a quantitative assessment of the biomechanical properties of the wound bed and surrounding tissue is important in understanding the process and factors that may contribute to wound healing. The present study found that the peri-ulcer area showed a greater magnitude of hardness and inelasticity, as reflected in the average stiffness coefficient, than did the ulcerated tissues. Indeed, the present study shows that both the central and peri-wound sites are stiffer as compared with healthy tissues. Chao et al.[23] reported that the stiffness coefficient of normal plantar tissues under the medial metatarsal heads ranges from 0.1 to 0.17 N/mm whereas the stiffness coefficient of wound tissues as shown in the present study ranges from 0.35 N/mm in central wound bed to 0.47 N/mm in peri-ulcer area. However, the normal reference value of stiffness of plantar tissues was only obtained at the metatarsal head,[23] and the stiffness of soft tissue is site specific, the stiffness can vary in other wound sites such as heel pad or toes as shown in the present study. Therefore, future studies should be performed to examine the normal reference value of stiffness coefficient obtained in specific sites of the foot. The stiffness measured from soft tissues with intact skin located adjacent to the skin ulcer can serve as a reference for wound repair.

The reduced elasticity at the edge of the wound as shown in the present study may scatter part of the contractile forces for wound contraction. Hinz[25] demonstrated that stiffer tissue may modulate the character of the more elastic tissue by driving the differentiation of a variety of precursor cells into fibrogenic myofibroblasts, and stated that this may provide clues to direct cell migration.[25] Wagh et al.[26] investigated the biomechanical properties of wound tissue at different locations of human bronchial epithelial cells. They found that the cells near the edge of the wound undergo localized changes in cellular stiffness, which may provide signals for the spreading and migration of cells. Williams and Harrison[15] showed different mechanical properties at different parts of a healing wound in pig incisional skin closed either by nylon suture or metal clips. They reported that the mechanical properties varied along the length of the wound in both cases and this could be explained in terms of the modification by the closure method of tension that is transmitted across the wound. Farahani and Kloth[8] suggested that the mechanical strain of the wound bed regulates the orientation of the collagen fibers and guides the contractile activity towards the restoration of the architecture of the original unwounded tissue and the functional activity of the previously wounded tissues.[8] Recent studies have also suggested that tissue stiffness plays a role in controlling the activity of transforming growth factor-beta1, which subsequently affects wound contraction. Eckes and Krieg[27] suggested that the mechanical tension can be regarded as an additional important regulatory parameter in modifying the metabolism and phenotype of cells in connective tissue, and that this should be better understood with respect to mechano-sensing cellular structures and specific or shared signaling pathways for the regulation of connective tissue homeostasis. Nevertheless, the mechanism of how different biomechanical properties at different locations of a wound influence wound healing remains uncertain, and further investigations into the matter are needed.

The present study shows that the newly developed air-jet indentation system is a reliable tool for characterizing the biomechanical properties of tissues around the wound in a noncontact way, which allows for better monitoring of wound tissues during the healing process. Information on the stiffness of wound tissue can provide insights for controlling the growth, remodeling, and function of cells. This new quantitative noncontact measurement method is a breakthrough in innovations relating to wound care. It may facilitate the carrying out of more high-quality evidence-based research in wound management.

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REFERENCES

1. Gamelli RL, He LK. Incisional wound healing. Model and analysis of wound breaking strength. *Methods Mol Med* 2003; 78: 37–54.
2. Mortazavi S, Rahmani M, Rahnama A, Saeed-Pour A, Nouri E, Hosseini N, Aghaiee M. The stimulatory effects of topical application of radioactive lantern mantle powder on wound healing. *Dose Response* 2009; 7: 149–59.
3. Shakespeare W. Natural course of wound repair versus impaired healing in chronic skin ulcers. In: Shai A, Maibach H, editors. *Wound healing and ulcers of the skin. Diagnosis and therapy — The practical approach*. Berlin, Heidelberg: Springer, 2005: 7–17.
4. Lee AR, Moon HK. Effect of topically applied silver sulfadiazine on fibroblast cell proliferation and biomechanical properties of the wound. *Arch Pharm Res* 2003; 26: 855–60.
5. McCauley RL, Li YY, Chopra V, Herndon DN, Robson MC. Cytoprotection of human dermal fibroblasts against silver sulfadiazine using recombinant growth factors. *J Surg Res* 1994; 56: 378–84.
6. Prakash S, Paul WE, Robbins PW. Fibrosin, a novel fibrogenic cytokine, modulates expression of myofibroblasts. *Exp Mol Pathol* 2007; 82: 42–8.
7. Desmoulière A, Chaponnier C, Gabbiani G. Tissue repair, contraction, and the myofibroblast. *Wound Repair Regen* 2005; 13: 7–12.
8. Farahani RM, Kloth LC. The hypothesis of 'biophysical matrix contraction': wound contraction revisited. *Int Wound J* 2008; 5: 477–82.
9. Hinz B, Mastrangelo D, Iselin CE, Chaponnier C, Gabbiani G. Mechanical tension controls granulation tissue contractile activity and myofibroblast differentiation. *Am J Pathol* 2001; 159: 1009–20.
10. Doillon CJ, Dunn MG, Bender E, Silver FH. Collagen fiber formation in repair tissue: development of strength and toughness. *Coll Relat Res* 1985; 5: 481–92.
11. Doillon CJ, Dunn MG, Silver FH. Relationship between mechanical properties and collagen structure of closed and open wounds. *J Biomech Eng* 1988; 110: 352–6.
12. Lee AR. Enhancing dermal matrix regeneration and biomechanical properties of 2nd degree-burn wounds by EGF-impregnated collagen sponge dressing. *Arch Pharm Res* 2005; 28: 1311–6.
13. Clark RA, Ghosh K, Tonnesen MG. Tissue engineering for cutaneous wounds. *J Invest Dermatol* 2007; 127: 1018–29.
14. Hamilton R, Apesos J, Korostoff E. Viscoelastic properties of healing wounds. *Plast Reconstr Surg* 1970; 45: 274–8.
15. Williams DF, Harrison ID. The variation of mechanical properties in different areas of a healing wound. *J Biomech* 1977; 10: 633–42.
16. Ksander GA, Vistnes LM, Rose EH. Excisional wound biomechanics, skin tension lines, and elastic contraction. *Plast Reconstr Surg* 1977; 59: 398–406.
17. McGuire MF. Studies of the excisional wound: I. Biomechanical effects of undermining and wound orientation on closing tension and work. *Plast Reconstr Surg* 1980; 66: 419–27.
18. Pickett BP, Burgess LP, Livermore GH, Tzikas TL, Vossoughi J. Wound healing. Tensile strength vs healing time for wounds closed under tension. *Arch Otolaryngol Head Neck Surg* 1996; 122: 565–8.
19. Edwards C, Marks R. Evaluation of biomechanical properties of human skin. *Clin Dermatol* 1995; 13: 375–80.
20. Perry LC, Connors AW, Matrisian LM, Nanney LB, Charles PD, Reyes DP, Kerr LD, Fisher J. Role of transforming growth factor-beta and epidermal growth factor in the wound-healing process: an in vivo biomechanical evaluation. *Wound Repair Regen* 1993; 1: 41–6.
21. Gingrass M, Perry L, Hill D, Wright T, Robson M, Fisher J. Nondisruptive, in vivo method for biomechanical characterization of linear incision wound healing: preliminary report. *Plast Reconstr Surg* 1998; 102: 801–6.
22. Huang YP, Zheng YP, Wang SZ, Chen ZP, Huang QH, He YH. An optical coherence tomography (OCT)-based air jet indentation system for measuring the mechanical properties of soft tissues. *Meas Sci Technol* 2009; 20: 1–11.
23. Chao CY, Zheng YP, Huang YP, Cheing GL. Biomechanical properties of the forefoot plantar soft tissue as measured by an optical coherence tomography-based air-jet indentation system and tissue ultrasound palpation system. *Clin Biomech (Bristol, Avon)* 2010; 25: 594–600.
24. Culav EM, Clark CH, Merrilees MJ. Connective tissues: matrix composition and its relevance to physical therapy. *Phys Ther* 1999; 79: 308–19.
25. Hinz B. Tissue stiffness, latent TGF-β1 activation, and mechanical signal transduction: implications for the pathogenesis and treatment of fibrosis. *Curr Rheumatol Rep* 2009; 11: 120–6.
26. Wagh AA, Roan E, Chapman KE, Desai LP, Rendon DA, Eckstein EC, Waters CM. Localized elasticity measured in epithelial cells migrating at a wound edge using atomic force microscopy. *Am J Physiol Lung Cell Mol Physiol* 2008; 295: L54–60.
27. Eckes B, Krieg T. Regulation of connective tissue homeostasis in the skin by mechanical forces. *Clin Exp Rheumatol* 2004; 22: S73–6.

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● *Original Contribution*

EPIDERMAL THICKNESS AND BIOMECHANICAL PROPERTIES OF PLANTAR TISSUES IN DIABETIC FOOT

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Abstract—Diabetic foot is a common complication for people with diabetes but it is unclear whether the change is initiated from the skin surface or underneath plantar tissues. This study compared the thickness of epidermis and the thickness and stiffness of the total plantar soft tissue among people with diabetes with or without complications. Seventy-two people with diabetes, including 22 people with neuropathies, 16 foot ulcerations, 34 pure diabetics without complications and 40 healthy controls participated in the study. The thickness of the epidermal layer of the plantar skin was examined using high-frequency ultrasonography; the thickness and stiffness of the total plantar soft tissue were measured by using tissue ultrasound palpation system at the big toe, the first, third and fifth metatarsal heads; and the heel pad. Compared with the control group, the average epidermal thickness of plantar skin was reduced by 15% in people with diabetic foot ulceration and 9% in people with neuropathy, but was increased by 6% in pure diabetics. There was an 8% increase in total thickness of plantar soft tissue in the 3 diabetic groups at all testing sites (all $p < 0.05$), except the first metatarsal head. The stiffness of plantar soft tissue was increased in all diabetic groups at all testing sites compared with the control (all $p < 0.05$). The epidermal plantar skin becomes thinner and plantar soft tissues stiffen in people with diabetes, particularly in persons who have neuropathy or ulceration, which increases the risk of tissue breakdown and ulceration formation. (E-mail: rsgladys@polyu.edu.hk) **© 2011 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Epidermal thickness, Plantar, Soft tissues, Biomechanical properties, Diabetic foot.**

INTRODUCTION

Foot disorder is one of the major complications of diabetes mellitus and constitutes up to 50% of all diabetes-related hospital admissions (Smith et al. 2004). The predisposing factors for diabetic foot ulceration include neuropathy, vascular insufficiency, plantar callus, elevated plantar pressures and limited joint range of motion (Bennett et al. 1996; Fernando et al. 1991; Mueller et al. 2005). Local changes in skin morphology and biomechanical properties of plantar soft tissue may further increase the risk for foot ulcer.

Plantar skin acts as the first line of biologic barrier in protecting the foot from mechanical trauma. Any pathologic changes in it place an important impact on tissue viability and hence tissue breakdown. However, evidence suggests that the process of injury in diabetic feet may not initiate entirely on the skin surface, but also in deeper plantar soft tissue layers (Gefen 2003; Thomas et al. 2003). The whole layer of plantar soft tissue is composed of a complex framework of skin, fat cells, fascia layers and muscles. These tissue layers work together and serves as a cushion for optimizing load-bearing during ambulation. How the local changes in skin morphology interact with the changes in biomechanical properties of the plantar soft tissue layers, and hence the overall cushioning effects of the whole layers of plantar soft tissues in a diabetic foot, remains unclear. It is worthwhile to examine both the plantar skin morphology and the biomechanical properties of the whole plantar soft tissue layers in persons with diabetes.

The morphology of the skin is composed of an outer thinner epidermal and an inner thicker dermal layer (Boulais and Misery 2008). The epidermis primarily consists of layers of densely packed keratinocytes, which serve as a barrier and give resistance to physical wear and tear (Hagisawa and Shimada 2005). The dermis consists of

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papillary and reticular layers of collagen and elastic fibers to provide mechanical strength, extensibility and elasticity (Hagisawa and Shimada 2005). Diabetic associated skin thickening is probably caused by excessive accumulation of advanced glycosylation end products in the collagen of the dermis (Seirafi et al. 2009). Ultrasound technology is one of the most popular techniques for assessing skin morphology or tissue properties in the last decade (Mofid et al. 2006; Vogt and Ermert 2005), but controversial findings have been reported in diabetic feet. Using ultrasonography imaging, Huntley and Walter (1990) demonstrated an increase in total skin thickness on the dorsum of the feet. Duffin et al. (2002) did not find any increase in total thickness of skin at the plantar surface of the foot among young diabetics. Conversely, Hashmi et al. (2006) showed that the plantar epidermal thickness was significantly thicker in type 2 diabetes without neuropathy compared with healthy subjects.

