A COMPREHENSIVE ASSESSMENT AND MANAGEMENT PROGRAM FOR DIABETIC ULCER

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CERTFICATE OF ORGINALITY

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

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RACHEL LAI-CHU KWAN (Name of student)

Abstract of thesis titled "A comprehensive assessment and management program for diabetic ulcer" submitted by RACHEL LAI-CHU KWAN for the degree of Master of Philosophy at the Hong Kong Polytechnic University in July, 2011.

ABSTRACT

Foot ulceration is extremely prevalent in patients with diabetes mellitus, particularly in the ageing population. The lifetime risk of developing a foot ulcer can be as high as 25 percent for people with diabetes (Boulton et al., 2005), in which 11.6% diabetic ulcer persisted unhealed (Jeffcoate et al., 2006). Diabetic foot ulceration is the major cause of high morbidity and mortality among people with diabetes due to high incidence of amputations (Boulton et al., 2005). Persistent hyperglycemia may lead to various complications including decreased sensation, which results in pathological changes in plantar soft tissues, which might stiffen its structure. The altered nerve and blood vessels function, as well as the biomechanical changes in the foot makes diabetic ulcers difficult to heal. Mechanical stresses at the wound site are hypothesized to affect the wound healing (Farahani and Kloth, 2008). It is suggested that the wound might become more extensible during healing process (Lee and Moon, 2003). Early treatment is vital for preventing serious complications such as lower limb amputation.

A systematic review was conducted to investigate the effectiveness of electrophysical modalities for managing diabetic ulcers, and found that electrophysical therapy can bring beneficial effects that may promote the healing of diabetic ulcers. Pulsed electromagnetic field was shown to promote nerve regeneration (Musaev et al., 2003) and microcirculation (Webb et al., 2003) in patients with diabetes, thus it is a potential treatment to be used for promoting healing of diabetic ulcer.

Therefore, this thesis consists of three inter-related studies. Study I investigated the biomechanical properties of plantar soft tissues in terms of stiffness and thickness

among different age groups of healthy people. Study II examined the biomechanical properties of plantar soft tissues in terms of stiffness and thickness among patients with diabetic ulceration and healthy control. Also, the relationship between these properties and tactile sensation were examined in the patients with diabetic ulceration. Specifically, a case study was done to monitor the change of biomechanical properties of the diabetic wound tissues across different stages of healing. Study III was a randomized controlled trial that examined the effectiveness of pulsed electromagnetic field energy (PEMF) in promoting healing of diabetic ulcer by restoring normal biomechanical properties (i.e. stiffness) in the wound tissues, and facilitating microcirculation in the nailfold and pulp of big toes.

In Study I, the effect of ageing on the biomechanical properties of plantar soft tissues was examined in sixty healthy volunteers without foot problems, aged from 41 to 83 years. The thickness and stiffness of the plantar soft tissues under the big toe, first metatarsal head, third metatarsal head, fifth metatarsal head, and heel were measured by using tissue ultrasound palpation system (TUPS). The mean stiffness of the plantar soft tissues at the big toe, first metatarsal head, third metatarsal head, fifth metatarsal head, and the heel significantly increased with age ($P<0.001$). The plantar soft tissues were thickest at the heel ($P<0.001$), with the thickness increasing with age, although there was no statistical significance between the groups. Significant and strong positive correlations between age and stiffness of the plantar soft tissues were found at the big toe ($r=0.608$), first metatarsal head ($r=0.549$), third metatarsal head ($r=0.657$), fifth metatarsal head ($r=0.633$), and heel ($r=0.584$) (all $P<0.001$).

In Study II, there were forty-three subjects (9 diabetic ulceration and 34 healthy controls). The biomechanical properties of the plantar soft tissues and the tactile sensation were compared between the two groups. The correlation between these properties and tactile sensation in the patients with diabetic ulceration was investigated. The mean stiffness and thickness of the plantar soft tissues at the big toe, first metatarsal head, third metatarsal head and the heel were significantly different between the diabetic ulceration and the healthy control groups (all $P<0.05$). In addition to the above measurement sites, the mean stiffness of the plantar soft tissues was also significantly different at the fifth metatarsal head between the two groups. The tactile sensation and mean stiffness of the plantar soft tissues at the big toe, first metatarsal head, third metatarsal head, fifth metatarsal head, and the heel was significantly different between groups (all $P<0.05$). Fair to strong correlations between tactile sensation and stiffness of the plantar soft tissues were found at the big toe ($r=0.618$), first metatarsal head ($r=0.498$), third metatarsal head ($r=0.484$), fifth metatarsal head ($r=0.626$), and the heel ($r=0.363$) (all $P<0.05$) in patients with diabetic ulceration. In the case study that monitor diabetic wound healing over 7 weeks, the normalized stiffness coefficient of the central wound bed area decreased 9.56% on day 7 and continued to decrease in day 14. It slightly increased by day 21, and finally increased by day 49. The decrease in stiffness of the wound bed tissue in the early stage of healing, allows considerable elasticity of the connective tissue fibers existing as a loose network. At the later stage, the differentiation of fibroblasts cause increase in the stiffness of the wound tissue due to extracellular matrix production and remodelling processes (Grinnell, 1994), and hence the wound bed were found to be stiffer.

In Study III, a randomised controlled trial that examined the effects of pulsed electromagnetic fields in promoting the healing of diabetic foot ulcer was conducted. Thirteen subjects (7 for PEMF group and 6 for sham PEMF group) diagnosed with Type II diabetes with unsatisfactory healing of ulcer(s) in the preceding four weeks were recruited. Subjects received either active pulsed electromagnetic field (Duration: 60 minutes; frequency: 12 Hz; and intensity: 12 Gauss) or sham PEMF for 14 sessions within three weeks. Assessment on microcirculation, biomechanical properties of wound tissue and wound healing stages were done at the baseline, after 14 sessions, and one-month follow-up. There was approximately 28% increase in nutritive capillary blood velocity and about 14% increase in capillary diameter in the PEMF group after 14 sessions. Upon the post treatment evaluation, the average stiffness coefficient of the wound bed decreased in both the PEMF group and sham PEMF group; while the PEMF group continued to decrease in average stiffness coefficient at the one-month follow-up, the sham PEMF group showed an increase. The stiffness measured from the wound tissues can serve as quantitative reference for healing stages, and to reflect the degree of growth, remodeling and function of the cells.

In conclusion, the biomechanical properties of the plantar soft tissues appear to change with age in healthy individuals. The stiffness of unloaded plantar soft tissues significantly increased with age, also there was a trend of increasing thickness of the plantar soft tissue with advancing age. In addition, the plantar soft tissues of the patients with diabetic ulceration were significantly thicker and stiffer than the healthy controls. Specifically, the change of biomechanical properties was found to correlate with the tactile sensation. The case study showed that during diabetic wound healing, the

stiffness of the wound tissues change at different stages. The normalized stiffness coefficient of the central wound bed area decreased at the inflammatory stage, then slightly returned on at the intermediate stage and increased in the later stage. Our randomized controlled trial demonstrated that pulsed electromagnetic field produced a trend in improving microcirculation, biomechanical properties of the wound tissue and wound healing status. As the sample size was small, a larger scale of clinical trial is needed to confirm that PEMF is an effective treatment for enhancing healing for diabetic foot ulcers.

PUBLICATIONS ARISING FROM THE THESIS

Journal articles

Kwan, R.L.C., Zheng, Y.P., Cheing, G.L.Y., 2010. The effect of aging on the biomechanical properties of plantar soft tissues. Clinical Biomechanics 25, 601-605. (Appendix IV)

Kwan, R.L.C., Cheing, G.L.Y., Vong, S.K.S., Lo, S.K. Electrophysical Therapy for Managing Diabetic Ulcers: A Systematic Review. (Under review by 'Journal of Diabetes and its Complications') (Appendix V)

Conference abstract

Cheing, G., Kwan R., Zheng, Y.P. The effect of aging on the biomechanical properties of plantar soft tissues. 16th International WCPT Congress June, 2011. Physiotherapy Volume 97 Supplement S1. (Appendix VI)

Kwan, R.L.C., Cheing, G.L.Y. Effectiveness of pulsed electromagnetic fields in promoting healing of diabetic foot ulcer. Diabetic Limb Salvage (DLS) Conference September, 2011. Dr. Robert W. Hobson II Award for Best DLS Abstract Poster Presentation 2nd Place. (Appendix VII)

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LIST OF ABBREVIATIONS

ABI	Ankle-Brachial Index
ADA	American Diabetes Association
ANOVA	Analysis of variance
DPN	Diabetic polyneuropathy
DU	Diabetic ulceration
ICC	Intraclass correlation coefficient
LDF	Laser Doppler flowmetry
MRI	Magnetic resonance imaging
OCT	Optical coherence tomography
PEMF	Pulsed electromagnetic field
SD	Standard deviation
SWM	Semmes-Weinstein monofilaments
TUPS	Tissue ultrasound palpation system
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

The World Health Organization (WHO) estimates that more than 346 million people have diabetes worldwide, and projects that deaths from diabetes will double between 2005 and 2030. It is estimated that China will lose $558 billion in foregone national income due to heart disease, stroke and diabetes alone (World Health Organization, 2011). In Hong Kong, approximately 277,500 people are diagnosed with diabetes mellitus, in which 30% of them are 65 years old or above (Census and Statics Department, 2010). As the population is increasing, the prevalence of diabetes related complications will continue to rise.

The lifetime risk of developing a foot ulcer could be as high as 25 percent for people with diabetes (Boulton et al., 2005), with 11.6% having persistent non-healing ulcer (Jeffcoate et al., 2006). Neuroischemic ulcers take longer to heal, and were three times more likely to lead to amputation (Oyibo et al., 2001). Over 70% of non-traumatic lower limb amputations were performed in patients with diabetes (Trautner et al., 1996). Diabetic foot ulceration is the main cause of high morbidity and mortality among people with diabetes due to high incidence of amputations (Boulton et al., 2005). The economical burden is enormous, and the management of diabetic foot problems costs £252 million per year in the United Kingdom (Boulton et al., 2005).

Approximately 45% to 60% of diabetic ulceration is purely caused by neuropathy, and about 45% were caused by both neuropathy and ischemia (Reiber et al., 1999). Patients with both peripheral arterial occlusive disease and diabetes have more serious functional disability than those with peripheral arterial occlusive disease alone (Dolan et al., 2002),

and more prone to ulceration (Brem et al., 2006). The nerve and microvascular dysfunction subsequently alter the foot biomechanics, and increase the chance of ulceration in the patients with diabetes.

Ageing usually lead to muscle weakness, as well as a decrease in range of motion in the ankle and foot region. It is common to have altered foot biomechanics or developing foot deformities, which results in pain and foot problems among elderly people. Diabetes is a common diagnosis found in the elderly population. The pathological changes of diabetes that lead to development of foot problems in old people are unclear. But the impact is even greater if patients also suffer from diabetic-associated systemic conditions such as peripheral vascular disease. Foot structure is a major determinant of plantar pressure that contributes to ulceration risk (Bus et al., 2005). Clawed toes and hammertoes are frequent clinical findings that ascribed to intrinsic muscle atrophy. This increases the likelihood of ulceration due to the distal displacement of metatarsal cushioning (Bus et al., 2004). In addition, the decreased ranges of movement in the joints of the foot and ankle (Fernando et al., 1991) and callus formation (Murray et al., 1996) were found related to elevated plantar foot pressure. It may end up with changes in plantar soft tissue, result in ulceration, then having lower limb amputation in severe cases. Recent research findings suggest that the change in biomechanical properties of plantar soft tissue is a potential risk factor for developing foot ulcer (Sopher et al., 2011). In order to provide suitable foot care for older people with or without diabetes, it is important to investigate the effect of aging and/ or diabetes on the biomechanical properties of plantar soft tissue. Therefore, this thesis examined the biomechanical

properties of plantar soft tissue first in a group of healthy older people, then further on people with diabetes ulcers.

Besides the biomechanical properties of plantar soft tissue, it is believed that the biomechanical changes on the wound tissue can reflect the healing stages of diabetic ulcer. In this thesis, a case study was done to investigate the changes of wound tissue biomechanical properties over time. The optical coherence tomography (OCT) based air jet indentation system is a potential quantitative and non-contact assessment for investigating the biomechanical properties of the wound. It can be used to monitor the healing stages of chronic wound.

This thesis also investigated several factors that may influence ulcer healing. It is well known that sufficient blood supply to the affected area is an essential factor for wound healing. This thesis made use of both laser Doppler flowmetry and the video capillaroscopy to examine the distribution of blood flow between the subpapillary microvascular compartments and the nutritional skin capillaries. The changes of each of these measurements at different healing stages were monitored in this thesis. This can also explain the unexplored etiology of diabetic ulcer healing.

In terms of clinical management of diabetic ulcer, offloading and debridement are common treatment methods, however, there is no evidence that these procedures were able to restore the sensory loss of lower limb, which is the main cause of infection, stress formation and ulceration (Smith, 2002). Various studies have reported that electrophysical modalities can promote chronic wound healing. This thesis conducted a systematic review to examine the evidence of the commonly used electrophysical

modalities in promoting wound healing (Appendix V). There are few studies conducted on electrophysical modalities such as ultrasound, lasers, and electromagnetic therapy. Among these modalities, pulsed electromagnetic field (PEMF) is a modality that emits low frequency and low intensity electromagnetic field, which is safe treatment delivered in a non contact manner that prevents the chance of infection. It has been used for promoting tissue healing over the last few decades (Ieran et al., 1990, Stiller et al., 1992). PEMF was shown to promote growth factor activity and levels, and to increase the rate of nerve regeneration in animal studies (Ito and Bassett, 1983, Longo et al., 1999). In human studies, it was found to reduce pain, improve vibration sense, muscle activities and spinal cord motor neuron excitability, as well as nerve conductive dysfunction caused by diabetic polyneuropathy (DPN) (Musaev et al., 2003), which is a major risk factor for developing diabetic ulceration. Also, PEMF was shown to increase the blood flow to the nerves that ease tissue hypoxia in patients with diabetes with no side effect reported (Webb et al., 2003). Therefore, pulse electromagnetic field can potentially be an effective and safe conservative treatment in promoting healing of diabetic ulcer in clinical practice.

1.2 OBJECTIVES

The present thesis consists of three inter-related studies. The objectives of the three studies were:

1. To investigate the biomechanical properties of plantar soft tissues in terms of stiffness and thickness among different age groups of healthy subjects, and to examine the relationship of these properties with ageing.

2. To investigate the biomechanical properties of human plantar soft tissues in terms of stiffness and thickness among people with diabetic ulceration and healthy control; and the relationship between these properties and tactile sensation in people with diabetic ulceration. Specifically to monitor the change of biomechanical properties of the diabetic wound tissues over time.
3. A randomized controlled trial was conducted to examine the effectiveness of pulsed electromagnetic field energy (PEMF) in promoting healing of diabetic ulcer in terms of restoring normal biomechanical properties (i.e. stiffness) in the wound tissue, and facilitating microcirculation in the foot.

CHAPTER 2

LITERATURE REVIEW

2.1 DIABETES MELLITUS

Diabetes is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. In 2003, the American Diabetes Association (ADA) Expert Committee revised the Diagnosis and Classification of Diabetes Mellitus for the two principal types of diabetes named as 'type 1 diabetes' and 'type 2 diabetes' (Genuth et al., 2003). Type 1, formerly named insulin-dependent diabetes mellitus, is characterized by autoimmune destruction of the pancreatic beta cells, results in insulin production deficiency and requires daily administration of insulin. Type 2, formerly named non-insulin dependent diabetes mellitus, comprises of 90% of people with diabetes around the worlds results from the body's ineffective use of insulin and a relative insulin deficiency, and is more prevalent in the elderly population aged over 65 years old (Centers for Disease Control and Prevention, 2011). Unfortunately, undetected type 2 diabetes is common, with 30% to 35% of patients who are unaware of undiagnosed diabetes that may have existed for 4 to 7 years before clinical recognition or till the development of severe complications (Harris et al., 1992). Therefore, early diagnosis and comprehensive foot examination are important to prevent further diabetic complications.

Diabetes is associated with numerous complications including changes in the microvascular and macrovascular system. Hyperglycemia is the major risk factor leading to serious damage to various body structures including the nerves and blood vessels which subsequently lead to complications in heart, eyes and kidneys. Diabetic neuropathy is associated with the damage to the nerves due to reduced blood flow.

Diabetic peripheral neuropathy may occur in the lower limb that increases the chance of developing foot complications. Ulcerations, infection, gangrene and amputations are the most serious diabetic associated complications, which subsequently lead to high morbidity and mortality among people with diabetes. Risk factors that contribute to diabetic ulceration include peripheral neuropathy, vascular disease and abnormal foot biomechanics.

2.1.1 PERIPHERAL NEUROPATHY

Patients with diabetic neuropathy have a 7-fold increase risk of developing diabetic ulcers (Reiber et al., 1999). Peripheral neuropathy may affect sensory, motor and/ or autonomic nerve fibers. Sensory neuropathy may result in loss of protective sensation in pain, pressure, temperature and proprioceptive awareness in the foot. People with peripheral neuropathy who walk with barefoot may encounter foot injury during routine ambulation as they have inadequate sensation to withdraw the foot from noxious stimuli (Boulton, 2004).

It is common for diabetic motor neuropathy to affect the foot. Motor neuropathy may result in muscle atrophy, leading to altered biomechanics and foot deformities. The support at the foot arch diminishes during weight bearing and the plantar fat pads move distally, leaving the metatarsal heads riding on the skin without padding effect (Bus et al., 2002). Eventually, ulceration occurs due to the callosities formation or weight bear skin being ruptured.

Diabetic neuropathy also leads to autonomic dysfunction that causes abnormal blood flow. Impaired sweat glands makes the foot dry and stiff, the fissure and crack provide a portal for infection (Brem et al., 2006). It alters the arteriovenous shunting and thermoregulatory function, and subsequently affects the prognosis of ulceration (Nabuurs-Franssen et al., 2002). The combination of sensory and motor neuropathy could cause abnormal stresses on the foot, and impair the microvascular circulation that contributes to the development of foot ulceration in the high pressure distribution region (Brem et al., 2006).

2.1.2 MICROVASCULAR DYSFUNCTION

Peripheral arterial occlusive disease plays a major role in non-healing diabetic foot ulcers, and it is a significant risk factor for amputation (Jeffcoate, 2005). It decreases the blood flow and disturbs microvascular and macrovascular circulation (Brem et al., 2006, Nazimek-Siewniak et al., 2002), and the oxygenation in the wound becomes impaired.

Besides peripheral arterial diseases, hyperglycemia may cause structural adaptation and remodeling that may lead to apparent capillary failure. A recent review stated that diminished capillary size and decrease microvascular blood flow both contribute to diabetic ulceration (Chao and Cheing, 2009). This thickening of the capillary basement membrane caused by the increased blood glucose level is common. It leads to reduced capillary exchange properties and reduced arteriolar vasoreaction followed by reduced capillary recruitment. The microvascular system of patients with diabetes fails to respond appropriately to stress and injury.

Considerable evidence indicates that abnormalities in the microvasculature contribute to nerve hypoxia (Malik et al., 2006). As a consequence, the blood supply to the sympathetic nerves controlling the arteriovenous shunts may become insufficient. This shunting increase venous pressure and consequently reduces the arteriovenous pressure over capillaries, which leads to a further reduction in nutritive capillary flow. It is common to have skin capillary circulation severely impaired in toes of patients with diabetes, who may or may not have other late diabetic complications. The blood bypasses the surface of the skin and causes a lack of integrity to the skin, subsequently, the skin becomes dry, and the cracks create a portal for the bacteria to grow.

2.2 BIOMECHANICAL PROPERTIES OF PLANTAR SOFT TISSUE

2.2.1 CHANGES IN BIOMECHANICAL PROPERTIES OF PLANTAR SOFT TISSUE AMONG ELDERLY PEOPLE

With the normal ageing process, various body systems are generally affected including the musculoskeletal system. The structure of the foot changes with advancing age. There is a reduction in the range of motion, tactile sensation, and strength of the foot, and a higher prevalence of foot deformities (Menz and Lord, 1999). The load-carrying ability under the plantar foot becomes impaired (Gefen, 2003). Musculoskeletal problems associated with ageing are further compounded by foot deformity. 50% of old aged people found with at least one foot deformity (Hung et al., 1985). In addition, compensation for these deformities might be responsible for accelerated wear and tear

on the joints (Whitney, 2003). These structural and functional changes in the foot may contribute to further complications in the elderly population.

Biomechanical examination of the plantar soft tissues provides a better understanding to the mechanics responsible for foot disorders in older people. Also, ageing effect could be one of the confounding factors that explain for the delay in diabetic wound healing.

In Chapter 4, a study done to examine the effects of ageing on biomechanical properties of plantar soft tissues was reported. In Chapter 5, the biomechanical properties of plantar soft tissue in the age-matched subjects with diabetic ulcers were investigated.

2.2.2 BIOMECHANICAL PROPERTIES OF PLANTAR SOFT TISSUE IN PEOPLE WITH DIABETES MELLITUS

Diabetes-related peripheral polyneuropathy results in both sensory and motor dysfunction, and subsequently alter the biomechanical properties of plantar soft tissue. It is common to have ulcers found at the sites of high pressure points of the foot including the big toe, plantar aspect of the metatarsal heads and the heel (Garcia et al., 2008, Singh et al., 2005). The support at foot arch diminishes during weight bearing and the plantar fat pads migrate distally, leaving the metatarsal heads riding on the skin without padding effect. The callosities formed or weight bear skin being ruptured, hence leads to ulceration. A study showed that the soft tissue thickness at the metatarsal heads contributes to the high plantar pressure (Cavanagh et al., 1997), in which the high content of non-enzymatic cross link in the plantar skin causes the diabetic feet to be

stiffened. The soft tissue stiffening under the metatarsal heads may account for the common location of foot ulcers reported in people with diabetes (Gefen, 2003).

The non-enzymatic glycosylation has been shown to affect structural and biomechanical properties of the plantar soft tissues in patient with diabetes, by limiting the range of motion in the joints of the foot and ankle, particularly at the first metatarsal head (Fernando et al., 1991). The heel and metatarsal head regions of the human foot are specially designed to provide cushioning and shock absorption to the underlying bone during all weight bearing tasks (Callaghan et al., 2008, Scott et al., 2007). The excessive callus formation at these peak pressure points might lead to irregular interaction of shear and compressive stresses at the skin surface that make tissue damage in patient with diabetes (Murray et al., 1996).

In Chapter 5, a study was conducted to investigate the correlation between the biomechanical properties of plantar soft tissues and tactile sensation for patients with diabetic ulceration. Specifically, the change of biomechanical properties of the diabetic wound tissues over time was monitored by a case study.

2.2.3 INSTRUMENT FOR ASSESSING BIOMECHANICAL PROPERTIES OF PLANTAR SOFT TISSUE

Careful monitoring of diabetic ulcer is essential to prevent lower limb amputation. In the last decade, there is a lack of quantitative outcome measure for assessing diabetic ulcer especially on the biomechanical properties. Hsu et al. commented that due to the

methodological weakness, the studies in the biomechanical testing over the foot showed contradictory results (Hsu et al., 1998). Ultrasonography allows a real time examination during the loading-unloading cycle, which provides direct visualization on the biomechanical properties of the foot. An ultrasound indentation system was developed, with a previous study showing a significant stiffer and thinner plantar soft tissues in elderly diabetic patients and healthy young subjects (Zheng et al., 2000). However, the effects of diabetes could not be distinguished in the study. As the sample size is small, and the healthy group and the diabetic group were not age-matched, further investigation on the effect of ageing and disease is needed.

Typical ultrasound indentation testing requires direct contact of the rigid transducer to the target tissue. For the assessment on intact skin in elderly and patients with diabetes, it is a safe assessment tool that provides real time information on the stiffness and thickness of plantar soft tissue, thus we incorporated this instrument as an assessment tool of our study. However, if the assessment is to be performed on wound tissue, this direct contact method may damage the delicate wound bed tissue during the indentation process or there is a potential chance of causing infection due to contamination. The deficiencies led us to explore the use of air jet optical indentation system that involves a non-contact measurement. This newly developed technology provides a quantitative and reliable monitoring on the biomechanical tissue property of diabetic ulcer during healing. A high value for test/retest reliability was demonstrated (ICC: 0.986; Pearson's correlation: r=0.972, $p<0.001$) by our research team (Chao et al., 2011), and were found highly comparable to the measurement made by direct contact methods including

conventional mechanical testing (r=0.89, $p<0.001$) (Huang et al., 2009) and Tissue Ultrasound Palpation System (TUPS) (Chao et al., 2010).

2.3 DIABETIC ULCERS

Diabetic foot ulcer is defined as any skin breakdown below the ankle of a patient with diabetes (Apelqvist et al., 2000). Foot deformity and neuropathy are common predisposing factors for inducing skin breakdown in persons with diabetes. Diabetic ulcer usually begins with minor skin lesion. This is particularly true for people with severe diabetes neuropathy are not aware of acute mechanical trauma or thermal trauma from foot baths or heating pads. These minor traumas can also destroy the skin in the neuropathic foot. Peripheral neuropathy and peripheral arterial disease commonly coexist in patients with diabetic foot ulcers (Kumar et al., 1994). If the microcirculation is inadequate to deliver the hyperemic response needed for healing, the minor wound can result in chronic ulcer, and gangrene may follow in severe cases. Besides, concentration of weight bearing force or excessive pressure under the metatarsals heads may lead to callus formation or ulceration. The most common sites of foot ulcers were the toes, followed by the plantar metatarsal heads and the heel (Reiber et al., 1998). Repetitive injury due to the insensitivity of the diabetic neuropathy, low degree of tissue oxygenation due to vascular disease and hyperglycemia, and ageing effects are factors that makes the wound difficult to heal and turn into chronic ulcer.