Klaesner et al. (2002) reported that the plantar tissue over the metatarsal head for people with diabetic neuropathy was significantly stiffer than age-matched control subjects. Zheng et al. (2000) suggested that the soft tissues on specific plantar pressure points for elderly people with diabetic neuropathy were significantly stiffer and thinner than that of the young healthy subjects. Duffin et al. (2002) reported that the plantar aponeurosis was significantly thicker in people with diabetes. Conversely, Robertson et al. (2002) did not demonstrate any significant difference in the plantar soft tissue thickness beneath the metatarsal heads between individuals with diabetes and healthy controls. The thickness of the plantar soft tissue affects the amount of cushioning, whereas the stiffness affects the stress or pressure distribution of the foot during weight bearing. Given a decrease in plantar soft thickness together with an increase in stiffness would predispose the foot to the risk for tissue breaking down during repetitive gait cycle.

Prevention of foot problems is an essential goal for people with diabetes. There is a lack of studies that examine the changes in plantar skin morphology and soft tissue properties in subjects with diabetes, and how they lead to skin breakdown and formation of foot ulcers is unclear. The present study aimed to examine the changes in epidermal thickness and biomechanical properties of plantar soft tissue in people with type 2 diabetes mellitus with or without neuropathy and ulceration.

MATERIALS AND METHODS

Participants

Seventy-two patients with type 2 diabetes, with or without complications, and 40 nondiabetic healthy control subjects participated in the study. All diabetic subjects were recruited from the diabetic clinic of a local hospital and had the confirmed medical diagnosis of types 2 diabetes mellitus. The group with diabetic peripheral neuropathy was identified using the 10g Semmes-Weinstein monofilament (unable to feel for 5 or more of the 10 testing points including the pulps of the first, third, and fifth digits; the plantar aspects of the first, third, and fifth metatarsal heads; the plantar medial and lateral sides of the midfoot; the plantar area of the heel; and the dorsal aspect of the midfoot) and vibration perception threshold of $>$25 Hz. The group with foot ulcerations either had history of previous or present diabetic foot ulceration below the malleoli level. Healthy control subjects who had no history of diabetes or any other forms of neuropathy and arterial diseases were recruited from the community. All of them passed the 8-h fasting glucose test. Subjects were excluded if they have peripheral vascular disease as determined by the absence of both posterior tibial and dorsal pedal pulses, the presence of intermittent claudication or symptoms and with ankle brachial index $<$0.9, unstable cardiac condition or malignancy.

The foot with history of or present foot ulceration was tested. If there was no history of diabetic ulcer, the right foot was tested. If the ulcerated foot had partial amputation or the presence of any skin lesions on the measuring points, the contralateral foot was tested. Ethical approval was obtained from a local university and hospital. Written consent was obtained from each subject.

Epidermal thickness measurement

A high-frequency ultrasound scanner with a *Vevo* 708 scanhead operating at a center frequency of 55 MHz (VisualSonics Inc., Toronto, Ontario, Canada) was used for imaging skin morphology. This frequency gives an axial resolution of 30 μm and a lateral resolution of approximately 70 μm, resulting in producing images with high resolution to a maximum depth of 8 mm. The system displays the information obtained in the form of a B-scan in a grey scale image. Ten ultrasound biomicroscopy scans were performed on the skin of the testing foot in five selected sites including the pulp of the big toe; the first, third and fifth metatarsal heads; and the heel pad, with two images captured at each measuring site. After applying ultrasound gel over the measuring sites, the *Vevo* 708 probe was placed perpendicular to the skin surface during image capturing to minimize the pressure of the transducer on the skin surface to avoid compressing the skin surface. By using fractal geometry, quantitative data on epidermal thickness was measured by analyzing the change in echogenicity of the ultrasound image on each sonogram. The first entry echo as shown in the sonograms corresponds to the interface between coupling gel and skin surface, followed by a broad echolucent band underneath that corresponds to the epidermis. This is followed by a thin echolucent band, the so-called *subepidermal low echogenic band*, that corresponds to

the upper dermis. After identifying the boundaries of different layers, the epidermal thickness as defined as the distance between the demarcation echo lines, was then calculated by the in-house *Vevo* image analysis software. The mean of the measurements for the two images obtained at each testing point was used for data analysis.

Measurement of thickness and stiffness of the total plantar soft tissue

A tissue ultrasound palpation system composed of of a pen-sized, hand-held indentation probe was used for measuring the thickness and stiffness of total plantar soft tissues. High reliability and validity (Zheng and Mak 1999, 1996) have been demonstrated. A 5-MHz ultrasound transducer in a diameter of 9 mm at the tip of the probe served as both the indenter and sensor for measuring thickness and deformation of tissue. It provides an axial resolution of approximately 310 μm. It was connected in series with a 10N compressive load cell, an ultrasound emitter and receiver, a strain gauge driver and amplifier and a personal computer (Fig. 1). Both the load signal and ultrasound signal obtained during the indentation test were digitized and collected by a personal computer *via* universal serial bus. The ultrasound signals were sampled at a rate of 100 MHz and a custom-developed computer program displayed the real-time ultrasound reflection signals, deformation transient and force transient (Fig. 2). The force response and ultrasound A-line signal were captured during the indentation test with an approximate rate of 25 frames per second. All data recorded were then synchronized and recorded for subsequent offline analysis. During the indentation test, the investigator applied manual loading and unloading of the probe on the plantar tissue surface and the ultrasound module continuously emitted ultrasound pulses into the soft tissue. The ultrasound echo signal was reflected back from the underlying bony interface and recorded. The time-of-flight of the ultrasound echo signal required to propagate from the plantar skin surface to that interface was used to calculate the tissue thickness. A uniform sound speed of 1540 m/s in soft tissue was assumed (Goss et al. 1980). The corresponding load applied to the probe was continuously sensed by the load cell and recorded by the program. Four to five cycles of loading and unloading, with a total duration of approximately 8 s duration was carried out for each indentation trial to ensure a stable manual indentation rate. The rate was estimated by monitoring the force and deformation curve shown in the computer screen (Fig. 2). The effective Young's modulus (E) was calculated by equation described by Hayes et al. (1972):

$$E = \frac{1-\nu^2}{2ak(\nu, a/h)} \frac{p}{w}. \quad (1)$$

In eqn (1), p is the applied force, w is the indentation depth, a refers to the radius of the indenter, ν is the Poisson's ratio, h is the tissue thickness and κ is a scaling factor that is a function of ν, a/h is numerically determined from an integral equation from Hayes results (Hayes et al. 1972) to provide a theoretical correction for the finite thickness of the elastic layer. A Poisson's ratio of 0.45 was used in this study assuming soft tissue to be nearly an incompressible material.

During the measurement, all subjects were positioned in supine lying position with the knees supported by a small towel. An ankle foot orthosis was used to support the lower leg and ankle of the subjects and maintained the ankle in a neutral position. A coupling gel was applied on the testing site. The selected testing sites included the pulp of big toe; the first, third and fifth metatarsal head; and the heel pad. Two trials of indentation measurements were done at each measuring site and the mean was obtained for subsequent data analysis.

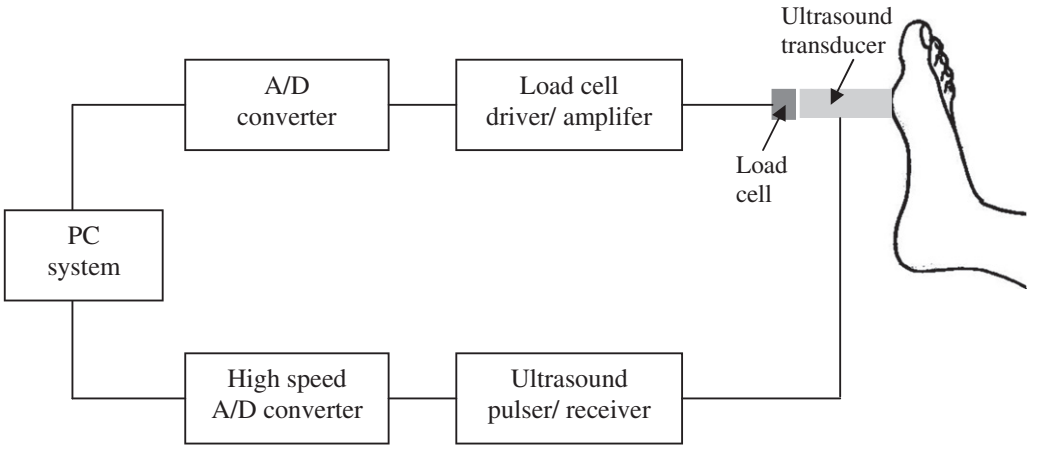

Fig. 1. A schematic representation of the tissue ultrasound palpation system.

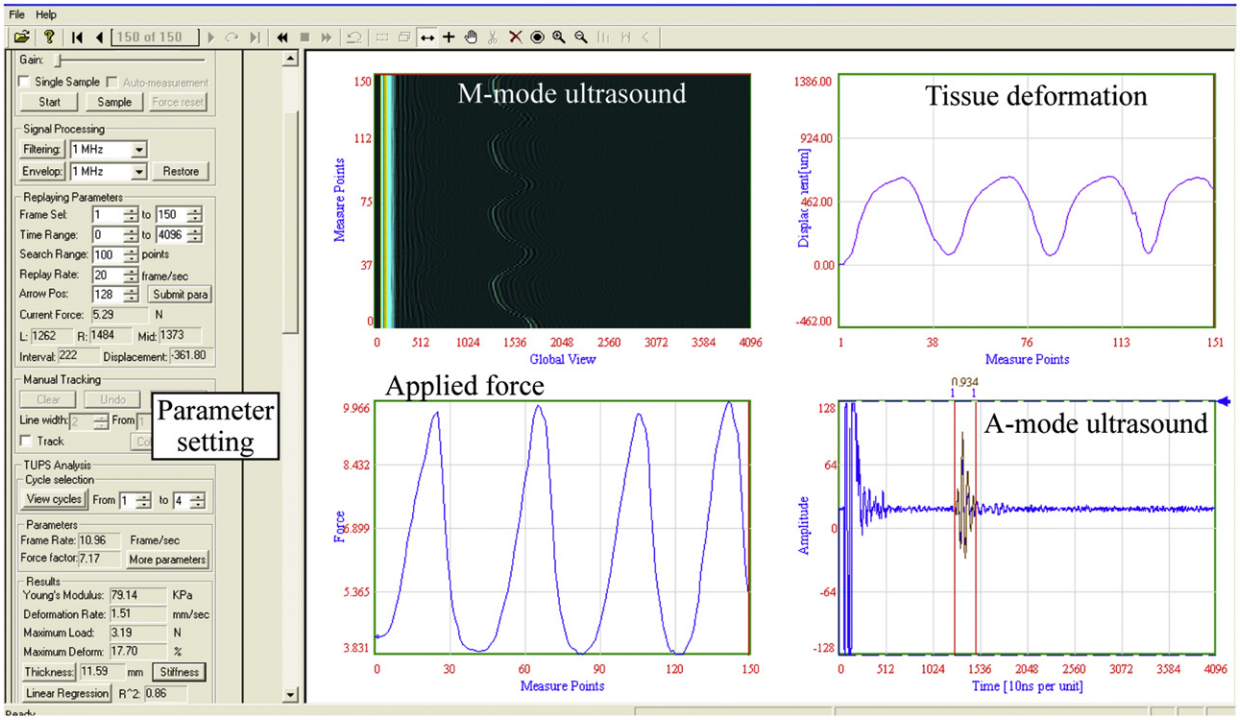

Fig. 2. The user interface of the signal processing software. The left column is the control panel; all of the parameters about data acquisition, signal processing, data replaying, manual tracking and thickness and stiffness measurements can be set in this area. In the main window, the right lower window displays the real-time ultrasound signal reflected from the soft tissue bone interface, the left lower window shows the force sensed by the load cell, the left upper window shows the M-mode ultrasound signals and the right upper window is the deformation of soft tissue extracted from the ultrasound signal using the cross-correlation tracking algorithm.

Statistical analysis

One-way analysis of variance was used to test for the demographic variables and all testing variables between the study groups. When significant results were detected, the analysis of variance was then followed by Hochberg's GT2 or Games-Howell post hoc multiple comparisons. If homogeneity of variance was violated, the F-statistics were adjusted by using the Brown-Forsythe test. The level of significance was set at 0.05 for all measurements.

RESULTS

Seventy-two people with type 2 diabetes, including 22 with diabetic peripheral neuropathies (DPN) (15 males and 7 females, age 67.77 ± 8.79 y), 16 with history of or present diabetic foot ulcerations (13 males and 3 females, age 60.29 ± 14.19 y) and 34 pure diabetes mellitus without complications (15 males and 19 females, age 64.38 ± 9.63 y); and 40 nondiabetic healthy controls (14 males and 26 females, age 67.07 ± 9.44 y) were included in the study. There were no differences in age, body mass index, diastolic blood pressure or ankle brachial index found between the groups. As expected, the fasting glucose was significantly higher in the diabetic groups than in the nondiabetic counterparts ($p = 0.002$). Subjects in the ulcer group demonstrated a higher systolic blood pressure than the pure diabetic and normal groups ($p = 0.007$). In addition, patients with sensory peripheral neuropathy and history of or present foot ulceration had a higher vibration perception threshold than pure diabetics and nondiabetic control subjects, ($p < 0.001$). Among the three diabetic groups, no significant differences were observed among the duration of diabetes and glycated hemoglobin level. Details of the subject demographics are shown in Table 1.

Epidermal thickness of plantar skin

The epidermal thickness of plantar skin was significantly different among people with or without diabetes or diabetic-related complications at the pulp of the big toe, $F(3, 108) = 3.38$, $p = 0.022$; the third metatarsal head $F(3, 108) = 3.83$, $p = 0.012$; the fifth metatarsal head, $F(3, 108) = 4.77$, $p = 0.004$; and the heel pad region, $F(3, 108) = 5.80$, $p = 0.001$ (Table 2); where $F(x, y)$ denotes an F-distribution with x degrees of freedom in the numerator and y denotes degrees of freedom in the denominator. Post hoc analyses indicated that the average epidermal thickness of plantar skin was significantly larger in pure diabetes without

Table 1. Subject characteristics

Group characteristics	DU (n = 16)	DPN (n = 22)	DM (n = 34)	Normal (n = 40)	p-value
Male/Female	13:3	15:7	15:19	14:26	0.004
Age (y)	60.29 ± 14.19	67.77 ± 8.79	64.38 ± 9.63	67.07 ± 9.44	0.067
Body mass index (kg/m^2)	25.25 ± 3.68	25.29 ± 4.30	24.63 ± 3.98	23.01 ± 3.54	0.059
Systolic blood pressure (mm Hg)	152.06 ± 21.91	142.81 ± 24.95	132.55 ± 15.10	133.93 ± 14.14	0.007†
Diastolic blood pressure (mm Hg)	83.17 ± 8.35	76.04 ± 14.31	77.33 ± 10.33	78.77 ± 8.97	0.166
Fasting glucose (mmol/L)	6.72 ± 2.70	6.87 ± 1.97	7.33 ± 2.14	5.54 ± 0.67	0.002*
Glycated hemoglobin (HbA_1c) (%)	7.37 ± 1.89	6.93 ± 1.32	7.23 ± 1.14	—	0.618
Duration of diabetes (y)	14.74 ± 12.31	13.58 ± 9.14	8.94 ± 7.28	—	0.106
Vibration threshold	31.93 ± 10.35	35.44 ± 8.37	16.23 ± 5.30	15.75 ± 6.74	< 0.001‡
Ankle brachial index (left)	1.12 ± 0.11	1.08 ± 0.11	1.10 ± 0.11	1.12 ± 0.09	0.420
Ankle brachial index (right)	1.14 ± 0.14	1.10 ± 0.13	1.15 ± 0.13	1.15 ± 0.11	0.380

Data are mean ± SD. $p < 0.05$.
DU = diabetic ulcer; DPN = diabetic peripheral neuropathy; DM = diabetes without complications.
* Normal group *vs*. ulcer, neuropathy and pure diabetic groups.
† Ulcer group *vs*. pure diabetic and normal groups.
‡ Normal group *vs*. neuropathy and ulcer groups.

complications but smaller for those with neuropathy and foot ulceration compared with the control (Figs. 3 and 4). No significant difference of epidermal thickness was observed between groups at the first metatarsal head ($F(3, 108)$, $p = 0.650$), but it followed a similar trend with the greatest thickness found in the pure diabetes followed by control subjects. On average, the epidermal thickness of plantar skin was reduced in diabetics with foot ulceration by 15% and those with neuropathy by 9%, but increased in people with pure diabetes by 6% compared with healthy control subjects.

Thickness and stiffness of total plantar soft tissue

Significant differences in thickness of total plantar soft tissues was found among the study groups at all testing sites except the first metatarsal head. The thickness of plantar soft tissue was increased in all diabetics compared with healthy control at the pulp of the big toe $F(3, 108) = 4.66$, $p = 0.004$; the third metatarsal head $F(3, 108) = 4.41$, $p = 0.006$; the fifth metatarsal head, $F(3, 108) = 3.69$, $p = 0.014$; and the heel pad region, $F(3, 108) = 3.40$, $p = 0.020$ (Table 3). Figure 5 shows the post hoc analyses among the testing sites and groups. On average, the thickness of plantar soft tissue was increased in diabetic groups by 8% at all testing sites. In addition, there was an increase in stiffness of plantar soft tissue in all diabetic groups at all testing sites (pulp of the big toe $F(3, 108) = 3.79$, $p = 0.012$; the first metatarsal head $F(3, 108) = 3.13$, $p = 0.029$); the third metatarsal head $F(3, 108) = 3.98$, $p = 0.010$; the fifth metatarsal head, $F(3, 108) = 5.14$, $p = 0.002$; and the heel pad region, $F(3, 108) = 5.08$, $p = 0.003$). This was particularly true for those with foot ulceration who demonstrated an average increase in stiffness by 30%, followed by neuropathy and pure diabetes (20%) compared with healthy controls (Fig. 6).

DISCUSSION

The present study demonstrated thickening of the epidermal layer of plantar skin in people with pure diabetes but thinning of the epidermal plantar skin for those with diabetes complicated by sensory peripheral neuropathy or foot ulceration. In addition, we found an increase in thickness and stiffness of total plantar soft tissues in people with diabetes but to a greater extent in persons with complications of neuropathy or foot ulceration.

Table 2. The epidermal thickness at different region of plantar foot as measured by high frequency ultrasonography

Epidermal thickness (mm)	DU (n = 16)	DPN (n = 22)	DM (n = 34)	Normal (n = 40)	F-ratio	p-value
Pulp of big toe	0.43 ± 0.14	0.45 ± 0.11	0.55 ± 0.18	0.51 ± 0.17	3.377	0.022‡
1st MTH	0.51 ± 0.16	0.51 ± 0.17	0.56 ± 0.18	0.55 ± 0.19	0.549	0.650
3rd MTH	0.51 ± 0.17	0.56 ± 0.18	0.66 ± 0.15	0.62 ± 0.15	3.829	0.012*
5th MTH	0.52 ± 0.19	0.58 ± 0.17	0.68 ± 0.14	0.61 ± 0.13	4.765	0.004*
Heel pad	0.53 ± 0.15	0.58 ± 0.14	0.68 ± 0.15	0.66 ± 0.13	5.702	0.001†

Data are mean ± SD.
DU = diabetic ulcer; DPN = diabetic peripheral neuropathy; DM = diabetes without complications; MTH = metatarsal head.
* Ulcer group *vs*. pure diabetic group.
† Ulcer group *vs*. pure diabetic and normal groups.
‡ Pure diabetic group *vs*. neuropathy and ulcer groups.

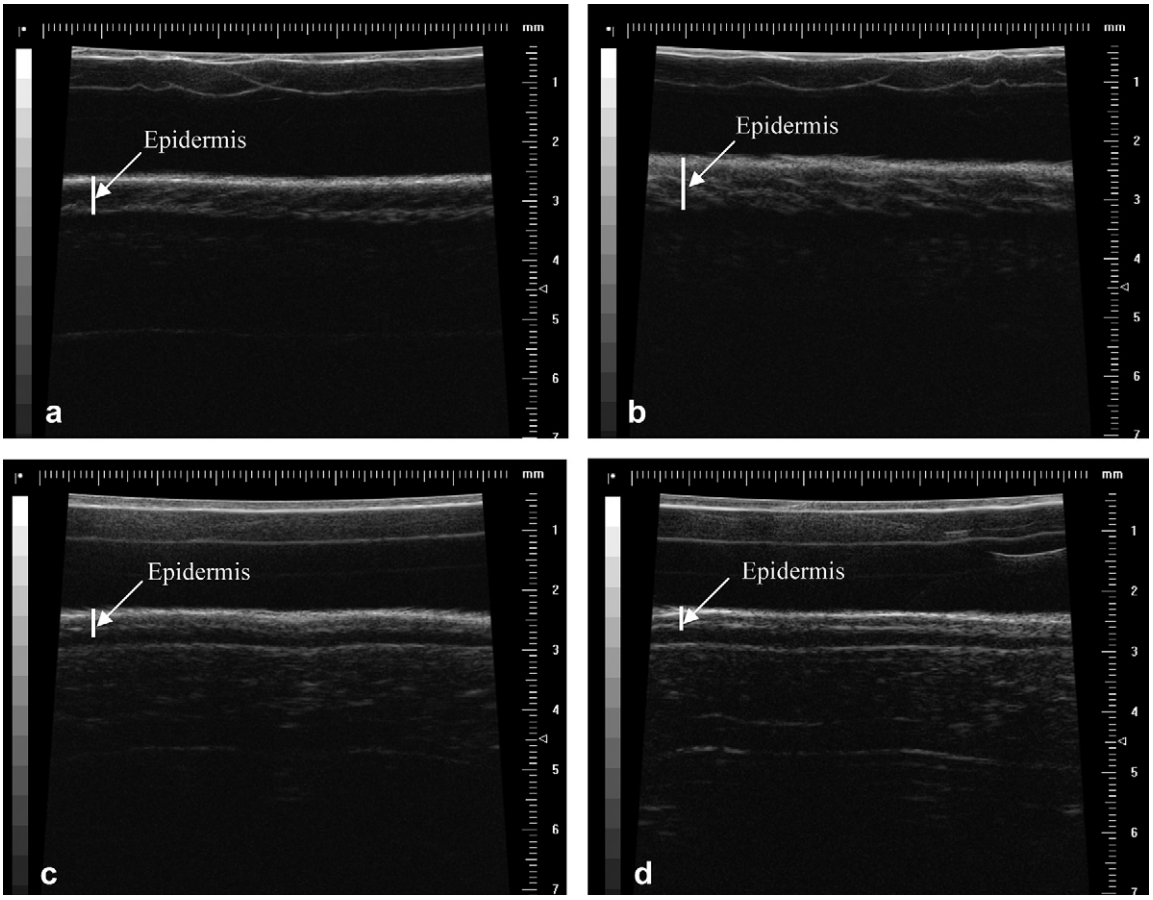

Fig. 3. Ultrasonic images of human plantar skin under metatarsal head showing different epidermal thickness (a) normal subjects, (b) pure diabetes without complications, (c) diabetes with peripheral neuropathy and (d) diabetes with active or history of foot ulceration. Images are taken by high-frequency ultrasound with central frequency at 55 MHz (Visualsonic Inc., Vevo 708).

Thinning of epidermal layer of plantar skin among people with diabetic neuropathy and foot ulceration

We found that the epidermal layer of plantar skin is hyperplastic in people with pure type 2 diabetes without foot complications. In contrast, the epidermis of the plantar skin of people with diabetic neuropathy and ulceration becomes atrophic compared with nondiabetic healthy controls. Epidermis is known to be a glycolytic tissue and is affected by the insulin level in the body for the regulation of the migration and proliferation of keratinocyte (Chen et al. 2009). The thickness of the epidermis is maintained by a delicate balance between proliferation, differentiation, and the cell death of keratinocytes (Truong and Khavari 2007). The hyperproliferative state of the epidermis in the plantar skin of pure diabetes is generally regarded to be the consequence of accumulation of advanced glycation products as induced by hyperglycemia. Studies have shown an increase in advanced glycosylation end products in the collagen of the dermis in diabetes (Kennedy and Baynes 1984; Sternberg et al. 1985), and these end products are postulated to lead to an increase in stiffness (Nikkels-Tassoudji et al. 1996) and thickness (Collier et al. 1986; Forst et al. 1994) of diabetic skin. In addition, Nikkels-Tassoudji et al.1996) demonstrated an increase in interaction between the fibrous networks of collagen through enriched intermolecular cross-linking in diabetic skin, which subsequently results in thickening and stiffening of the skin. Therefore, the increase in cross-linking of collagen among people with diabetes may account for the observed skin thickening in diabetes.