Clinical management of diabetic foot ulcers result in huge costs for both the society and patients. The economical burden is enormous especially for those with lower limb

amputation and prolonged hospitalization. The estimated annual management cost of diabetic foot problem reaches 116 billion per year in United States (Driver et al., 2010). Evidence based management and prevention in diabetic ulcer is essential to reduce the incidence and morbidity rate.

2.3.1 STAGES IN WOUND HEALING

Normal wound healing process is divided into three stages that are partially overlapped, namely inflammation stage, tissue formation stage and tissue remodeling stage.

2.3.1.1 Inflammation stage

The inflammation stage begins immediately upon injury. The injured blood and lymphatic vessels undergo rapid vasoconstriction to prevent leakage of blood. Later on, ruptured cells release inflammatory cytokines that initiate vasodilation with increased capillary permeability, and thus facilitate the entry of macrophages and neutrophils into the wound site from the bloodstream (Dinh and Veves, 2005). These cells act against pathogenic organisms and debride necrotic tissue, protecting the wound against infection, as well as coagulation, angiogenesis and secreting various growth factors that activate tissue formation stage (Shai and Maibach, 2005, Dinh and Veves, 2005).

2.3.1.2 Tissue formation stage

The main processes that take place in this stage are angiogenesis and granulation tissue formation, re-epithelialization, and extracellular matrix formation. Collagen type III and ground substance are embedded, fibroblasts then migrate to the wound bed. The

migration of the fibroblasts is dependent on the peripheral innervations in the wound. Re-epithelialization and proliferation then follow (Shai and Maibach, 2005).

2.3.1.3 Tissue remodeling stage

Collagen type III is now replaced by the more stable collagen type I, and are arranged in a desired alignment, leading to the formation of new scar. This would gradually increase the strength of the healing wound. However, the healed wound tissue might not be able to regain the original strength, and prone to a higher risk of re-injury (Shai and Maibach, 2005).

2.3.2 QUANTITATIVE ASSESSMENT FOR DIABETIC ULCERS

An objective and accurate assessment provides information on baseline data that can assist in monitoring the process of wound healing (Keast et al., 2004). The current clinical measurement for ulcer is subjective, and has poor inter-rater or intra-rater reliability (Defloor et al., 2006). Goldman and Salcido (2002) commented that there is a lack of sophisticated methods to provide quantitative assessment of the wound healing condition including physiological changes of skin morphology, tissue elasticity and vascular perfusion (Goldman and Salcido, 2002).

Accurate and precise area measurement is a crucial part of wound assessment and predicting the healing time (Rodeheaver and Stotts, 1995). Measuring method making use of ruler and acetate tracing plus manual square counting method are the most commonly used clinical method for wound size measurement. However, these

techniques were found inconsistent and not reliable especially with decreasing wound size (Thawer et al., 2002). These also carry a chance of wound contamination as the acetate tracing sheet sticks directly onto the wound during measurement. The use of non contact computer wound measurement software is preferable because it eliminates the risk of wound infection and the possibility of procedure-related damage to the wound (Williams, 2000).

Ankle-Brachial Index (ABI) is a common clinical vascular test to check for peripheral arterial occlusive disease. The pressure cuff is placed on the upper arm and inflated until no brachial pulse is detected by the Doppler device. The cuff is then slowly deflated until a Doppler-detected pulse returns (the systolic pressure). The whole process is repeated on the leg. The ankle systolic pressure divided by the brachial systolic pressure gives the Ankle-Brachial Index. However, this test has been faulted for underestimating the severity of arterial insufficiency, especially in people with Diabetes Mellitus where blood vessels are non-compressible (Armstrong and Lavery, 1998).

Mechanical stresses at the wound site are thought to guide the collagen fibrillogenesis, control contractile activity of the granulation tissues and myofibroblast differentiation. Any altered tension during wound closure will affect healing progress (Farahani and Kloth, 2008, Hinz et al., 2001). The elasticity of the wound tissue might reflect the extent of tissue organization, and thus provide information on the wound healing progress. The noncontact air jet indentation system allows a quantitative assessment on the mechanical properties of the soft tissue, and to monitor the effectiveness of the treatment (Chao et al., 2011).

2.3.3 CLINICAL MANAGEMENT FOR DIABETIC ULCERS

Non healing wounds could be stuck at the inflammation stage, by increasing cytokine response and impaired growth factor activity (Tarnuzzer and Schultz, 1996). Collagen synthesis in the patients with diabetes is markedly decreased, due to the collagen peptide production and posttranslational modification of collagen degradation dysfunction (Dinh and Veves, 2005). These impairments of the cellular mediators during the wound healing process cause a delayed wound healing or unhealed at all. The primary treatment goals for diabetic foot ulcers are to resolve the wound and to lower the recurrence rate. Prolonged inflammation and failure to re-epithelialize could make the chronic wound further breakdown and get infected.

Clinicians explore various therapeutic options to accelerate the healing of diabetic foot ulcers including drug therapy, non-surgical ulcer care or surgery. A non-surgical treatment approach, like hyperbaric oxygen and offloading is usually recommended for neuropathic ulceration (Millington and Norris, 2000) while surgical revascularization is suggested for selected cases of ischemic ulceration (Brem et al., 2006). Failure of non-surgical or surgical treatment will end up with performing amputations. Various new types of dressings such as calcium alginates and collagen dressings have also been used, unfortunately, even with the best available wound care procedures, chronic diabetic wounds tend to heal very slowly, or unheal over months or even years. This is particularly true for patients who have history of previous ulceration or amputation, they are at a higher risk of developing ulceration, infection and subsequent lower limb amputation (Armstrong and Lavery, 1998).

2.3.4 ELECTROPHYSICAL THERAPY FOR DIABETIC ULCERS: A SYSTEMATIC REVIEW

Electrophysical therapy consists of a variety of physical modalities used by physical therapists ranging from electrical stimulation to the use of sound waves (ultrasound) and light (laser) to electromagnetic energy (Pope et al., 1995, Watson, 2000). These modalities have been used to promote tissue repair, to enhance fibroblast activity (Webb and Dyson, 2003) and angiogenesis (Goldman et al., 2004). However, the findings of these studies were inconclusive, and the clinical use of electrophysical modalities on promoting diabetic wound healing is still under investigation. Many of the conclusions from the earlier published works were in vitro (Aaron et al., 2004, Cho et al., 2000, Dalecki, 2004, Merriman et al., 2004), from animal studies (Gal et al., 2009, Stadler et al., 2001) or on wounds caused by venous insufficiency and pressure sores (Alster and Nanni, 1998, Gardner et al., 1999, Hopkins et al., 2004, Lundeberg and Malm, 1991, Lundeberg et al., 1990, Maeshige et al., 2010, Peters et al., 2001, Stiller et al., 1992). Research on electrophysical therapy has mostly been done on animal wounds consisting of surgically excised skin. These experimental wounds excluded common problems associated with delays in healing such as ischemia, infection, necrotic debris, or sinus formation. Therefore, these animal wound models may not be ideal for studying the effect of electrophysical therapy on human diabetic ulcer healing (Sullivan et al., 2001) The choice of treatment parameters and the dosage of various electrophysical therapies for managing various human wounds are still uncertain.

Thus far, there have been no published reviews or meta-analyses evaluating the efficacy of electrophysical modalities in the treatment of diabetic ulcers, whether sufficient proof has been found in human diabetic ulcers are still unknown. Therefore, a systematic review on this topic was conducted to examine the current evidence of the use of electrophysical therapies on promoting healing of diabetic ulcer. The full report of the systematic review in a manuscript format is shown in Appendix V. After performing a systematic search, seven clinical trials fulfilled the search criteria and were identified. There were four trials on electrical stimulation (Baker et al., 1997, Isakov et al., 1996, Peters et al., 2001, Petrofsky et al., 2007), one trial on ultrasound (Ennis et al., 2005), one trial on electromagnetic fields (Isakov et al., 1996), and one trial on phototherapy (Minatel et al., 2009). The three studies on electrical stimulation included in the meta-analysis are either underpowered or methodologically weak, and only a few studies were conducted on other electrophysical modalities such as ultrasound, and lasers. Nevertheless, the positive effects of electrical stimulation, LASERS, ultrasound and electromagnetic therapy interventions are sufficient to encourage high-quality randomized controlled trial with larger sample sizes in these areas.

2.3.4.1 Ultrasound

Therapeutic ultrasound has been widely used to manage many musculoskeletal conditions in clinical settings, but with conflicting results. The clinical use of ultrasound to promote wound healing is still under investigation. The possible mechanism of ultrasound in promoting tissue repair is likely due to its mechanical effect, with

micromassage changing the permeability of membranes and stimulating the proliferation of fibroblasts (Dalecki, 2004).

Ennis et al. (2006) reported significant improvements in the proportion of wounds healed and the time required to heal with ultrasound treatment, and the effects were also considered strong by a modified Jadad scale. The researchers compared the efficacy of a 40 KHz ultrasound device with a sham device that delivered a saline mist without producing ultrasound for managing diabetic ulcers. Both the experimental group and sham group showed improvement from pre-treatment to post-treatment in the proportion of wounds healed, with the healing of the treatment group differing significantly from that of the control group. In addition, the experimental group healed significantly more quickly than did the sham group.

2.3.4.2 Phototherapy

Laser treatment has been used for managing venous ulcers (Lundeberg and Malm, 1991), open wounds (Hopkins et al., 2004), and burns (Alster and Nanni, 1998). It has been considered a non-invasive treatment with no reported side effects. However, there is still little agreement on protocol on wound healing.

Minatel et al. (2009) performed a study that examined the efficacy of phototherapy, with a combination of 660 and 890nm lights, in promoting the healing of chronic diabetic ulcers as compared to a sham group (Minatel et al., 2009). The mean ulcer granulation and healing rates were significantly higher for the experimental group than the sham

group throughout the course of the treatment. The author concluded that the combination of 660 and 890nm lights promotes tissue granulation and the rapid healing of diabetic ulcers.

2.3.4.3 Electrical stimulation

Electrical stimulation has been used to treat diabetic ulcers that have not responded to standard wound treatments (Baker et al., 1997, Lundeberg et al., 1992, Peters et al., 2001, Petrofsky et al., 2007). The studies by Lunderberg and co-workers and Peter and collaborators, investigated the effect of electrical stimulation on diabetic ulcers and trial quality was high assessed by the modified Jadad scale (Lundeberg et al., 1992, Peters et al., 2001). The waveform used in the four trials included symmetrical biphasic, monophasic, or square-wave pulse (Baker et al., 1997, Lundeberg et al., 1992, Peters et al., 2001, Petrofsky et al., 2007). The frequency used was 8Hz, 10Hz, 30Hz, 50Hz, and 300Hz, respectively. However, in the study by Baker et al., the control group refers to subjects either receiving no stimulation or low-level stimulation, whereas we cannot rule out the effect of low-level stimulation (Baker et al., 1997). Three out of four trials found significance between-group differences in healing rates, with the experimental groups in all of these studies shown to be more favorable than the control groups (Baker et al., 1997, Lundeberg et al., 1992, Petrofsky et al., 2007). The exception was the trial reported by Peters and collaborators, which showed no significance between-group differences in healing rates (Peters et al., 2001).

The results of our meta-analysis support the efficacy of electrical stimulation on the healing of diabetic ulcers in human subjects (Baker et al., 1997, Lundeberg et al., 1992, Petrofsky et al., 2007). However, as a high-quality study (Peters et al., 2001) was excluded from the meta-analysis and a beneficial effect was not demonstrated in this study, this exclusion may have some influence on the final results.

However, there is still a need for additional evidence to support the use of electrophysical therapy for promoting the healing of diabetic ulcers because current studies are few and tend to have small sample sizes.

Based on the systematic review done (detail referred in Appendix V), the evidence of the clinical effectiveness of electromagnetic therapy in managing of diabetic ulcers is low, indeed the use of electromagnetic therapy does not elicit any complications from direct contact with the electrodes that are adopted by other electrophysical modalities. Previous studies showed that PEMF is an effective treatment in promoting nerve regeneration (Musaev et al., 2003) and improving microcirculation (Webb et al., 2003) in patients with diabetes, which is a major risk factor for developing diabetic ulceration. The PEMF could also enhance the transcutaneous partial pressure of oxygen measurements and Doppler flow in patients with diabetes without complications (Webb et al., 2003). Hence PEMF might be a potential modality to restore microcirculatory dysfunction and biomechanical properties changes associated with diabetic ulcers.

2.3.5 PULSED ELECTROMAGNETIC FIELD FOR MANAGING DIABETIC ULCERS

The use of physical modality is an alternative treatment approach for promoting healing of diabetic ulcer, limited clinical researches have been done. Among all the modalities, the more popular one is electrical stimulation that requires the placement of electrodes directly on the wound bed, and this increases the chance of contamination of the wound.