The present study did not demonstrate an increase in epidermal thickness in plantar skin for all groups with diabetes. Instead, we found that epidermal thickness of plantar skin becomes thinner when the disease continues to progress up to a point where clinical manifestations of neuropathy and ulceration occur. Apart from being a physical barrier, the epidermis layer is regarded as a sensory organ innervated by afferent nerve fibers (Hsieh et al. 1997). The sensory innervations can influence epidermal thickness and ultimately the health of

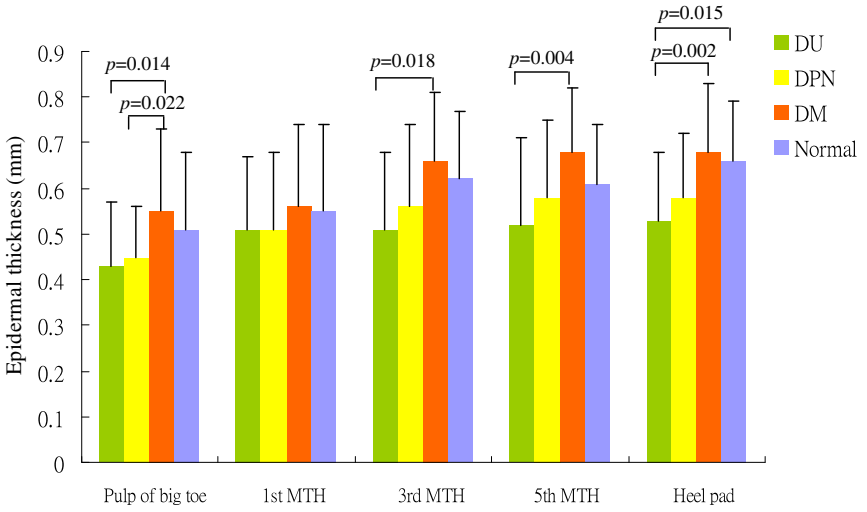

Fig. 4. Epidermal thickness of plantar skin at different sites of the foot. *DU*, Diabetic ulcer; *DPN*, diabetic peripheral neuropathy; *DM*, diabetes without complications; *MTH*, metatarsal head.

the skin (Hsieh et al. 1996, 1997; Huang et al. 1999). Sensory nerves interact with keratinocytes and exhibit trophic influences on the epidermis (Chiang et al. 1998). Alterations in keratinocytes influence the production of nerve growth factor and ultimately the maintenance of sensory nerves terminating in the epidermis (Chiang et al. 1998). Studies *in vitro* demonstrated that denervated skin caused epidermal thinning (Chiang et al. 1998; Hsieh et al. 1996; Hsieh and Lin 1999). Possible explanations may include reduced keratinocyte proliferation, speeding of keratinocyte differentiation and enhanced programmed cell death. Hsieh and Lin (1999) demonstrated a 30–40% reduction in epidermal thickness after depletion of epidermal nerves in the rat. This is also supported by the findings from the present human study. Our subjects in the diabetic neuropathic or ulceration groups experienced sensory neuropathy as demonstrated in the monofilament examination during the screening test in the inclusion procedures. Comparatively, we found a 15% and 9% reduction in epidermal thickness in the ulceration group and neuropathy group, respectively. It has been reported that in patients with sensory neuropathy as in diabetic peripheral neuropathy, the amount of epidermal nerves is substantially lower than that in normal subjects, as shown in the skin biopsies (Hsieh et al. 1997, 2000; Kennedy et al. 1996; Umapathi et al. 2007). This may explain the observed epidermal thinning in our diabetic groups that had complications

Table 3. The total thickness and stiffness of plantar soft tissues at different sites of the foot as measured by tissue ultrasound palpation system

Tissue properties	DU (n = 16)	DPN (n = 22)	DM (n = 34)	Normal (n = 40)	F-ratio	p-value
Total thickness (mm)						
Pulp of big toe	8.64 ± 1.15	8.04 ± 1.37	7.71 ± 1.58	7.11 ± 1.63	4.663	0.004¶
1st MTH	10.07 ± 2.26	10.21 ± 2.67	9.95 ± 1.63	9.43 ± 1.88	0.863	0.463
3rd MTH	10.92 ± 1.52	10.92 ± 1.62	10.51 ± 2.00	9.58 ± 1.55	4.410	0.006*
5th MTH	9.53 ± 2.35	10.02 ± 1.68	8.87 ± 2.08	8.38 ± 2.06	3.689	0.014§
Heel pad	25.12 ± 3.10	25.02 ± 3.19	25.73 ± 2.54	23.65 ± 3.29	3.399	0.020‡
Young's modulus (kPa)						
Pulp of big toe	84.24 ± 35.77	77.05 ± 18.53	77.50 ± 24.07	62.29 ± 27.85	3.788	0.012¶
1st MTH	107.10 ± 33.41	91.40 ± 34.96	99.75 ± 31.61	80.06 ± 34.04	3.125	0.029¶
3rd MTH	134.69 ± 44.94	121.45 ± 34.20	121.60 ± 31.80	99.42 ± 46.17	3.982	0.010¶
5th MTH	104.30 ± 23.87	99.47 ± 27.26	104.09 ± 26.19	81.81 ± 36.05	5.136	0.002†
Heel pad	87.56 ± 21.36	85.41 ± 15.64	79.37 ± 13.38	69.78 ± 19.74	5.075	0.003*

Data are mean ± SD.
DU = diabetic ulcer; DPN = diabetic peripheral neuropathy; DM = diabetes without complications; MTH = metatarsal head.
* Normal group *vs*. neuropathy and ulcer groups.
† Normal group *vs*. pure diabetic group and ulcer groups.
‡ Normal group *vs*. pure diabetic group.
§ Normal group *vs*. neuropathy group.
¶ Normal group *vs*. ulcer group.

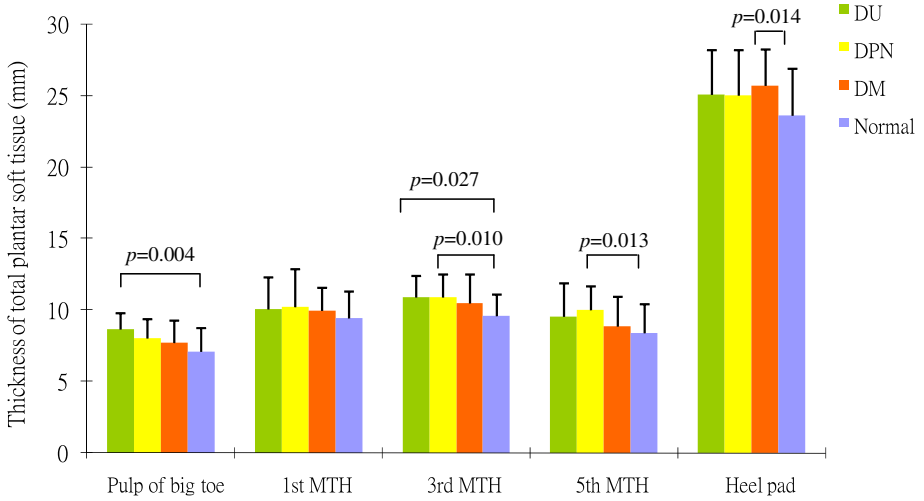

Fig. 5. Total thickness of plantar soft tissues at different regions of the foot. *DU*, Diabetic ulcer; *DPN*, diabetic peripheral neuropathy; *DM*, diabetes without complications; *MTH*, metatarsal head.

of neuropathy or foot ulceration. In addition, we also demonstrated that the extent of epidermal thinning was more obvious in people with ulceration than with neuropathy alone. This may explain why people with diabetic neuropathy or history of ulceration may be prone to have a higher incidence of ulceration or re-ulceration.

Thickening and stiffening of plantar soft tissue in people with diabetes with or without complications

Plantar soft tissues are rich in collagen and are therefore susceptible to nonenzymatic glycation of the collagenous component. The present study demonstrated an increase in thickness and stiffness of plantar soft tissue for all subjects with diabetes, to a greater extent in those with complications of neuropathy and ulceration compared with nondiabetic healthy controls. The observed increased thickness and stiffness of plantar soft tissue properties in diabetes is thought to be associated with high glucose levels in the tissues, which promotes nonenzymatic glycation of structural proteins in the collagen, elastin and keratin, hence facilitating intermolecular cross-linking of the soft tissue and subsequently causing tissue thickening and stiffening (Huntley 1993). Among the five measurement points, the third metatarsal head was the stiffest. This is in agreement with those reported by Klaesner et al. (2002) who demonstrated that the third metatarsal head was stiffer than the first and fifth metatarsal heads. This may be because the second and third metatarsal heads withstand

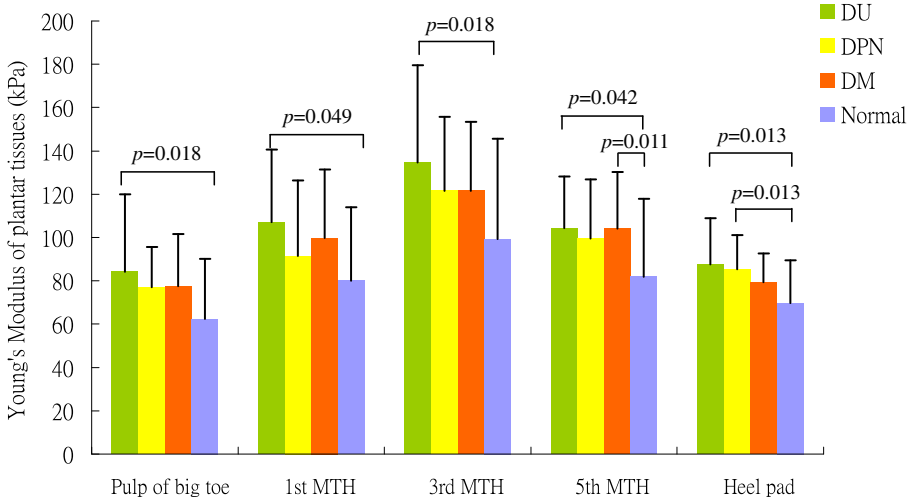

Fig. 6. Stiffness of plantar soft tissues at different region of the foot. *DU*, Diabetic ulcer; *DPN*, diabetic peripheral neuropathy; *DM*, diabetes without complications; *MTH*, metatarsal head.

high-peak plantar pressure during ambulation and result in stiffening of the plantar soft tissues (Martínez-Nova et al. 2008).

Unlike epidermal thickness of plantar skin, there is an increase in thickness of total plantar soft tissue among diabetic subjects with neuropathy or ulceration. These groups of patients had impaired sensation and may exert more pressure on the insensate plantar skin and soft tissues unwittingly, which in turn would lead to more hardening and lead to formation of ulcers. In fact, the plantar skin and soft tissues need to be very mobile and stretchable to accommodate different shapes and the nature of the ground. The changes in soft tissue biomechanical properties of plantar tissues in diabetes results in abnormal loading during ambulation, and subsequently leads to a breakdown of the tissues (Edsberg et al. 2000). Such an increase in stiffness of the plantar soft tissue may cause stress concentration and diminish its ability of the sole to effectively distribute foot-ground contact force during repetitive load bearing, hence causing abnormal stress concentrations that could lead to microtears of the tissues and ulcer development. This is the first study that reported both the thickness and stiffness of plantar skin and total plantar soft tissue among people with diabetes. To further explore whether there is any relationship between thickness and stiffness of the plantar skin and plantar soft tissue, we performed Pearson correlation, but no statistically significant correlation was found. It appears that there are multiple factors that may determine the thickness or stiffness of plantar tissues.

The present study demonstrated a decrease in epidermal thickness of plantar skin and reduced flexibility of total plantar soft tissues in people with diabetic neuropathy and foot ulceration. This implies that diabetes-associated changes in biomechanical properties of plantar skin and soft tissues may probably be important risk factors leading to foot ulceration in people with diabetes. Therefore, regular examination of the sole of the foot in people with diabetes and proper shoe wear should be reinforced to prevent foot complications. Also, regular exercise can be an effective intervention for controlling blood sugar level and delay the onset of neuropathy (Balducci et al. 2006; Thomas et al. 2006).

CONCLUSIONS

People with pure type 2 diabetes tend to have thicker epidermal plantar skin than their nondiabetic counterparts. In contrast, epidermal thinning of plantar skin occurs in people who have clinical manifestation of diabetic neuropathy and ulceration. On the other hand, the total thickness and stiffness of plantar soft tissue were increased in all subjects with diabetes, in which a greater increase was found in those complicated with neuropathy and ulceration. Such a decrease in the epidermal thickness of plantar skin, but an increase in the stiffness of total plantar soft tissue, makes the diabetic foot prone to tissue breakdown and hence ulcer formation.

Acknowledgments—This project was supported by the General Research Fund provided by the Research Grants Council of the Hong Kong SAR Government (PolyU 5126/07E, PolyU5128/08E).

REFERENCES

Balducci S, Iacobellis G, Parisi L, Di Biase N, Calandriello E, Leonetti F, Fallucca F. Exercise training can modify the natural history of diabetic peripheral neuropathy. J Diabetes Complications 2006;20:216–223.

Bennett PJ, Stocks AE, Whittam DJ. Analysis of risk factors for neuropathic foot ulceration in diabetes mellitus. J Am Podiatr Med Assoc 1996;86:112–116.

Boulais N, Misery L. The epidermis: A sensory tissue. Eur J Dermatol 2008;18:119–127.

Chen X, Lin W, Lu S, Shi Y, Zou J, Liu Z, Liao W. Insulin prevents latent skin lesions by inhibiting the generation of advanced glycation end products in streptozotocin-induced diabetic rats. Endocrinol Pathol 2009;20:163–169.

Chiang HY, Huang IT, Chen WP, Chien HF, Shun CT, Chang YC, Hsieh ST. Regional difference in epidermal thinning after skin denervation. Exp Neurol 1998;154:137–145.

Collier A, Matthews DM, Kellett HA, Clarke BF, Hunter JA. Change in skin thickness associated with cheiroarthropathy in insulin dependent diabetes mellitus. BMJ 1986;292:936.

Duffin AC, Lam A, Kidd R, Chan AK, Donaghue KC. Ultrasonography of plantar soft tissues thickness in young people with diabetes. Diabetes Med 2002;19:1009–1013.

Edsberg LE, Cutway R, Anain S, Natiella JR. Microstructural and mechanical characterization of human tissue at and adjacent to pressure ulcers. J Rehabil Res Dev 2000;37:463–471.

Fernando DJ, Masson EA, Veves A, Boulton AJ. Relationship of limited joint mobility to abnormal foot pressures and diabetic foot ulceration. Diabetes Care 1991;14:8–11.

Forst T, Kann P, Pfützner A, Lobmann R, Schäfer H, Beyer J. Association between "diabetic thick skin syndrome" and neurological disorders in diabetes mellitus. Acta Diabetol 1994; 31:73–77.

Gefen A. Plantar soft tissue loading under the medial metatarsals in the standing diabetic foot. Med Eng Phys 2003;25:491–499.

Goss SA, Johnston RL, Dunn F. Compilation of empirical ultrasonic properties of mammalian tissues. II. J Acoust Soc Am 1980;68: 93–108.

Hagisawa S, Shimada T. Skin morphology and its mechanical properties associated with loading. In: Bader D, Bouten C, Colin D, *et al*., (eds). Pressure ulcer research: Current and future prospectives. Berlin: Springer-Verlag; 2005. p. 161–185.

Hashmi F, Malone-Lee J, Hounsell E. Plantar skin in type II diabetes: An investigation of protein glycation and biomechanical properties of plantar epidermis. Eur J Dermatol 2006;16:23–32.

Hayes WC, Keer LM, Herrmann G, Mockros LF. A mathematical analysis for indentation tests of articular cartilage. J Biomech 1972;5:541–551.

Hsieh ST, Chiang HY, Lin WM. Pathology of nerve terminal degeneration in the skin. J Neuropathol Exp Neurol 2000;59:297–307.

Hsieh ST, Choi S, Lin WM, Chang YC, Mcarthur JC, Griffin JW. Epidermal denervation and its effects on keratinocytes and Langerhans cells. J Neurocytol 1996;25:513–524.

Hsieh ST, Lin WM. Modulation of keratinocyte proliferation by skin innervation. J Invest Dermatol 1999;113:579–586.

Hsieh ST, Lin WM, Chiang HY, Huang IT, Ko MH, Chang YC, Chen WP. Skin innervation and its effects on the epidermis. J Biomed Sci 1997;4:264–268.

Huang IT, Lin WM, Shun CT, Hsieh ST. Influence of cutaneous nerves on keratinocyte proliferation and epidermal thickness in mice. Neuroscience 1999;94:965–973.

Huntley AC. Cutaneous manifestations of diabetes mellitus. Diabetes Metab Rev 1993;9:161–176.

Huntley AC, Walter RM Jr. Quantitative determination of skin thickness in diabetes mellitus: relationship to disease parameters. J Med 1990; 21:257–264.

Kennedy L, Baynes JW. Non-enzymatic glycosylation and the chronic complications of diabetes: An overview. Diabetologia 1984;26: 93–98.

Kennedy WR, Wendelschafer-Crabb G, Johnson T. Quantitation of epidermal nerves in diabetic neuropathy. Neurology 1996;47: 1042–1048.

Klaesner JW, Hastings MK, Zou D, Lewis C, Mueller MJ. Plantar tissue stiffness in patients with diabetes mellitus and peripheral neuropathy. Arch Phys Med Rehabil 2002;83:1796–1801.

Martínez-Nova A, Pascual Huerta J, Sánchez-Rodríguez R. Cadence, age, and weight as determinants of forefoot plantar pressures using the Biofoot in-shoe system. J Am Podiatr Med Assoc 2008;98: 302–310.

Mofid Y, Ossant F, Imberdis C, Josse G, Patat F. In-vivo imaging of skin under stress: Potential of high-frequency (20 MHz) static 2-D elastography. IEEE Trans Ultrason Ferroelectr Freq Control 2006; 53:925–935.

Mueller MJ, Zou D, Lott DJ. "Pressure gradien" as an indicator of plantar skin injury. Diabetes Care 2005;28:2908–2912.

Nikkels-Tassoudji N, Henry F, Letawe C, Pierard-Franchimont C, Lefebvre P, Pierard GE. Mechanical properties of the diabetic waxy skin. Dermatology 1996;192:19–22.

Robertson DD, Mueller MJ, Smith KE, Commean PK, Pilgram T, Johnson JE. Structural changes in the forefoot of individuals with diabetes and a prior plantar ulcer. J Bone Joint Surg Am 2002; 84-A:1395–1404.

Seirafi H, Farsinejad K, Firooz A, Davoudi SM, Robati RM, Hoseini MS, Ehsani AH, Sadr B. Biophysical characteristics of skin in diabetes: A controlled study. J Eur Acad Dermatol Venereol 2009;23:146–149.

Smith D, Cullen MJ, Nolan JJ. The cost of managing diabetic foot ulceration in an Irish hospital. Irish J Med Sci 2004;173:89–92.

Sternberg M, Cohen-Forterre L, Peyroux J. Connective tissue in diabetes mellitus: Biochemical alterations of the intercellular matrix with special reference to proteoglycans, collagens and basement membrane. Diabetes Metab 1985;11:27–50.

Thomas DE, Elliott EJ, Naughton GA. Exercise for type 2 diabetes mellitus. Cochrane Database Syst Rev 2006;19:CD002968.

Thomas VJ, Patil KM, Radhakrishnan S, Narayanamurthy VB, Parivalavan R. The role of skin hardness, thickness, and sensory loss on standing foot power in the development of plantar ulcers in patients with diabetes mellitus—a preliminary study. Int J Low Extrem Wounds 2003;2:132–139.

Truong AB, Khavari PA. Control of keratinocyte proliferation and differentiation by p63. Cell Cycle 2007;6:295–299.

Umapathi T, Tan WL, Loke SC, Soon PC, Tavintharan S, Chan YH. Intraepidermal nerve fiber density as a marker of early diabetic neuropathy. Muscle Nerve 2007;35:591–598.

Vogt M, Ermert H. Development and evaluation of a high-frequency ultrasound-based system for in vivo strain imaging of the skin. IEEE Trans Ultrason Ferroelectr Freq Control 2005;52:375–385.

Zheng Y, Mak AF. Effective elastic properties for lower limb soft tissues from manual indentation experiment. IEEE Trans Rehabil Eng 1999;7:257–267.

Zheng YP, Choi YK, Wong K, Chan S, Mak AF. Biomechanical assessment of plantar foot tissue in diabetic patients using an ultrasound indentation system. Ultrasound Med Biol 2000;26:451–456.

Zheng YP, Mak AF. An ultrasound indentation system for biomechanical properties assessment of soft tissues in-vivo. IEEE Trans Biomed Eng 1996;43:912–918.

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ORIGINAL ARTICLE

The Association Between Skin Blood Flow and Edema on Epidermal Thickness in the Diabetic Foot

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Abstract

Background: Skin blood flow plays an important role in maintaining the health of the skin. The development of interstitial edema may impede oxygen diffusion to the skin. The aim of this study was to evaluate the association of skin blood flow and edema and epidermal thickness in the feet of people with and without diabetic neuropathy compared with a healthy control group.

Subjects and Methods: Eighty-seven subjects (19 people with diabetic neuropathy and foot ulceration, 35 people with diabetes but without neuropathy, and 33 healthy controls without diabetes) participated in the study. High-frequency ultrasonography was used to measure the epidermal thickness and edema in papillary skin at the big toe as reflected by the thickness of the subepidermal low echogenic band (SLEB). The capillary nutritive blood flow was measured by the use of video capillaroscopy, and skin blood flux was monitored by laser Doppler flowmetry.

Results: There was a 7.2% increase in epidermal thickness in those with diabetes but without neuropathy and a 16.5% decrease in people with diabetic neuropathy and foot ulceration compared with the healthy controls (all $P<0.05$). The SLEB thickness increased in all subjects with diabetes to a greater degree in those with neuropathy and ulceration than in those without (64.7% vs. 11.8%, $P<0.001$). Skin blood flux was shown to be higher in the diabetes groups than in the controls (all $P<0.05$), but no significant differences were found in the resting nutritive capillary blood flow ($P>0.05$). A significant negative correlation ($P=0.002$, $r=-0.366$) was demonstrated between the SLEB and epidermal thickness at the pulp of the big toe, whereas no significant correlation was demonstrated between skin blood flow and epidermal thickness (all $P>0.05$).

Conclusions: An increase in subepidermal edema was demonstrated in people with diabetic neuropathy and ulceration, which may partly contribute to reduced epidermal thickness at the pulp of the big toe. This may subsequently lead to the breaking down of skin in the diabetic foot.

Introduction

Foot ulcer is a common complication seen in people with diabetes mellitus. There are many predisposing factors for diabetic foot ulceration, including impaired blood supply.[1–3] Skin blood flow provides nourishment and removes waste, which plays a vital role in maintaining the general health of the skin. Any pathogenesis that takes place in the skin may result in a change in the thickness of the skin. An example is the breakdown of skin that may occur in a diabetic foot, leading to foot ulcerations. Indeed, it has been suggested that the earliest manifestation of a microcirculatory disorder is edema.[4] Yet the relationship among skin blood flow, edema, and skin thickness in a diabetic foot remains unclear.

Human skin is composed of an outer thinner epidermis and an inner thicker dermis. The epidermis consists of keratin with no blood supply, and nutrition is provided by the papillary layer of the dermis. The microvascular network located in the dermal layer of the skin is composed of both nutritive capillary blood flow and thermoregulatory arteriovenous (AV) shunt flow. The glabrous (hairless) skin is mainly involved in thermoregulation, in which large numbers of AV shunts are maintained in the constricted state by sympathetic tone. In contrast, the main function of nonglabrous (hairy) skin is primarily to provide nutrition. Its regulation involves the intrinsic myogenic, sympathetic, and endothelial control.[5] In people with diabetes, especially if the disease is complicated by neuropathy, an increase in foot swelling is a commonly observed feature that precedes the development of noticeable

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skin lesions. However, the impact of swelling on skin thickness and on the risk of skin breakdown is unclear. Under normal circumstances, the development of distal interstitial edema is prevented by the venoarteriolar reflex by limiting the rise of capillary hydrostatic pressure during leg dependency.[6] Such a vasoconstriction reflex response is impaired in people with diabetic neuropathy and ulceration.[7–9] The reduction or absence of effective precapillary vasoconstriction upon standing will expose the capillary bed to a high hydrostatic load, leading to edema and thickening of the capillary basement membrane. Very few studies have performed a quantitative assessment of the degree of foot swelling that occurs in a diabetic foot. It is unclear whether foot edema could lead to a change in skin thickness, a key factor contributing to foot ulceration.