Pulsed electromagnetic field (PEMF) is an athermal physical modality that has been used to treat orthopaedic and related conditions since 1979. Electromagnetic waves could travel in space without the need of a propagation medium, and thus could be delivered to the target tissue in a non contact way.

Recent animal studies demonstrated that PEMF could elicit arteriolar vasodilation (Callaghan et al., 2008) and increased blood flow velocity (Xu et al., 2001). Histological examination showed that PEMF could stimulate the growth of capillaries, fibroblasts, and connective tissue at the inflammation stage of normal wound healing (Callaghan et al., 2008). Besides, it increases the collagen fibers and enhance the re-epithelialization at the later stage of healing (Athanasiou et al., 2007). PEMF is also able to promote wound closure and prevents necrosis in diabetic mice (Callaghan et al., 2008, Patino et al., 1996). It has been shown to accelerate wound healing in mice under diabetic and normal conditions by up-regulation of fibroblast growth factor FGF-2 mediated angiogenesis, and prevented tissue necrosis in response to a standardized ischemic insult, suggesting that non-invasive angiogenic stimulation by PEMF may be useful to prevent ulcer

formation, necrosis, and amputation in diabetic patients (Callaghan et al., 2008, Tepper et al., 2004). Research evidences have shown that PEMF can also enhance the healing of venous ulcer (Kenkre et al., 1996, Ravaghi et al., 2006), but limited research has been done on managing human neuropathic ulcer. Only one small scale clinical trial that recruited diabetic patients with diabetic stump wound was identified. Isakov et al. (1996) examined the efficacy of using electromagnetic stimulation to manage stump wounds for diabetic amputees as compared with a conservative treatment (Isakov et al., 1996). This study was considered to be methodologically strong by modified Jadad scale. There was no significant mean difference between the estimated and actual healing times, and no significant improvement in the mean healing time with the experimental groups. Although no significant improvement was found in the mean healing time, the etiology and pathology behind stump wound is different from diabetic wound commonly due to neuropathic or ischemic cause. Also, the use of electromagnetic therapy does not elicit any complications from direct contact with the electrodes that are adopted by other electrophysical modalities. Indeed, electromagnetic therapy can be applied in the presence of casts or wound dressings, with a low risk of infection. As there is only one study on electromagnetic therapy included in this review, further research is warranted before dismissing any beneficial effects from this therapy.

At present, there is insufficient clinical evidence from randomized controlled trials to support the use of electromagnetic therapy in managing diabetic ulcer. As PEMF has been shown to improve the healing in venous ulcers, as well as promoting nerve regeneration and circulation in animal studies, we hypothesize that PEMF could enhance

the healing of diabetic ulcer in human. Further research is needed to investigate the beneficial effects from the pulsed electromagnetic therapy for managing diabetic ulcers.

CHAPTER 3

INSTRUMENTATION

This chapter describes the instrumentation and treatment modalities used in the studies of this thesis. Semmes-Weinstein monofilaments were used to detect the sensation of the feet; laser Doppler flowmetry and video capillaroscopy were used to examine the microcirculation; tissue ultrasound palpation system was used to examine the biomechanical properties of the plantar soft tissues via a direct contact method; optical coherence tomography based non-contact air jet compression system was used to measure the biomechanical properties of diabetic ulcer in a non contact method. In addition, ultrasound biomicroscopy and clinical assessment were used to monitor the diabetic ulcer healing.

All assessments for the 3 inter-related studies reported in this thesis were conducted in the same investigation room throughout the study period. The room temperature was kept at 24±1°C, the relative humidity maintained at around 40% to 60%.

3.1 SENSORY EXAMINATION

3.1.1 SEMMES-WEINSTEIN MONOFILAMENTS

Tactile sensation was assessed with the nylon Semmes-Weinstein monofilaments (Rolyan* Monofilaments, Smith & Nephew, Inc., Germantown, USA). A set of 20 monofilaments were calibrated to deliver different ratings from 1.65 to 6.65, which represents the decimal log of 10 times of the linear bowing force (Table 3.1). There is international consensus that monofilament is advocated as a screening test for sensory neuropathy in diabetic foot, which is an important part of the practical guidelines on the

management and the prevention of the diabetic ulcer and amputation (Apelqvist et al., 2000). These 4.17, 5.07 and 6.10 filaments produced a 100% Sensitivity and 77.7% Specificity in screening patients who are at risk of diabetic foot ulcer (Kumar et al., 1991).

Table 3.1 The Semmes-Weinstein Monofilament ratings (Tanenberg and Donofrio, 2008)

Colour code	Rod marking	Target Force (grams)	Plantar thresholds
Green	A 1.65	0.008	Normal
	B 2.36	0.02	
	C 2.44	0.04	
	D 2.83	0.07	
Blue	E 3.22	0.16	
	F 3.61	0.4	
Purple	G 3.84	0.6	
	H 4.08	1	Diminished
	I 4.17	1.4	light touch
	J 4.31	2	
Red	K 4.56	4	Diminished
	L 4.74	6	protective
	M 4.93	8	sensation
	N 5.07	10	
	O 5.18	15	Loss of
	P 5.46	26	protective
	Q 5.88	60	sensation

	R 6.10	100	
	S 6.45	180	
	T 6.65	300	Deep pressure sensation only

The subjects were requested to lie in a supine position. The monofilaments were applied perpendicularly to the skin until a first-order buckling occurred, and the threshold was determined by a subject's verbal response to say 'yes' each time he or she senses the application Three trials were taken at each measurement site, alternately with one sham application in which no filament is applied (Apelqvist et al., 2000). When the patient was unable to respond correctly in more than one trial, a heavier monofilament was taken.

3.2 MICROCIRCULATION

All subjects were acclimatized for at least 30 min before the investigation on microcirculation started.

3.2.1 VIDEO CAPILLAROSCOPY

A number of optical and non-invasive techniques can provide information about blood flow. The most direct method is capillary microscopy, where the capillaries in the nail fold, oriented parallel to the skin surface, are studied individually. The blood velocity can be estimated by inspecting the spatial dislocation of the red blood cell frame by

frame. Another variant of capillary microscopy studies the number of active (visible) capillary loops in a certain area.

The microcirculation of the capillaries over the big toe nail fold was examined by video capillaroscopy system (CapiScope, Version 3.33.0.0, KK Technology, Devon, England). The system equipped with an x6.3 magnification microscope objective (KK Technology, Devon, England) and a1/2" imaging sensor (KK Technology, Devon, England), which provided an approximately x200 image on a 14-inch computer monitor. The subjects were positioned in supine position and were told to relax during the entire study. The lower leg and ankle was supported by a tailor-made stainless-steel foot stand padded with towels, so that the subject could maintain this posture throughout the assessment. A thin layer of paraffin oil layer was applied on the nail fold to improve the optical properties of the skin. The microscope was attached to a heavy stand, minor adjustment on three dimensions were available. Two recordings of one minute flow were made for each measurement. Capillaries with good optical signals of visible erythrocyte aggregates and plasma gaps were chosen for investigation (Pangratis, 1997). Capillary blood velocity was measured by frame to frame technique. The diameters and mean capillary blood flow was determined for the three selected capillaries (Figure 3.1). Video capillaroscopy is a reliable tool assessing the localized blood cell flow through a direct view of the capillary loops of the nailfold parallel to the surface of the skin (coefficient of variation=4.2%) (Netten et al., 1996).

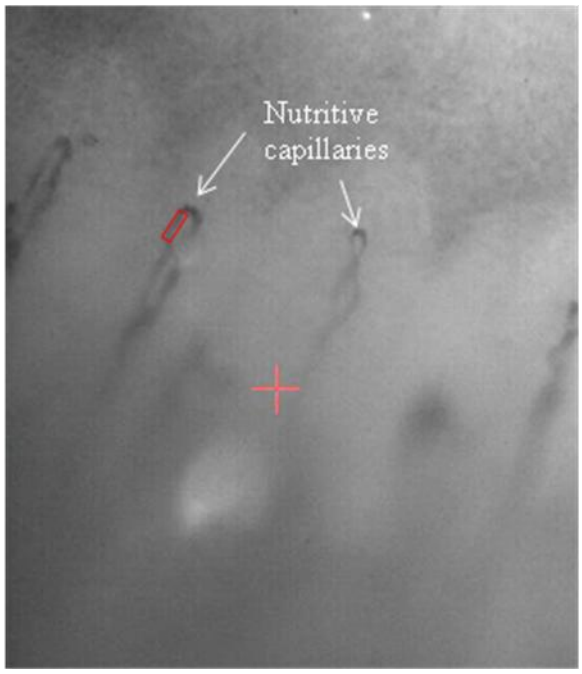


Figure 3.1 Capillaries at big toe nailfold taken by Video Capillaroscopy. Velocity of the nutritive capillaries is measured by putting the red line along the vessel. The grey level profile along the line is taken for each field once every 1/50 of the second. The grey level pattern along each line is compared to the pattern from the next field. The comparison is performed by calculating the correlation coefficient for every possible shift of the previous grey level profile relative to the new profile.

3.2.2 LASER DOPPLER FLOWMETRY

Laser Doppler flowmetry (LDF) is a non-invasive technique that permits continuous measurement of microcirculatory blood flow in living tissue. It measures the flow associated with nutritional blood capillaries close to the skin surface and the underlying

arterioles, venules and arteriovenous shunts that are involved in regulation of skin temperature (Figure 3.2).

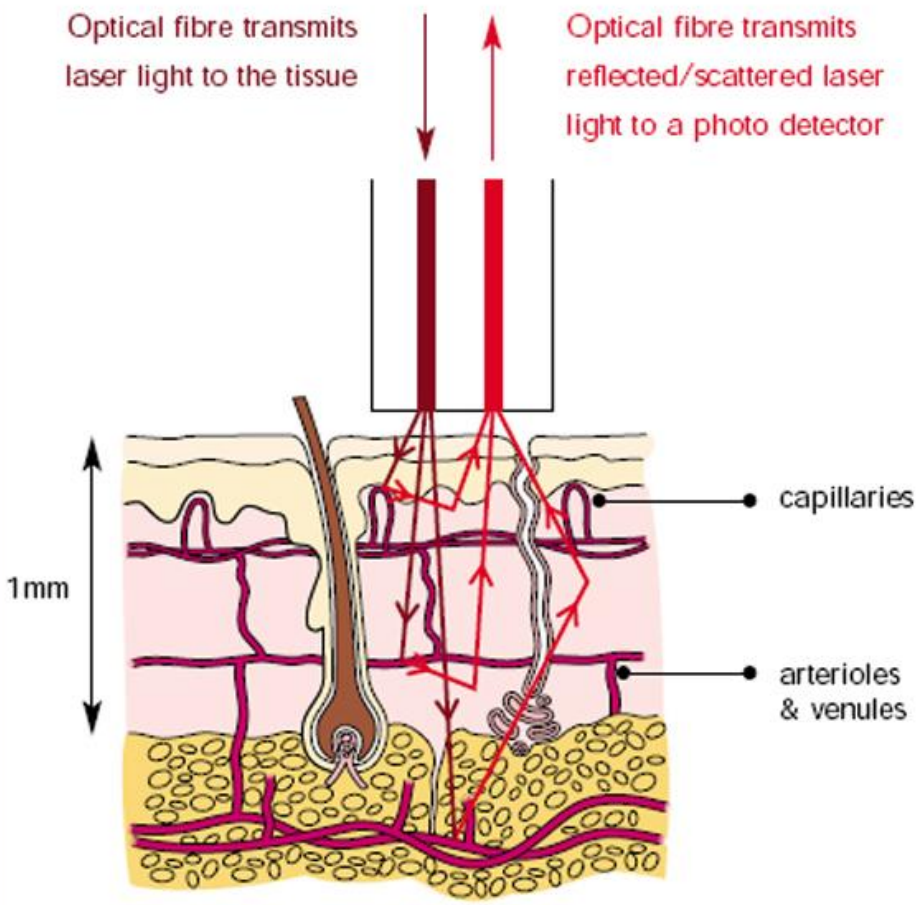


Figure 3.2 The use of laser Doppler flowmetry in assessing the microcirculation in the skin, in which the flux represents the moving particles inside the capillaries, arterioles, venules, as well as the arteriovenous shunts.

The principle of this method is to measure the Doppler shift – the frequency change that light undergoes when reflected by moving scatters, such as red blood cells. Optical fibers are used to guide light from the laser to the probe attached to the skin. The backscattered and Doppler broadened light is subsequently picked up by the same probe and brought back to the photodetector unit in the instrument. An output signal that scales linearly with the perfusion in the tissue is extracted by processing the Doppler signal and is fed to the display for continuous tracking of the perfusion.

The term commonly used to describe blood flow measured by the laser Doppler technique is 'flux', a quantity proportional to the product of the average speed of the blood cells and their number concentration. This is expressed in arbitrary 'perfusion units'. Some studies indicate increased perfusion/oxygen saturation at the site of an ulcer can lead to improved healing (Newton et al., 2002), and the control of inflammation can aid diabetic ulcer healing rate (Lau et al., 2009). Monitoring of the microcirculation of wounds is used in numerous wound healing studies (Newton et al., 2002) and is of significant importance.