Given that a rich microvascular network is located in the dermal layer of the skin, any change in skin blood flow or in the formation of interstitial edema would definitely affect the local tissue tension inside and hence the tissue properties of skin layers in response to external pressure. This is particularly true for plantar skin that is located in weight-bearing areas. Edema is an extremely important factor affecting the oxygenation of tissue in that it increases the intercellular spaces, hence causing an increase in the distance for oxygen to diffuse to the most distant cell.[10] Skin edema may lead to ulcers, but very few studies have examined the presence of edema in the skin of the diabetic foot.

Contradictory findings on morphological changes associated with diabetes in the skin of the diabetic foot have been reported.[11–13] Some studies have found thickening of the skin and others thinning of the skin occurring in the feet of people with diabetes. A study suggested that such thickening of the skin may be due to an excessive accumulation of advanced glycosylation end-products in the collagen of the dermis.[14] We postulate that skin blood flow and edema may influence the thickness of the skin. Ultrasound technology is a commonly used technique for assessing skin morphology.[15,16] The echogenicity of the skin tissues is supposed to be inversely related to the amount of fluid contained in the ultrasound images, and the subepidermal low echogenic band (SLEB) is presumed to represent either the edema in the papillary dermis or the photodamage of the skin.[17] High-frequency ultrasonography can be used to measure skin thickness and edema in the papillary skin layer.[18,19]

Skin health is reliant on an adequate perfusion of blood supply. Edema may disrupt the microcirculation system, thereby impairing the supply of nutrients to the skin. This can eventually lead to changes in skin morphology and to the breakdown of the skin. The aim of this study was to evaluate the association between skin blood flow and edema and epidermal thickness in the feet of people with and without diabetes.

Subjects and Methods

Participants

Eighty-seven subjects (35 people with diabetes but without neuropathy [DM group], 19 with diabetic neuropathy and a history or present condition of foot ulceration [DU group], and 33 healthy controls without diabetes) participated in the present study. All diabetes subjects were recruited from the diabetes clinic of a local hospital in the period of January 2009–November 2010 and had the confirmed medical diagnosis of type 2 diabetes mellitus. Diabetic peripheral neuropathy was identified using the 10-g Semmes–Weinstein monofilament (lack of feeling in at least five of the following 10 testing points: the pulp of the first, third, and fifth toes; the plantar aspects of the first, third, and fifth metatarsal heads; the plantar medial and lateral sides of the midfoot; the plantar area of the heel; and the dorsal aspect of the midfoot) and a vibration perception threshold of above 25 volts. The group with foot ulcerations all had diabetic neuropathy: they either had a history of diabetic foot ulceration below the malleoli level or were currently suffering from that condition. Healthy control subjects who had no history of diabetes or any other form of neuropathy or arterial disease were recruited from the community by convenience sampling. All of them passed the 8-h fasting glucose test. Subjects were excluded if they had peripheral vascular disease as determined by the absence of both posterior tibial and dorsalis pedis pulses and the presence or symptoms of intermittent claudication and with an ankle brachial index smaller than 0.9, unstable cardiac condition, or malignancy.

Testing was undertaken on those with a history or present condition of foot ulceration. For subjects who had no history of diabetic ulcers, the right foot was tested. If the ulcerated foot had been partially amputated or if any skin lesions were found on the measuring sites, the contralateral foot was tested. Ethical approval for the test was obtained from a local university and hospital. Written consent to participate in the test was obtained from each subject.

Measurement of the thickness of the epidermal and subepidermal low echogenic band (SLEB)

A high-frequency ultrasound scanner with a Vevo model 708 scanhead operating at a center frequency of 55 MHz (VisualSonics Inc., Toronto, ON, Canada) was used for imaging skin morphology. This frequency gives an axial resolution of 30 μm and a lateral resolution of approximately 70 μm, producing high-resolution images to a maximum depth of 8 mm. The system displays the information obtained in the form of a B-scan in a gray scale image. In total, four ultrasound biomicroscopy scans were performed on the skin of the foot being tested, at the nailfold and pulp of the big toe. After ultrasound gel was applied over the measuring sites, the Vevo model 708 probe was placed perpendicular to the surface of the skin during the capturing of the image. The pressure of the transducer on the surface of the skin was minimized to avoid compressing the surface of the skin. By using fractal geometry, quantitative data on epidermal thickness were measured by analyzing the change in the echogenicity of the ultrasound image on each sonogram. The first entry echo as shown in the sonograms corresponds to the interface between the coupling gel and surface of the skin, followed by a broad echo-rich band underneath corresponding to the epidermis. This is followed by a thin echolucent band, the so-called SLEB, corresponding to the upper dermis (Fig. 1). After the boundaries of different layers were identified, the thickness of the skin at various layers, as defined as the distance between the demarcation echo lines, was then calculated by the in-house Vevo image analysis software. The mean of the measurements for the two images obtained at each testing point was used to analyze the data.

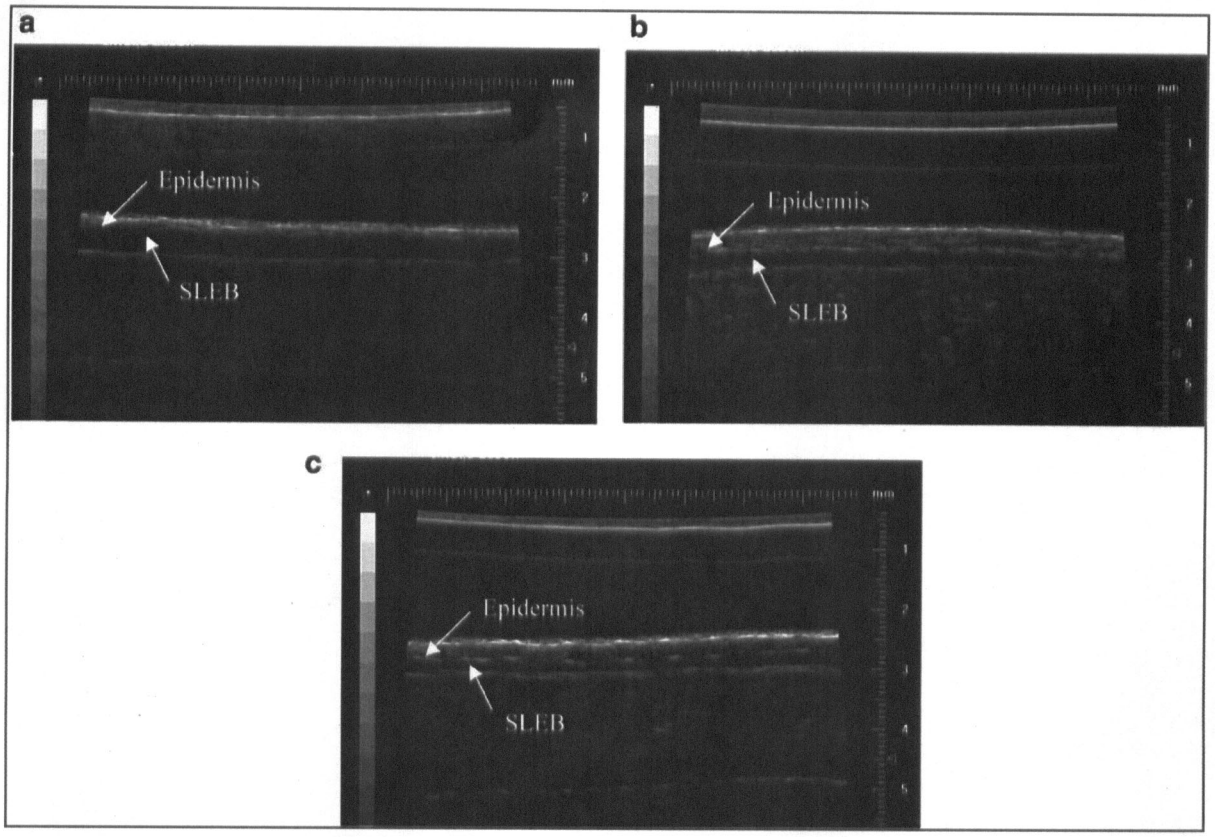

FIG. 1. Ultrasound images showing the epidermal and subepidermal low echogenic band (SLEB) thickness at the pulp of big toe among the study groups: **(a)** diabetic neuropathy and ulceration, **(b)** diabetes without neuropathy, and **(c)** normal healthy controls. Images are taken by high-frequency ultrasound with central frequency at 55 MHz.

Measurement of skin blood flow

Capillary diameter and capillary blood cell velocity. Using CapiScope capillaroscopy (KK Technology, Honiton, Devon, UK), capillary blood cell velocity (in mm/s) representing skin nutritive blood flow was monitored in the nailfold of the big toe while the subjects were in a supine position. The subjects were positioned comfortably in a supine position with the foot being tested placed on the platform of a custom-made foot stand. The subjects were acclimatized for 20 min prior to the actual recording. A drop of liquid paraffin oil was applied to the testing area to maximize the translucency of the keratin layer and decrease reflection. Images were subsequently videotaped, coded, and stored using CapiScope videocapillaroscopy analysis software for an off-line analysis using a computerized, videophotometric, cross-correlation technique (KK Technology). The diameter of the capillaries and the capillary blood cell velocity were measured. Capillary blood cell velocity was recorded for 1 min in five different erythrocyte-perfused capillaries identified at the measuring sites, and the mean value was obtained for subsequent analysis.

Skin blood flux. The skin blood flux over the skin of the nailfold and the pulp of the big toe was recorded using laser Doppler flowmetry (model DRT4, Moore Instruments, Milway, Devon, UK). This instrument mainly measures the flow of blood in arteriovenous anastomoses, arterioles, and venous plexuses.[20] The measurements were carried out in a quiet environment with the room temperature controlled at 24 ± 0.2°C. Caution was taken to minimize external disturbances. The testing sites of the skin were cleaned with alcohol prep. The laser Doppler probe was then gently applied on each of the testing sites using an adhesive pad to avoid vascular compression. The subjects were then comfortably positioned in a supine position for at least 20 min to acclimatize to the room's temperature. Baseline resting skin blood perfusion was represented in arbitrary units, calculated as a product of mean blood cell velocity and the concentration of blood cells in the recording area in terms of flux. Continuous measurements were made for 5 min in each of the measuring sites, and the averaged data obtained in the last 2 min were recorded. All of the data were stored on a computer for off-line analysis using the Moorsoft software package (Moor Instruments).

Statistical analyses

One-way analysis of variance was used to test for group differences in the demographic variables and all testing variables. When a significant group difference was detected, Hochberg's GT2 or Games–Howell post hoc multiple comparisons were performed to identify pairwise group differences. Correlations among epidermal thickness, the thickness

TABLE 1. SUBJECT CHARACTERISTICS

Group characteristic	*Normal* (n=33)	*DM* (n=35)	*DU* (n=19)	P *value*
Male/female	11:22	16:19	14:5	0.019
Age (years)	69.00±4.7	65.17±9.0	63.32±12.7	0.103
Body mass index (kg/m^2)	23.29±3.62	24.55±4.15	25.75±4.05	0.111
Blood pressure (mmHg)				
Systolic	136.18±14.03	133.90±14.69	144.11±20.43	0.079
Diastolic	78.83±9.25	77.31±10.84	80.23±9.49	0.587
Fasting glucose (mmol/L)	5.55±0.61	7.25±2.13	6.90±3.11	<0.001[a]
Glycated hemoglobin (%)	—	7.27±1.12	8.07±2.32	0.323
Duration of diabetes (years)	—	9.29±7.48	13.67±12.09	<0.001[b]
Vibration threshold (volts)	15.62±6.69	16.46±5.35	34.06±10.59	<0.001[a]
Ankle brachial index				
Left	1.11±0.08	1.10±0.12	1.12±0.11	0.789
Right	1.11±0.10	1.14±0.14	1.15±0.14	0.695

Data are mean±SD values.
[a]Normal group versus both diabetes groups.
[b]Ulcer group versus diabetes-only group.
DM, with diabetes but without neuropathy; DU, diabetic neuropathy and foot ulceration.

of the SLEB, and skin blood flow were calculated using Pearson's correlation analysis. The level of significance was set at 0.05 for all measurements.