The Laser Doppler Flowmetry (DRT 4, Moor Instruments Ltd, Devon, England) was used to assess the skin blood flow (flux) of microcirculation at the pulp and the nail fold of the big toe. This laser Doppler unit consists of a control box and two optical probes. A piece of optical fibre 100 mm in length connects each probe to the control box. A laser aperture and light sensor (with a distance of 0.25 mm between them) were fixed at the end of each probe. During assessment, the patients were placed in a comfortable supine position, and the probe of LDF was directly adhered to the targeted location by

using a double-sided adhesive disc. This equipment produced a laser light with a 785 nm wavelength, and the power output from this laser aperture was set at approximately 0.5 to 1.5 micro-Watts. The data acquisition rate was set at 40 Hz (40 data points per second), with an integration time of 0.1 second. Data were obtained in a wave format, which was translated automatically by computer software into an average value of flux.

3.3 BIOMECHANICAL PROPERTIES OF PLANTAR SOFT TISSUE

3.3.1 TISSUE ULTRASOUND PALPATION SYSTEM: A DIRECT CONTACT METHOD

The tissue ultrasound palpation system (TUPS) comprised of a pen size, hand-held indentation probe that was used to investigate the thickness and the Young's modulus of the plantar soft tissue (Zheng et al., 1999, Zheng and Mak, 1996). A 5 MHz and 9 mm ultrasound transducer was installed at the tip of the probe, and served as the indenter. A 10-N compressive load cell was connected in a series with the ultrasound transducer to record the corresponding force response. The thickness and indentation depth of the soft tissue layer were determined from the time of flight of the ultrasound echo signal that reflected from the soft tissue-bone interface. The load signal and the ultrasound signal were both digitized and collected by a computer, and shown in real-time with a program (Figure 3.3).

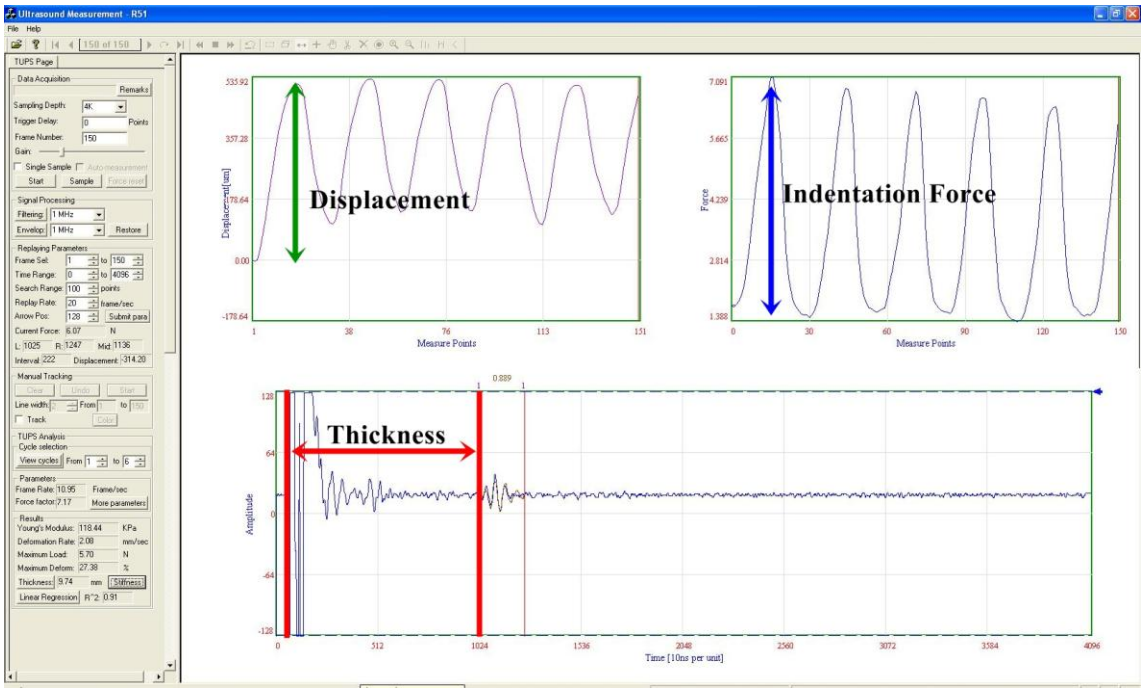


Figure 3.3 The software interface of TUPS. The window allows tracking of the ultrasound echo, thus displacement and the force applied on the soft tissue could be obtained for plotting the load-deformation curve.

The deflection of the ultrasound echo due to the deformation of soft tissue was determined using a cross-correlation technique. The effective Young's modulus was calculated by the computer program that showed the stiffness or elasticity of the plantar soft tissue by the following equation:

$$E=\frac{(1-\nu^2)}{2a\kappa(\nu, a/h)}\frac{P}{w'}$$

Where *h* is the tissue thickness, *a* is the indentor radius, *v* is the Poisson's ratio, and κ is a scaling factor. The ratio *P/w* was determined by the linear regression of the load-indentation responses.

Each subject was placed in a supine position with the ankle maintained in a neutral position and the knee in a straight position. The lower leg and ankle was supported with towels and a foot stand, so that the subject could maintain this posture throughout the assessment. The plantar soft tissues covering the big toe, first metatarsal head, third metatarsal head, fifth metatarsal head, and the heel of the right foot were palpated and marked. A water-soluble acoustic gel was applied on the testing site to minimize the reflective loss of ultrasound. The plantar soft tissue was preconditioned by loading and unloading the probe on the testing site for a few times. The probe was then held with minimal force in a suitable alignment to obtain the maximum ultrasound echo signal. The load was then gradually applied manually and repeated for 5 cycles in each trial at a controlled indentation rate of 1-2 mm/s. The maximum indentation depth was kept to within 10% of the initial thickness (Zheng et al., 2000). The above trial was repeated twice at each testing site. The mean of the two trials was taken for a subsequent analysis.

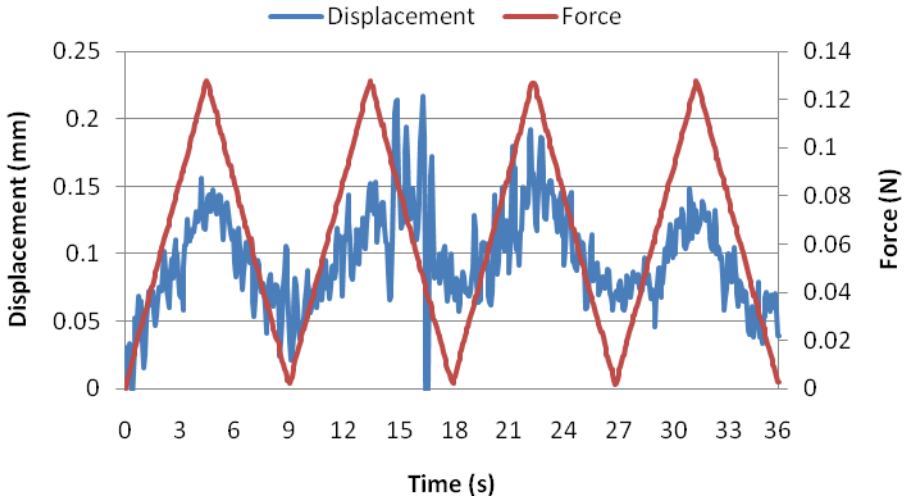
3.3.2 OPTICAL COHERENCE TOMOGRAPHY (OCT) BASED AIR JET INDENTATION SYSTEM: A NON CONTACT EVALUATION METHOD

Typical ultrasound indentation testing requires direct contact of the rigid transducer to the target tissue. In this case, the wound tissue might be disrupted during the indentation process or there is a potential chance of causing infection. To address these issues, the

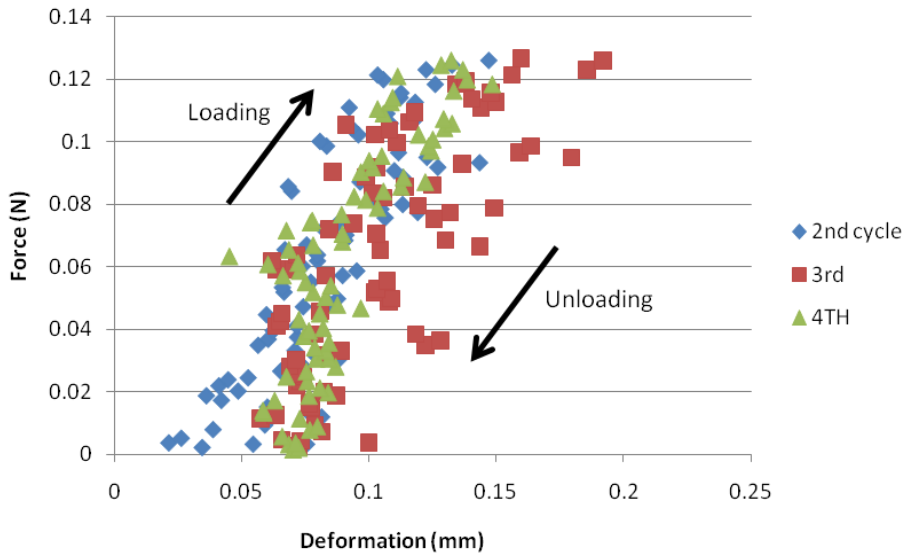
stiffness of the wound tissue was assessed by the optical coherence tomography (OCT) based air jet indentation system, which was developed by our research collaborators (Huang et al., 2009). The validity of the instrument was demonstrated in the silicon tissue-mimicking phantoms (Huang et al., 2009), as well as the healthy young subjects and elderly (Chao et al., 2010). And the test/retest reliability was demonstrated in the patients with diabetic foot ulcer (Chao et al., 2011). The probe consisted of a time domain OCT system (Lab of Optical Imaging and Sensing, Graduate School at Shenzhen, Tsinghua University, China) and an air jet bubbler that contains a super luminescent diode light source (Dense Light, DL-CS3055 A, Singapore) with a central wavelength of 1310nm, a nominal -3dB spectral bandwidth of 50nm, and a nominal output power of 5mW. This provides an axial resolution of 18 μm and an imaging depth of approximately 2-3mm in highly scattered materials. And electronic proportional valve with pressure feedback (ITV 1030-311L-Q, SMC Corporation, Toyko, Japan) at a measurement range of 0.5MPa was installed before the bubbler to continuously control and monitor the pressure of the air jet by voltage control. A visible red light beam was used together with the invisible infrared beam in order to guide the target point. The 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 1mm. A pipeline with maximally constant air pressure was connected to the system to provide the air jet for the indentation. 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 anterioposterior 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

assessment. During the indentation process, the signals from the OCT and the pressure sensor were displayed in real time by the computer software as same as the TUPS interface. Four cycles of loading and unloading with a total duration of approximately 36 seconds at an indentation rate of around 0.08mm/s was carried out for each indentation trial loading cycle (Figure 3.4a). The maximum indentation force used was 0.14N and it gave a maximum displacement of about 0.35mm. The stiffness coefficient *k* (N/mm), that is the slope of the force-deformation relationship, was then calculated from each trial, and it was used to represent the biomechanical properties of the wound tissue (Figure 3.4b). Only the loading phase at the second to fourth cycle was utilized to calculate the stiffness coefficient (Figure 3.4c).

(a)



(b)



(c)

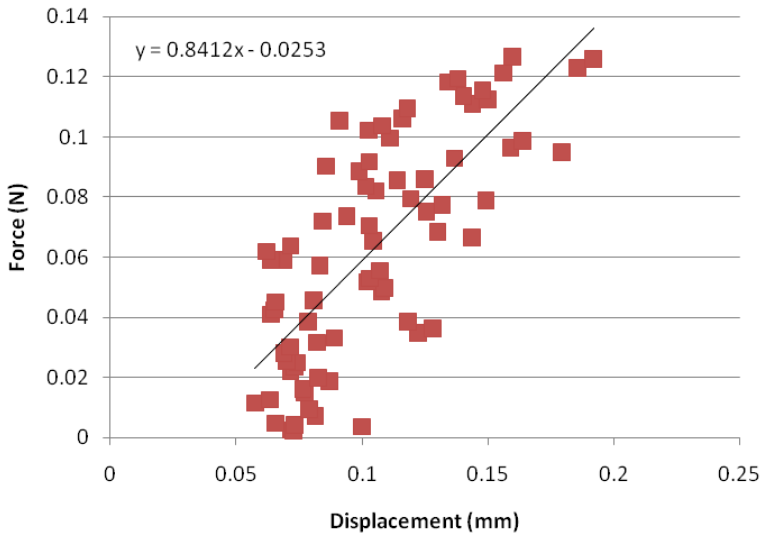


Figure 3.4 (a) A typical indentation curve obtained on one subject on the peri-ulcer area with four loading and unloading cycles by the air jet indentation system. (b) A scatter plot of the force-deformation relationship by the air jet indentation system for the 2nd to 4th loading and unloading cycles. (c) The stiffness coefficient of the wound tissues were estimated by the loading phase for the 2nd to 4th cycle.