Results

Details of the demographics of the subjects are shown in Table 1. Nineteen subjects with a history or present condition of diabetic foot ulcerations (DU group) (14 males and 5 females, 63.32±12.7 years old), 35 diabetes subjects without neuropathy or ulceration (DM group) (16 males and 19 females, 65.17±9.0 years old), and 33 healthy controls without diabetes (11 males and 22 females, 69.00±4.7 years old) participated in the study. No significant differences in demographic data, diastolic blood pressure, and ankle brachial index were found among the groups. In line with the group definition, the fasting glucose was significantly higher in the diabetes groups than in the healthy controls without diabetes ($P<0.001$). Subjects in the DU group demonstrated a longer duration of diabetes and higher vibration perception threshold than subjects in the DM group and healthy control subjects ($P<0.001$). No significant difference in the glycated hemoglobin level was found between the two diabetes groups.

Epidermal thickness and edema in papillary dermal skin

The epidermal thickness of plantar skin was significantly different among the study groups at both measuring sites (Table 2). Post hoc analyses indicated that the average epidermal thickness of plantar skin was significantly thicker in the DM group and thinner in the DU group compared with the control group (Fig. 2). Specifically, epidermal thickness had decreased by 16.5% in the DU group but increased by 7.2% in the DM group compared with the healthy controls. The SLEB was observed only at the pulp of the big toe, but not at the nailfold. We demonstrated that the SLEB thickness had increased in both diabetes groups ($P<0.001$). Compared with the control group, a 12% increase was found in the DM group, and a 65% increase was observed in the DU group (Fig. 2). A significant negative correlation ($P=0.002$, $r=-0.366$) was demonstrated between SLEB and epidermal thickness at the pulp of the big toe (Fig. 3).

Skin blood flow

At the nailfold of the big toe, the resting capillary diameter, nutritive blood flow, and skin blood flux were similar in all of the study groups (all $P>0.05$) (Table 3 and Figs. 4

TABLE 2. EPIDERMAL THICKNESS AND SUBEPIDERMAL LOW ECHOGENIC BAND THICKNESS AT DIFFERENT REGIONS OF THE PLANTAR FOOT AS MEASURED BY HIGH-FREQUENCY ULTRASONOGRAPHY

Skin thickness (mm)	*Normal* (n=33)	*DM* (n=35)	*DU* (n=19)	F *ratio*	P *value*
Epidermal thickness					
Nailfold of big toe	0.22±0.07	0.25±0.08	0.19±0.06	3.993	0.022[a]
Pulp of big toe	0.50±0.16	0.54±0.18	0.40±0.13	5.246	0.009[b]
SLEB thickness					
Pulp of big toe	0.17±0.08	0.19±0.07	0.28±0.09	9.544	<0.001[c]

Data are mean±SD values.
[a]Diabetes-only group versus ulcer group.
[b]Diabetes-only group versus ulcer and normal groups.
[c]Ulcer group versus diabetes-only and normal groups.
DM, with diabetes but without neuropathy; DU, diabetic neuropathy and foot ulceration; SLEB, subepidermal low echogenic band.

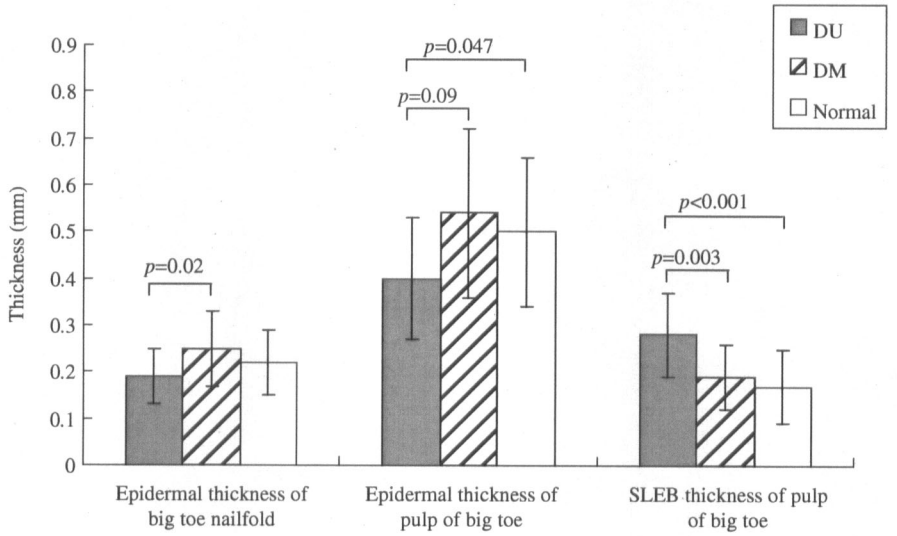

FIG. 2. Epidermal and subepidermal low echogenic band (SLEB) thickness at different regions of the big toe in people with or without diabetes. DM, with diabetes but without neuropathy; DU, diabetic neuropathy and foot ulceration.

and 5). At the pulp of the big toe, the resting skin blood flux was significantly higher in the DM group, followed by the DU group and the control group, respectively (161.08 ± 100.51 vs. 139.46 ± 79.23 vs. 93.53 ± 63.26 arbitrary units, respectively, $P = 0.009$). The skin blood flux was significantly higher at the pulp than at the nailfold. No significant correlation was demonstrated between skin blood flow and epidermal thickness (all $P > 0.05$).

Discussion

We found thickening of the epidermal layer of plantar skin in the DM group, but thinning in the DU group. In addition, there was an increase in papillary dermal edema over the pulp of the big toe, as reflected by the presence of SLEB in all subjects with diabetes but to a greater degree in those with foot ulceration. We found no difference in capillary diameter and nutritive capillary blood flow among the groups, but we observed an increase in skin blood flux in the diabetic foot. A significant negative correlation was demonstrated between the SLEB and epidermal thickness. However, we did not find any correlation between skin blood flow and the thickness of the epidermal skin.

Compared with the healthy control group, the epidermal thickness had decreased by 16.5% in the DU group but increased by 7.2% in the DM group. The epidermis is known to be a glycolytic tissue that is affected by the level of insulin in the body for regulating the migration and proliferation of keratinocytes.[21] The observed hyperproliferative state of the epidermis in the DM group is believed to be the consequence of an accumulation of advanced glycation end-products induced by hyperglycemia[22,23] or an increase in the cross-

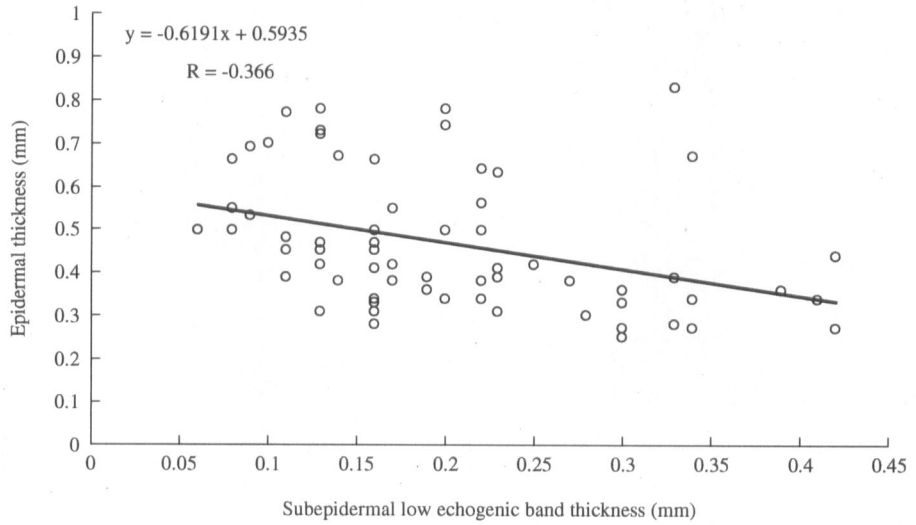

FIG. 3. Correlation curve between epidermal and subepidermal low echogenic band thickness at the pulp of big toe.

TABLE 3. NUTRITIVE BLOOD FLOW OVER THE BIG TOE NAILFOLD AS MEASURED BY CAPILLAROSCOPY AND SKIN BLOOD FLUX AT THE NAILFOLD AND PULP OF THE BIG TOE AS MEASURED BY LASER DOPPLER FLOWMETRY

Skin blood flow	*Normal* (n=33)	*DM* (n=35)	*DU* (n=19)	F *ratio*	P *value*
Nutritive capillary blood flow					
Capillary blood cell diameter (mm)	8.90±1.77	9.07±1.93	8.58±1.38	0.438	0.647
Capillary blood cell velocity (mm/s)	42.54±11.76	46.24±12.12	42.78±10.16	0.971	0.383
Skin blood flux (arbitrary units)					
Big toe nailfold	44.93±26.70	46.66±26.26	45.22±24.37	0.038	0.963
Pulp of big toe	93.53±63.26	161.08±100.51	139.46±79.23	5.069	0.009[a]

Data are mean±SD values.
[a]Diabetes-only group versus normal group.
DM, with diabetes but without neuropathy; DU, diabetic neuropathy and foot ulceration.

linking of collagen fibers.[24] However, we did not observe an increase in epidermal thickness in all of our subjects with diabetes. We found epidermal atrophy when diabetes is complicated by neuropathy and ulceration. Previous studies using skin biopsies have shown that the amount of epidermal nerves in patients with sensory neuropathy, such as diabetic peripheral neuropathy, is substantially lower than in normal subjects.[25–28] Such a reduction in epidermal nerves may induce a reduction in the proliferation of keratinocyte, speed up keratinocyte differentiation, and enhance programmed cell death, hence causing epidermal thinning.[29–31] This may partly explain the epidermal atrophy that was observed in our diabetes group with neuropathy and ulceration (the DU group). The epidermal layer of plantar skin serves as the front line in the biological barrier protecting the foot from physical wear and tear.[32] This may explain why people with diabetic neuropathy or a history of ulceration have a higher incidence of ulceration or re-ulceration. However, one should bear in mind that the present study used the 10-g monofilament test and vibration perception threshold as screening tools for neuropathy and that subjects in our DU group may have relatively severe neuropathy. Our findings might not be generalized to people with mild neuropathy.

We detected a SLEB at the pulp of big toe but not at the nailfold region. The pulp of the big toe is not an area that is subject to exposure from the sun, so the observed SLEB is not likely to have been due to ultraviolet exposure or photodamage of the skin. Instead, it may represent the degree of edema in the papillary dermis. We found an increase in SLEB thickness in all subjects with diabetes, to a greater degree in those with foot ulceration. This indicates that people with diabetes develop foot swelling, particularly those with a history or present condition of foot ulceration. In addition, a significant negative correlation was demonstrated between the SLEB and epidermal thickness, meaning that the epidermal layer becomes thinner with an increase in papillary dermal edema. Therefore, diabetes associated with subepidermal edema may have pathophysiologic consequences. The efficiency of the delivery of oxygen to the tissues depends on various factors, including the nature of the intercellular matrix, cell density, and whether the intercellular spaces are affected by edema.[10] Edema impairs skin oxygen consumption by increasing the volume of interstitial tissue, hence increasing the distance for oxygen to diffuse between capillaries and target tissues and leading to less efficient delivery of oxygen to the tissue. Therefore, the presence of edema in the

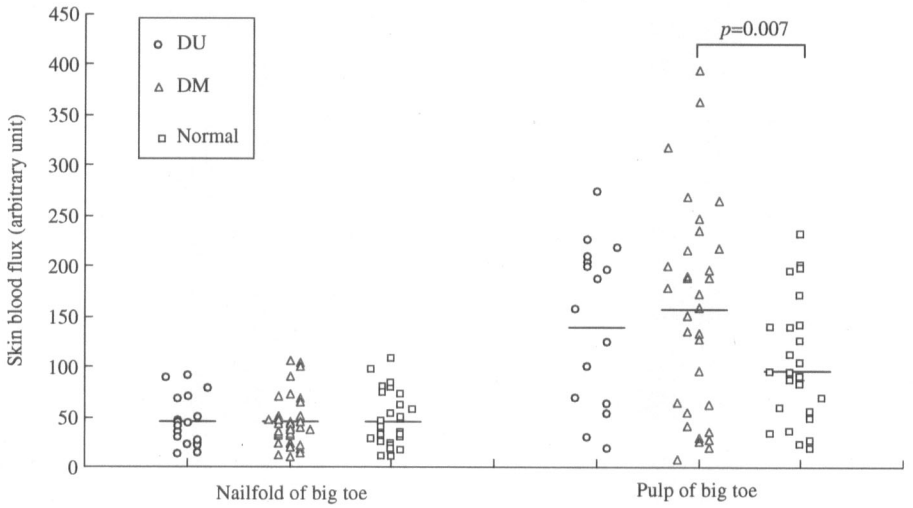

FIG. 4. Resting skin blood flux at the nailfold and pulp of the big toe as measured by laser Doppler flowmetry. The horizontal bar represents the averaged mean value. DM, with diabetes but without neuropathy; DU, diabetic neuropathy and foot ulceration.

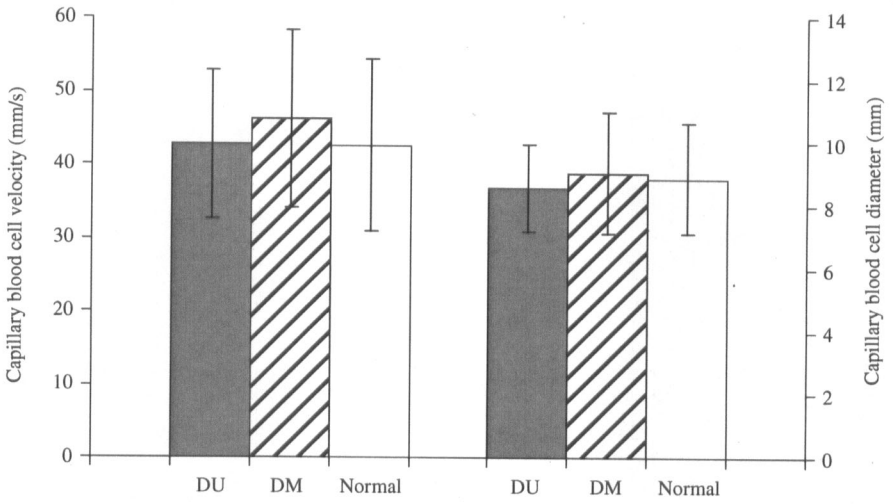

FIG. 5. Capillary blood cell velocity and diameter at the nailfold of the big toe. DM, with diabetes but without neuropathy; DU, diabetic neuropathy and foot ulceration.

subepidermal region where the capillary loops are located could significantly impair epidermal nutrition and metabolism. This ultimately leads to epidermal atrophy and predisposes the skin in a diabetic foot to breaking down. This may explain the epidermal atrophy observed in people with diabetic foot ulceration. We found that water tends to accumulate in the subepidermal papillary skin layer. This may due to be the fact that the papillary dermis is rich in collagen fibers and hence is a site for pronounced collagen damage due to the pathological changes induced by diabetes. With an increase in compactness and in the degree of the folding of the proteins, the water-binding capacity for fluid accumulation also increases.[17]

Skin blood flow in patients with diabetes mellitus has been studied extensively in the last few decades. However, conflicting results have been found. The present study found no significant differences in the resting nailfold nutritive capillary blood flow and capillary diameters but a marked increase in the skin blood flux at the pulp of all people with diabetes. Laser Doppler flowmetry measures the flow of blood in both the capillaries and the AV shunts. We demonstrated that the skin blood flux was significantly higher at the pulp (glabrous skin) than in the nailfold (nonglabrous skin), as the pulp contains a large number of AV anastomoses. Houben et al.[33] also reported similar findings, namely, that the resting skin blood flux increased whereas the capillary blood cell velocity remained unchanged at the forearm in people with diabetes compared with healthy controls. However, they did not investigate the change in skin blood flow in the diabetic foot.

The present study presented an unexplored area on the association of skin blood flow and edema with epidermal thickness in diabetes and in the diabetic foot, and this subject so far has received inadequate attention. We demonstrated in the present study subepidermal edema and a reduced epidermal thickness in people with diabetic neuropathy and ulceration. Our subjects in the DU group had a longer duration of diabetes than did the DM group, which suggests that a longer history of diabetes may lead to greater impact on microangiopathy. Instead of using clinical examination scores such as the Neuropathy Disability Score[34] for screening neuropathy, the present study made use of monofilament for screening neuropathy, which can be more stringent for detecting neuropathy. Therefore, our findings may be more applicable to people with more severe diabetic neuropathy but may not be generalized to people who have mild diabetic neuropathy. Also, our sample size is relatively small. These are the limitations of the present study. Further studies should be conducted in people with diabetes who have a shorter history of the disease, with or without foot ulceration, who have different severity of neuropathy with a larger sample size.

The present study did not find any significant correlation between skin blood flow and epidermal thickness. Given that the microvascular network is located only in the dermal layer of the skin and that both an increase in skin blood flux and edema formation in a restricted volume of the papillary skin layer occurred, it is conceivable that there was a remarkable increase in local tissue tension inside the layers of the skin, especially the plantar skin, in an area subject to repetitive weight bearing during ambulation. This could further impede the skin blood flow during ambulation, ultimately affecting the health of the skin and predisposing it to breaking down and forming ulcers.

Conclusions

People with diabetes tend to have thicker epidermal skin in the foot. However, if they develop diabetic neuropathy and ulceration, epidermal thinning occurs. Our findings demonstrated that the AV shunt flow increases in all subjects with diabetes. In addition, we observed an increase in subepidermal edema in all people with diabetes, with a greater increase occurring in those whose condition was complicated by neuropathy and ulceration. This may contribute to the reduction in epidermal thickness that makes the diabetic foot prone to tissue breakdown and hence ulcer formation.

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Author Disclosure Statement

No competing financial interests exist.

References

1. Mueller MJ, Zou D, Lott DJ: "Pressure gradient" as an indicator of plantar skin injury. Diabetes Care 2005;28:2908–2912.
2. Bennett PJ, Stocks AE, Whittam DJ: Analysis of risk factors for neuropathic foot ulceration in diabetes mellitus. J Am Podiatr Med Assoc 1996;86:112–116.
3. Fernando DJ, Masson EA, Veves A, Boulton AJ: Relationship of limited joint mobility to abnormal foot pressures and diabetic foot ulceration. Diabetes Care 1991;14:8–11.
4. Zimny S, Dessel F, Ehren M, Pfohl M, Schatz H: Early detection of microcirculatory impairment in diabetic patients with foot at risk. Diabetes Care 2001;24:1810–1814.
5. Urbancic-Rovan V, Stefanovska A, Bernjak A, Azman-Juvan K, Kocijancic A: Skin blood flow in the upper and lower extremities of diabetic patients with and without autonomic neuropathy. J Vasc Res 2004;41:535–545.
6. Flynn MD, Tooke JE: Diabetic neuropathy and the microcirculation. Diabet Med 1995;12:298–301.
7. Belcaro G, Nicolaides AN: The venoarteriolar response in diabetics. Angiology 1991;42:827–835.
8. Belcaro G, Nicolaides AN, Volteas N, Leon M: Skin flow the venoarteriolar response and capillary filtration in diabetics. A 3-year follow-up. Angiology 1992;43:490–495.
9. Iwase M, Imoto H, Murata A, Nakamura U, Nohara S, Uchizono Y, Iino K, Iida M: Altered postural regulation of foot skin oxygenation and blood flow in patients with type 2 diabetes mellitus. Exp Clin Endocrinol Diabetes 2007;115:444–447.
10. Silver IA: Local factors in tissue oxygenation. J Clin Pathol Suppl (R Coll Pathol) 1977;11:7–13.
11. Duffin AC, Lam A, Kidd R, Chan AK, Donaghue KC: Ultrasonography of plantar soft tissues thickness in young people with diabetes. Diabet Med 2002;19:1009–1013.
12. Hashmi F, Malone-Lee J, Hounsell E: Plantar skin in type II diabetes: an investigation of protein glycation and biomechanical properties of plantar epidermis. Eur J Dermatol 2006;16:23–32.
13. Huntley AC, Walter RM Jr: Quantitative determination of skin thickness in diabetes mellitus: relationship to disease parameters. J Med 1990;21:257–264.
14. Seirafi H, Farsinejad K, Firooz A, Davoudi SM, Robati RM, Hoseini MS, Ehsani AH, Sadr B: Biophysical characteristics of skin in diabetes: a controlled study. J Eur Acad Dermatol Venereol 2009;23:146–149.
15. Vogt M, Ermert H: Development and evaluation of a high-frequency ultrasound-based system for in vivo strain imaging of the skin. IEEE Trans Ultrason Ferroelectr Freq Control 2005;52:375–385.
16. Mofid Y, Ossant F, Imberdis C, Josse G, Patat F: In-vivo imaging of skin under stress: potential of high-frequency (20 Mhz) static 2-D elastography. IEEE Trans Ultrason Ferroelectr Freq Control 2006;53:925–935.
17. El-Zawahry MB, Abdel El-Hameed El-Cheweikh HM, Abd-El-Rahman Ramadan S, Ahmed Bassiouny D, Mohamed Fawzy M: Ultrasound biomicroscopy in the diagnosis of skin diseases. Eur J Dermatol 2007;17:469–475.
18. Gniadecka M: Non-invasive methods for determination of oedema and water behavior in the skin. Skin Res Technol 1995;1:55–60.
19. Gniadecka M: Localization of dermal edema in lipodermatosclerosis, lymphedema, and cardiac insufficiency. High-frequency ultrasound examination of intradermal echogenicity. J Am Acad Dermatol 1996;35:37–41.
20. Chao CY, Cheing GL: Microvascular dysfunction in diabetic foot disease and ulceration. Diabetes Metab Res Rev 2009;25:604–614.
21. Chen X, Lin W, Lu S, Shi Y, Zou J, Liu Z, Liao W: Insulin prevents latent skin lesions by inhibiting the generation of advanced glycation end products in streptozotocin-induced diabetic rats. Endocr Pathol 2009;20:163–169.
22. Kennedy L, Baynes JW: Non-enzymatic glycosylation and the chronic complications of diabetes: an overview. Diabetologia 1984;26:93–98.
23. Sternberg M, Cohen-Forterre L, Peyroux J: Connective tissue in diabetes mellitus: biochemical alterations of the intercellular matrix with special reference to proteoglycans, collagens and basement membrane. Diabete Metab 1985;11:27–50.
24. Nikkels-Tassoudji N, Henry F, Letawe C, Pierard-Franchimont C, Lefebvre P, Pierard GE: Mechanical properties of the diabetic waxy skin. Dermatology 1996;192:19–22.
25. Kennedy WR, Wendelschafer-Crabb G, Johnson T: Quantitation of epidermal nerves in diabetic neuropathy. Neurology 1996;47:1042–1048.
26. Umapathi T, Tan WL, Loke SC, Soon PC, Tavintharan S, Chan YH: Intraepidermal nerve fiber density as a marker of early diabetic neuropathy. Muscle Nerve 2007;35:591–598.
27. Hsieh ST, Lin WM, Chiang HY, Huang IT, Ko MH, Chang YC, Chen WP: Skin innervation and its effects on the epidermis. J Biomed Sci 1997;4:264–268.
28. Hsieh ST, Chiang HY, Lin WM: Pathology of nerve terminal degeneration in the skin. J Neuropathol Exp Neurol 2000;59:297–307.
29. Chiang HY, Huang IT, Chen WP, Chien HF, Shun CT, Chang YC, Hsieh ST: Regional difference in epidermal thinning after skin denervation. Exp Neurol 1998;154:137–145.
30. Hsieh ST, Choi S, Lin WM, Chang YC, Mcarthur JC, Griffin JW: Epidermal denervation and its effects on keratinocytes and Langerhans cells. J Neurocytol 1996;25:513–524.
31. Hsieh ST, Lin WM: Modulation of keratinocyte proliferation by skin innervation. J Invest Dermatol 1999;113:579–586.
32. Hagisawa S, Shimada T: Skin morphology and its mechanical properties associated with loading. In: Bader D, Bouten C, Colin D, Oomens C, eds. Pressure Ulcer Research: Current and Future Perspectives. Berlin: Springer-Verlag, 2005: 161–185.
33. Houben AJ, Nieuwenhuijzen Kruseman AC, Bouhouch E, Slaaf DW, Schaper NC: Peripheral macro- and microcirculation in short-term insulin-dependent diabetes mellitus: the role of prostaglandins in early haemodynamic changes. Eur J Clin Invest 1993;23:662–667.
34. Young MJ, Boulton AJ, MacLeod AF, Williams DR, Sonksen PH: A multicentre study of the prevalence of diabetic peripheral neuropathy in the United Kingdom hospital clinic population. Diabetologia 1993;36:150–154.

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