The subjects were positioned in sitting comfortably and were told to relax during the entire study. The ankle was kept in a neutral position and the knee in a slightly flexed position. The lower leg and ankle was supported by a tailor-made stainless-steel foot stand padded with towels, so that the subject could maintain this posture throughout the assessment (Figure 3.5). The central portion of the ulcer, and the proximal, distal, medial and lateral edges of peri-wound areas were tested. Two trials were conducted and averaged to represent the data for each testing sites.

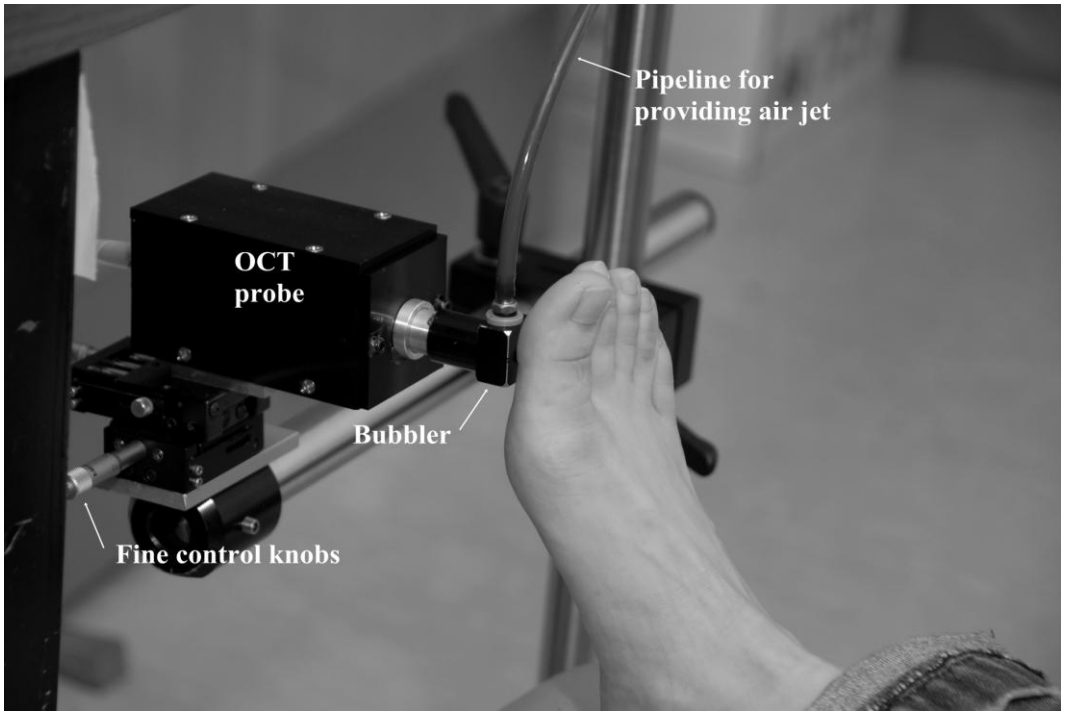


Figure 3.5 The experimental setup for the wound biomechanical properties assessment using the OCT based air jet indentation system

3.4 ASSESSMENT OF WOUND HEALING

3.4.1 ULTRASOUND BIOMICROSCOPY

Clinicians have had to rely on clinical expertise and invasive methods to inspect the wound etiology and wound complications. Using diagnostic ultrasound, the wound can be evaluated objectively and noninvasively. Unlike computerized tomography and magnetic resonance imaging (MRI), the use of diagnostic ultrasound allows clinicians to scan soft tissues in real time in clinical setting, avoiding potential delays due to MRI scheduling and follow-up consultations.

The ultrasound beam comes into contact with the targeted tissues, which causes the reflection of ultrasound signal. An ultrasound detector connected with a computer is designed to monitor the backscattered ultrasound. The software calculated the echo time delay of sound reflected from the tissue at different depths, data that can be used to build a cross-sectional image of the tissue. Transducer frequency has a direct effect on image resolution, whereas the higher the frequency the higher the resolution. In 'Chapter 6', longitudinal and transverse scans were obtained by placing the probe in either direction. An ultrasound biomicroscopy (Vevo 770 High Resolution Imaging System, VisualSonics, Canada) utilized a linear array transducer with a central frequency of 55MHz. B-mode was utilized in this study, that allowed a two-dimensional measurement performed on nearly exact (to the nearest 0.01mm) depth. The wound depth was calculated by the use of the computer software.

Prior to scanning, the wound were cleansed with normal saline. The wound cavity was then filled with a wound ultrasound transmission gel (SonoView^{TM} Wound Mapping

Gel, Hudson Diagnostic Imaging, Elmwood Part, New Jersey) and then covered with an acrylic adhesive film dressing (Opsite™ Flexigrid™, Smith & Nephew, Hull, UK). The ultrasound transmission gel (in 20 ml individual package) is used to fill the wound cavity and to eliminate any dead space or air pockets. The dressing seals the wound containing the gel and protects the wound from the transducer (Figure 3.6). Additional transmission gel is applied to the surface of the transducer and placed at a 90-degree angle to the wound (Figure 3.7).

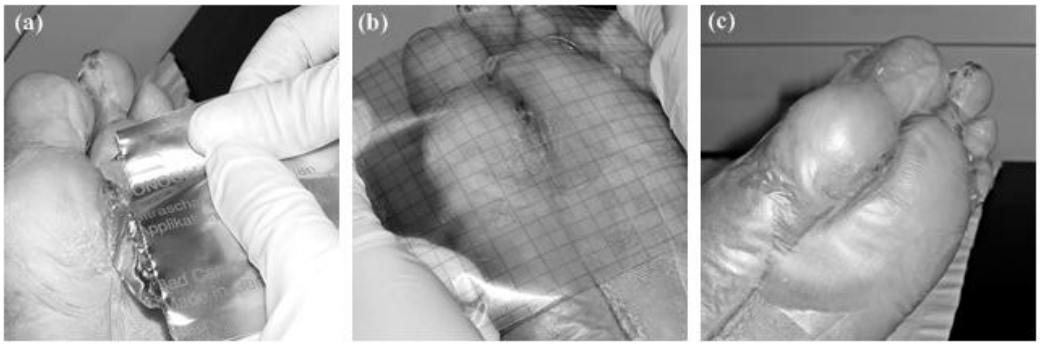


Figure 3.6 Wound ultrasound transmission gel applied with adhesive film dressing covered. (a) the wound gel is applied. (b) the wound is covered with dressing. (c) No air bubble is allowed between the dressing and the wound.

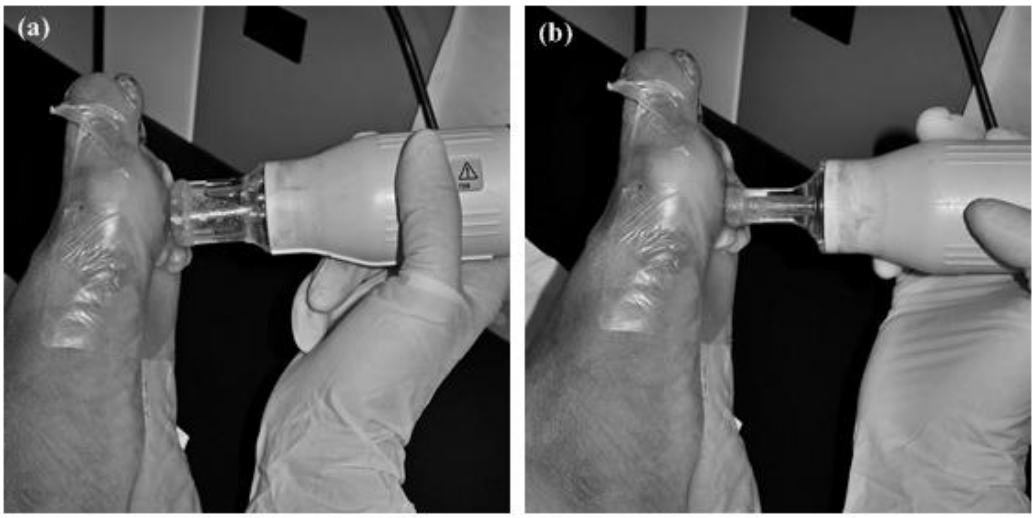


Figure 3.7 The transducer is placed over the wound in the (a) longitudinal axis and (b) transverse axis. The ultrasound scan demonstrates the skin surface, the ulcer, the base of the wound, and the depth can be measured.

A typical image scanned by the ultrasound biomicroscopy is shown in figure 3.8. The ultrasound scan illustrates the ulcer on the plantar foot of a patient with diabetes mellitus. Note that the bright band is caused by the high acoustic impedance of the adhesive wound dressing, which indicates the wound surface. The depth of the wound can be measured accurately.

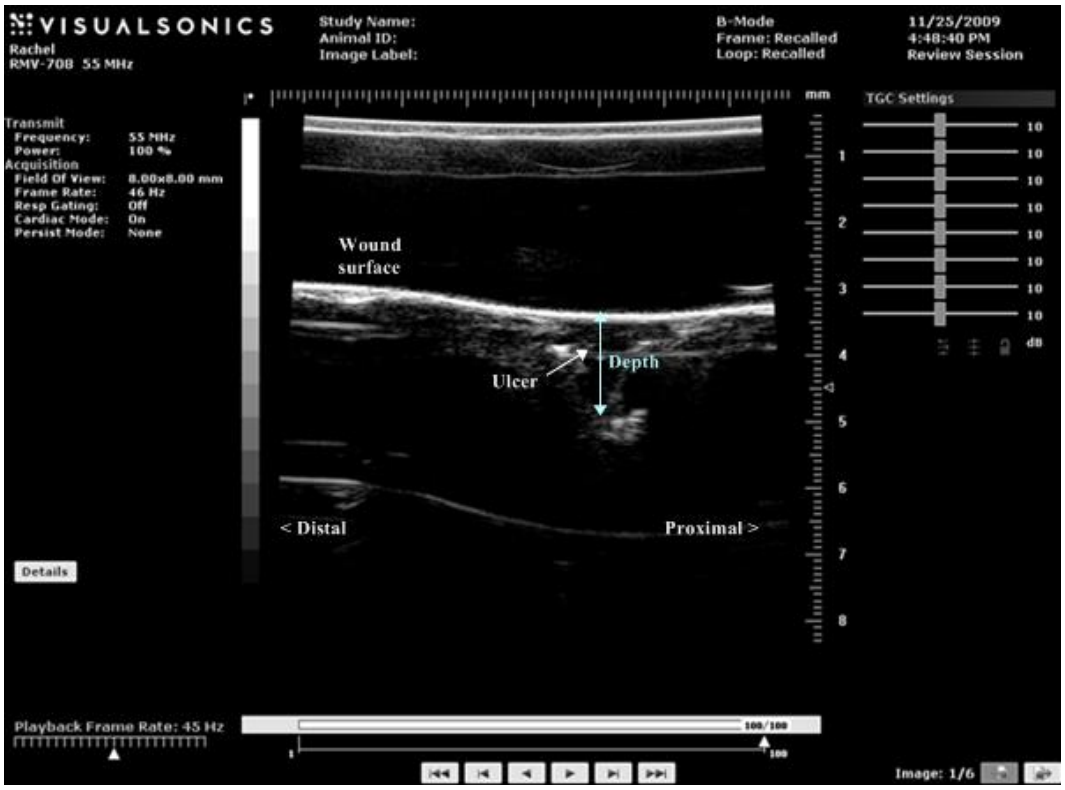


Figure 3.8 This image indicates the diabetic ulcer over the plantar surface of the foot scanned by the ultrasound biomicroscopy. The wound surface and the ulcer can be identified, and the depth of the wound bed is measured.

3.4.2 CLINICAL ASSESSMENT FOR CHRONIC WOUND

Digital images of the wound were obtained by one assessor after the wound cleansing, by using a CANON digital single-lens reflex camera (Model EOS-40D, Canon Inc. Operations, Tokyo, Japan). The camera equipped with a 22.2 x 14.8 mm CMOS image sensor permits still image taking of approximately 10.1 million pixels, with a lens (F2.8, f=100mm) (Model EF 100mm f/2.8L Macro IS USM, Canon Inc. Operations, Tokyo,

Japan), an external flash (Model Macro Ring Lite MR-14EX, Canon Inc. Operations, Tokyo, Japan) and a full colour live LCD (TFT) monitor. A square-shape target plate (VeV Measurement Documentation. Vista Medical, Winnipeg, Manitoba, Canada) of 3 x 3 cm^2 was positioned adjacent to, and in the plane of the wound before capturing the image. The distance of the camera was fixed by a stand and the light supply environment was kept constant. The photos were taken with standard settings (ISO 100, exposure time 1/250 sec, aperture 8.0, white balance 5700K, resolution 3888 x 2592), and output as Joint Photographic Experts Group (JPEG) format. Each digital image (72 dpi; 24-bit colour) was visualized on the screen (14 inch; 1024 x 768 pixels) of the laptop. The image of the ulcers together with the target plate was taken by the digital camera. The width, length and the size of the ulcers were calculated by tracing the boundaries with VeV wound measurement system (VeV Measurement Documentation. Vista Medical, Winnipeg, Manitoba, Canada) (Figure 3.9). A previous study showed that the VeV wound measurement system was shown to have excellent intrarater reliability (ICC3,1=0.99) and interrater reliability (ICC2,1=0.94) (Thawer et al., 2002). It also demonstrated that the computerized technique is comparable with the well-established manual tracing technique when used by equally trained assessors (ICC 2,1=0.94; ICC2,2=0.98) (Thawer et al., 2002).

Figure 3.9 The VeV wound measurement system. The software automatically detects the 3x3cm target plate in a correct orientation. The software then trace margin of the wound on the image of the screen. The length, width and area can be calculated.

3.5 TREATMENT MODALITY

PULSED ELECTROMAGNETIC FIELD (PEMF) UNIT

The patients enrolled in the study were randomized assigned in the active PEMF or control (sham) group by using a computer-generated randomization table. The PEMF System (model XKC-600W, manufactured by Magnetopulse International, Australia) was used. The electromagnetic field is generated by passing electrical current through

eight concentric coils. There are 200 turns of coil, with each coil 30 cm in diameter. The intensity of the electromagnetic field provided by the unit ranged from 1 to 99 Gauss, with a frequency ranging from 1 to 50 Hz. The frequency of PEMF was delivered at 12Hz, with magnetic flux density of 12G. Each treatment session lasted for 1 hour, which repeated for 14 daily sessions within three weeks. Subjects remained in the study until the wound healed or 14 daily sessions were completed, whichever came first. The internal circuit of the sham PEMF unit was disconnected by the control of a switch box (channel A and channel B) (Figure 3.10). Although the device was switched to the "sham PEMF" setting, it did not direct any pulsed electromagnetic field to the wound bed. After the data analyses were done, the investigator was informed that channel B was the active PEMF channel.

Figure 3.10 The pulsed electromagnetic field unit and a switch box, and one of the channels was the sham channel in which the internal channel was disconnected and there was no active PEMF output. The subjects were randomly allocated into active PEMF or control (sham) group while both the subjects and investigators were blinded from the group allocation until data analyses were completed.

CHAPTER 4

THE EFFECT OF AGING ON BIOMECHANICAL PROPERTIES OF PLANTAR SOFT TISSUES

This Chapter was published as

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and

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Refer to Appendix IV for published manuscript and Appendix VI for published conference abstract.

Elevated plantar pressure (Reiber et al., 1999) is the main cause of foot ulceration, depends on the biomechanical properties of the plantar soft tissues, as well as the foot structure and the footwear (Burnfield et al., 2004). Previous study showed a significant stiffer and thinner plantar soft tissues in elderly patients with diabetes and healthy young subjects (Zheng et al., 2000). However, the effects of diabetes on biomechanical property of plantar soft tissues remained unclear as the healthy group and the diabetic group in that study was not age-matched. Diabetes is more prevalent in the elderly population, and ageing effect could be one of the confounding factors that explain for the delay in wound healing. Thus, we first want to investigate the contribution of ageing on the changes in biomechanical properties of plantar soft tissues among healthy elderly.

4.1 INTRODUCTION

The heel and metatarsal head regions of the human foot are specially designed to provide cushioning and shock absorption to the underlying bone during all weight bearing tasks (Callaghan et al., 2008, Scott et al., 2007). Menz and Lord (1999) stated that with advancing age, the structure of the foot changes. There is a reduction in the range of motion, tactile sensation, and strength of the foot, and a higher prevalence of foot deformities (Menz and Lord, 1999). The load-carrying ability under the plantar foot becomes impaired (Gefen, 2003). These structural and functional changes over time may contribute to foot pain.

Foot problems are extremely prevalent in the aging population (Menz and Lord, 1999). The stiffened plantar soft tissues due to aging under the metatarsal heads may impair the

reaction of the tissues to different impact velocities, eventually developing into metatarsalgia in the elderly (Hsu et al., 2005). The altered mechanical properties of the heel pad also leads to shock-induced injuries such as heel pain and Achilles tendinitis (Hsu et al., 1998). Various orthopedic foot deformities and metatarsal problems may lead to inactivity, and subsequently to weakness and falls (Menz and Lord, 1999). The soft tissue stiffening under the metatarsal heads may account for the common location of foot ulcers reported in people with diabetes (Gefen, 2003).

Ultrasonography has been used to evaluate the thickness of the plantar soft tissues under the metatarsal heads (Wang et al., 1999). Zheng et al. (1999) developed an ultrasonic indentation probe to examine the total thickness and stiffness (elastic modulus) of the plantar soft tissues (Zheng et al., 1999). The ultrasound technique was employed to determine the change in the thickness of the soft tissue layer in the indented site of the tested body part. The measurements of thickness and stiffness would reflect the geometric effects and the mechanical properties of the soft tissue (Zheng et al., 2000), which would subsequently influence the adaptability of the plantar soft tissues during weight bearing situations. Ledoux et al. (2004) examined the damping properties of the plantar soft tissue at the primary regions, including the heel, the five metatarsal heads, and the big toe (Ledoux et al., 2004). However, they used fresh frozen cadaveric tissue rather than live specimens, in which the loading-unloading properties might have been altered. Most other biomechanical tests of the plantar soft tissue have focused on the heel region (Hsu et al., 1998, Ozdemir et al., 2004). It is unclear how aging would influence the change in the properties of the plantar soft tissue at various testing sites of the foot.

Understanding the influence of aging on the biomechanics of plantar soft tissue is of great importance for designing footwear and preventing injuries. It is hypothesized that the biomechanical properties of human plantar soft tissues can be altered with aging, by an increase in thickness and stiffness. The present study compared the biomechanical properties of human plantar soft tissues, in terms of thickness and stiffness, among four age groups. In addition, the relationship of these properties with aging was examined.

4.2 METHODS

A total of 60 healthy volunteers (46 females and14 males) were recruited from the community by convenience sampling. The subjects were assigned into four age groups: 40-49, 50-59, 60-69, and >70 years. Subjects with any foot lesions, major medical conditions including diabetes, peripheral vascular disease or stroke, or a history of orthopedic or neurological disorders were excluded. The purpose of study, procedures of measurement, and risks associated with the study were explained to all of the subjects before their written informed consent to participate in this study was obtained.

The tissue ultrasound palpation system (TUPS) (Research Institute of Innovative Products and Technologies, HKSAR, China) consisted of a pen-size probe that served as the indenter with a 5 MHz and 9 mm ultrasound transducer installed at the tip of the probe, which was used to investigate the thickness and the Young's modulus of the plantar soft tissue (Zheng et al., 1999, Zheng and Mak, 1996). Each subject was asked to lie in a supine position with the ankle maintained in a neutral position and the knee in a straight position. The subject was told to relax during the entire study, and the head was

supported with a pillow to maintain a neutral cervical spine. The lower leg and ankle was supported by a foot stand padded with towels, so that the subject could maintain this posture throughout the assessment. The plantar soft tissues covering the big toe, first metatarsal head, third metatarsal head, fifth metatarsal head, and the heel of the right foot were palpated and marked (Zheng et al., 2000) (Figure 4.1). The metatarsal heads were chosen because they have been recognized as areas of high plantar pressures and at risk of ulceration (Garcia et al., 2008, Singh et al., 2005). The direction of indentation was carefully monitored by the real time visual feedback of the indentation response displayed on the computer monitor, which allowed maintaining the maximum ultrasound echo signal throughout the test. The above trial was repeated for two trials at each testing site and the mean was taken for subsequent analysis.

The thickness and indentation depth of the soft tissue layer were determined from the time of flight of the ultrasound echo signal that reflected from the soft tissue-bone interface. The load signal and the ultrasound signal were both digitized and collected by a computer, and shown in real-time with a program. The deflection of the ultrasound echo due to the deformation of soft tissue was determined using a cross-correlation technique. The effective Young's modulus was calculated by the computer program that showed the stiffness or elasticity of the plantar soft tissue. Details of TUPS refer to Chapter 3 Section 3.3.1.

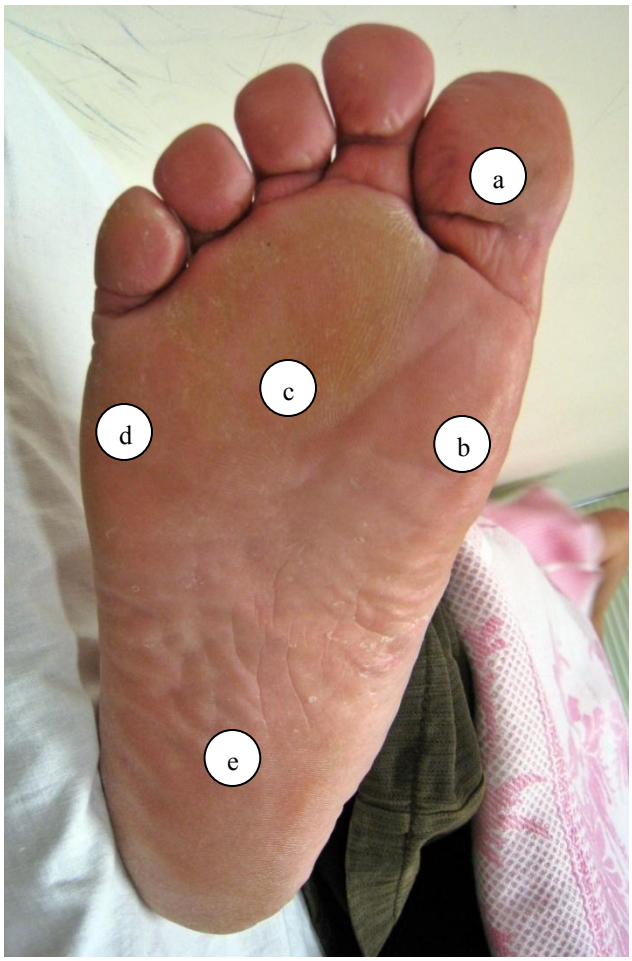


Figure 4.1 The biomechanical properties of the plantar soft tissues were measured by the tissue ultrasound palpation system at (a) pulp of big toe, (b) first metatarsal head, (c) third metatarsal head, (d) fifth metatarsal head, and (e) heel.

Statistical analyses were performed using the software package IBM® SPSS® Statistics 17.0 (IBM Corporation, Chicago, IL). The differences between the age groups with regard to the thickness and stiffness of the plantar soft tissue were calculated by a one-way analysis of variance (ANOVA) test. Significant findings were followed by

Bonferroni post hoc analyses to examine group differences, and independent t-test to investigate the gender effect. The relationship between age and the thickness or stiffness of the plantar soft tissue was calculated using the Pearson correlation coefficient. The level of significance was set at $P<0.05$.

4.3 RESULTS

Descriptive statistics on the height, weight, and body mass index of different age groups are presented in Table 4.1. No statistical difference in any of these demographic characteristics was found among the groups.

Table 4.1 Demographic characteristics (mean (SD)) for various age groups

	Age Group			
	41-50 (n=7)	51-60 (n=19)	61-70 (n=17)	>71 (n=17)
Age (years)	45.1 (3.3)	56.4 (2.4)	66.6 (2.8)	74.3 (3.3)
Gender (male:female)	0:7	3:16	4:13	7:10
Height (cm)	157.3 (6.7)	158.4 (6.4)	154.9 (7.6)	155.9 (8.7)

Weight (kg)	54.9 (5.8)	61.1 (9.5)	57.0 (6.6)	58.1 (9.2)
Body Mass Index (kg/m^2)	22.2 (2.2)	24.3 (3.1)	23.8 (2.5)	23.9 (3.4)

4.3.1 Stiffness of plantar soft tissues

The mean stiffness (Young's modulus) of the big toe, first metatarsal head, third metatarsal head, fifth metatarsal head, and the heel were significantly different between the four age groups (P <0.001 each) (Table 4.2).

The post hoc analyses indicated that over 71 years age group had a significantly stiffer big toe, first metatarsal head, third metatarsal head, fifth metatarsal head, or heel plantar soft tissue than did the group aged 51-60 years or the group aged 41-50 years (P <0.01 each). However, the over 71 years age group was not differ from the group aged 61-70 years (P>0.05).

The plantar soft tissues of the big toe and fifth metatarsal head of the 61-70 years age group were significantly stiffer than those of the 51-60 years age group and the 41-50 years age group, and the tissues of the first metatarsal head and the third metatarsal head were significantly stiffer than those of the group aged 51-60 years (P <0.05 each). However, the 41-50 years age group was not differ from the group aged 51-60 years at any measurement sites (P>0.05).

Table 4.2 Comparisons of the stiffness and thickness of the plantar soft tissues among different age groups (mean(SD)).

	41-50	51-60	61-70	>71	*F*-value	*P*-value
Stiffness (kPa)						
Big toe	21.7(6.6)	32.8(18.4)	62.0(34.5)	77.8(40.9)	9.723	<0.001*
1st MTH	36.8(11.6)	53.6(36.6)	94.9(54.6)	107.3(53.8)	6.756	0.001*
3rd MTH	31.7(13.0)	50.0(28.5)	87.3(48.7)	122.9(51.9)	12.719	<0.001*
5th MTH	29.8(15.1)	41.6(19.3)	79.2(46.1)	93.8(45.2)	9.495	<0.001*
Heel	32.4(11.9)	55.0(16.42)	70.7(22.1)	76.7(21.8)	10.854	<0.001*
Thickness (mm)						
Big toe	5.37(0.86)	7.18(2.96)	6.29(1.55)	6.74(1.25)	1.631	0.193
1st MTH	9.19(2.77)	8.90(2.73)	9.35(2.27)	8.75(2.29)	0.130	0.942
3rd MTH	9.97(0.94)	9.14(1.56)	9.52(1.77)	8.73(1.85)	1.046	0.380
5th MTH	6.39(1.86)	8.84(4.00)	8.39(2.51)	8.62(2.04)	1.294	0.285
Heel	20.88(1.82)	20.77(3.37)	22.66(3.83)	22.49(3.49)	1.125	0.347

MTH: metatarsal head

*$P \leq 0.001$

Independent t-test was performed to compare gender difference in plantar soft tissue stiffness only in the sub-groups whose age were over 71 years age group, because of the similar number of male and female subjects in that sub-group. The male and female distributions are uneven in other age sub-groups. Although we found a trend that the female subjects tended to have stiffer plantar soft tissue, but only the stiffness of the heel was statistically significant between male and female. Therefore, our findings did not support that the effect of stiffening with age is gender-dependent. The mean stiffness of plantar soft tissues (kPa) is shown in the table below (Table 4.3).

Table 4.3 Comparisons of the stiffness of the plantar soft tissues between male and female subjects (mean(SD)).

	Male (kPa)	Female (kPa)	*P*-value
Big toe	70.3±42.7	83.1±41.0	0.542
1st metatarsal head	89.3±44.2	119.9±58.4	0.262
3rd metatarsal head	98.1±58.6	140.3±41.0	0.100
5th metatarsal head	79.1±43.4	104.2±45.7	0.273
Heel	64.2±18.5	85.4±20.3	*0.044

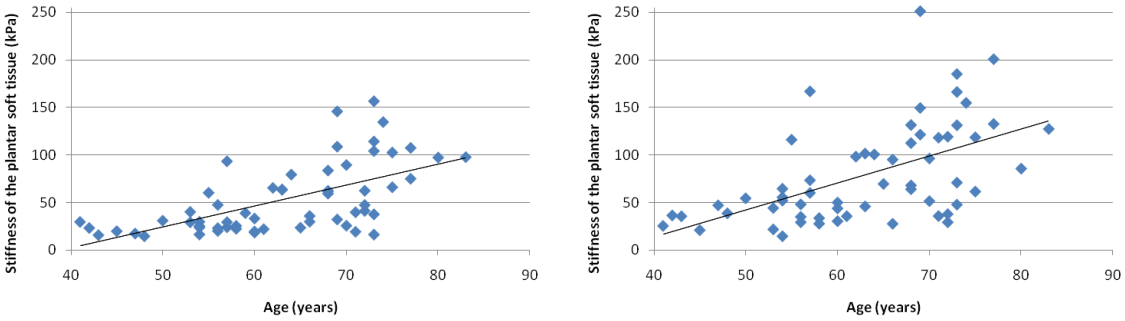
4.3.2 Thickness of plantar soft tissues

For each age group, the plantar soft tissue at the heel region was significantly thicker than that at the big toe and the metatarsal heads (P<0.001). The plantar soft tissues at the big toe, the fifth metatarsal head, and the heel tended to be thicker with increasing age, from the thinnest for the group aged 41-50 years to the thickest for the over-71 years age group. However, this difference did not reach the level of significance (Table 4.2).

4.3.3 Correlation between age and the stiffness of the plantar soft tissue

The Pearson correlation coefficients for a comparison of age with the stiffness of the plantar soft tissue are presented in Figure 4.2. There is a strong positive correlation between age and the stiffness of the plantar soft tissues at the big toe (r=0.608), first metatarsal head (r=0.549), third metatarsal head (r=0.657), fifth metatarsal head (r=0.633), and heel (r=0.584) (all P <0.001).

(a) (b)



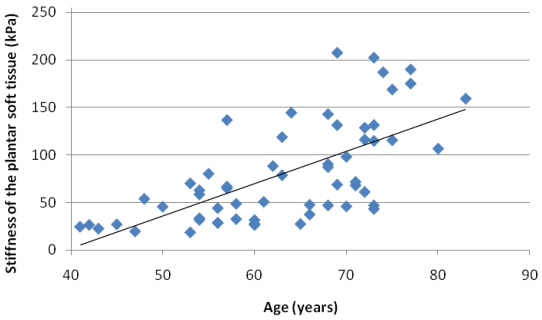
Big toe

r=0.608, P<0.001^{+}

1st MTH

r=0.549, P<0.001^{+}

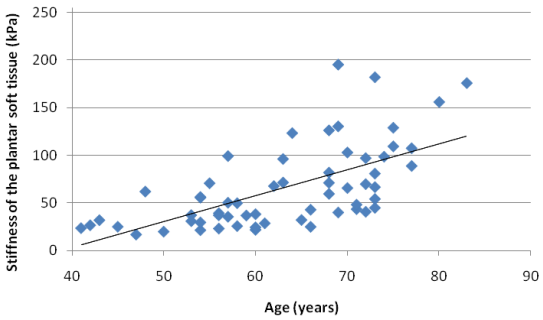
(c)



3rd MTH

r=0.657, *P*<0.001+

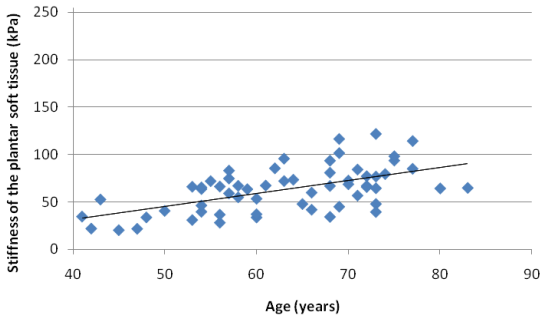
(d)



5th MTH

r=0.633, *P*<0.001+

(e)



Heel

r=0.584, *P*<0.001+

Figure 4.2 The linear correlation between age and the stiffness of plantar soft tissues. + *P*<0.001, a significant correlation was found between age and stiffness at (a) the big toe, (b) first metatarsal head, (c) third metatarsal head, (d) fifth metatarsal head, and (e) heel.

4.4 DISCUSSION

As skin ages, the collagen and elastic fibers in the cell matrix become less elastic and more fragile. As a result, the elasticity of the skin diminishes and its ability to resist an external shearing force is reduced (Edelstein, 1992). Our findings show that the stiffness of the plantar soft tissue under the metatarsal heads and heel increases linearly with age. The stiffened soft tissue may reduce the adaptability of the tissue to respond to sudden or repetitive stress, which may lead to foot problems in elderly people (Hsu et al., 2005). Degeneration related to repetitive microtrauma causes a gradual loss of collagen in the fat pad of the heel, a decrease in water content and elastic fibrous tissue, and thus a decrease in the elasticity of the heel's fat pad (Ozdemir et al., 2004). Elderly people tend to lose propulsive force during the late stance phrase of walking (Edelstein, 1992). The inelastic and weakened soft tissue may then contribute to the instability of the foot in stance phrase of walking, making it less capable of achieving postural control in weight-bearing positions.

Increased stiffening of collagen-rich soft tissue has been found in patients with diabetes (Reihsner and Menzel, 1998). The soft tissues become less elastic and less able to distribute pressure through deformation. Thus, the cushioning property of the plantar soft tissue is impaired, especially at high-pressure sites such as the metatarsal heads (Gefen, 2003). With age, the micro-tears that are caused by repetitive shear stress during walking are expected to increase for patients with diabetes. This is particularly true for people suffering from peripheral neuropathy, who are prone to developing foot ulcerations.

However, there was uneven gender distributions in most of the sub-groups including the group aged 41-50 years, the group aged 51-60 years and the group aged 61-70 years, subsequent analysis of gender effect on plantar soft tissue stiffness was performed only in the group whose age was over 71 years old. Although the female subjects tended to have stiffer plantar soft tissue, significant difference in the stiffness of plantar tissue was found between male and female only in the heel region (P <0.05), but not in other measuring sites (all P >0.05).

The thickness of soft tissue is an important predictor of peak plantar pressure, whereas high pressure during walking could lead to skin breakdown or other injuries (Mueller et al., 2003). Although the thickness of the plantar soft tissue was not significantly different between the four age groups in this study, nevertheless there was a trend of thicker plantar soft tissue in the older groups. There are many factors behind the change in the total thickness of the plantar tissue of the elderly, including choice of footwear, systemic diseases, and gender (Menz and Lord, 1999). Socioeconomic and occupational factors could also potentially affect the thickness of the plantar soft tissues. Those people endure long hour of continuous standing in their jobs are likely to have thickened plantar soft tissues compared to those are mostly sedentary. As the male and female ratio was significantly different in most age sub-groups, we could not examine the gender effect of plantar soft tissues in the present study. This may explain why our result is not statistically significant. An increase in the distribution of body fat may be the cause of the increased thickness of the plantar soft tissues, and may lead to increased pressure in the sealed fibrous compartment, resulting in the stiffer soft tissues noted in the older subjects (Hsu et al., 2005).

The plantar soft tissues at the heel were the thickest, compared with those at the big toe and the metatarsal heads. The functional thickness of the cushioning layer under the big toe and the metatarsal heads is supposed to be thinner than the values we obtained, as we measured the total thickness of all soft tissue layers, including the flexor tendon, which is not responsible for shock absorption. The peak plantar pressure sustained more energy distributed at the forefoot region than at the heel in both walking and running (Wang et al., 1999). Thus, the metatarsal head region is more susceptible to injuries and prone to ulceration than the other sites of the foot.

We postulate that an increase in the overall thickness of the plantar soft tissue may lead to a decrease in tactile sensation and hinder afferent sensory feedback. Together with changes in the stiffness of the plantar soft tissue and motor function, this may partly explain why elderly people tend to have difficulties controlling their posture, and are thus prone to injurious falls (Boyd and Stevens, 2009). However, since no sensory tests for plantar soft tissues were performed in our study, further investigation should be done to confirm this hypothesis.

The use of orthotics and supportive devices to relieve contact pressure is likely to re-distribute the contact pressures and reduce the stresses under the metatarsal heads. Clinicians may consider using ultrasound technology to examine the biomechanical properties of the plantar soft tissues, and enhancing their clinical prognosis and prescription of footwear for those who are aging or suffering from diabetes. Since the function and mobility of the plantar fat pads are not well known and our study emphasized the vertical compression of plantar soft tissues in a non-weight bearing

position, the shear displacements of the underlying soft tissues were not studied. Also, our findings might not be fully transferrable to the measurement performed in a standing position, which is a weight bearing position with the force of gravity presented. A future study can be conducted to evaluate the relationship between the altered tissue properties and the development of forefoot ulcers for patients with diabetes in weight bearing position.

4.5 CONCLUSIONS

Our findings demonstrated that the biomechanical properties of plantar soft tissues appear to change with age in healthy individuals. The stiffness of unloaded plantar soft tissues significantly increased with age. Moreover, there is a trend of increasing thickness of the plantar soft tissue with advancing age. The altered biomechanical properties in plantar soft tissues may be responsible for the higher incidence of foot problems in elderly individuals. Therefore, adequate orthotics and supportive devices are suggested for ageing people to prevent foot problems.

This chapter demonstrated that the biomechanical properties of plantar soft tissues changed with ageing in healthy subjects, in the next chapter, we will further examine the biomechanical properties of plantar soft tissue in patients with diabetic ulceration, as compared to age-matched subjects. Also, we shall explore the relationship between the biomechanical properties of plantar soft tissues and tactile sensation in patients with diabetic ulceration. Both of these factors are risk factors for developing diabetic ulcer.

CHAPTER 5

THE BIOMECHANICAL PROPERTIES OF PLANTAR SOFT TISSUES AND WOUND TISSUE OF PATIENTS WITH DIABETIC ULCER

Our previous chapter has demonstrated that the biomechanical properties of plantar soft tissues change with ageing in healthy individuals. The present chapter conducted a cross sectional study that examines the biomechanical properties of plantar soft tissue in patients with diabetic ulcers as compared to aged-match subjects; and its relationship with the tactile sensation was explored. Specifically, a case study was done to investigate the change of biomechanical properties of the diabetic wound tissue across healing stages.

5.1 INTRODUCTION

Neuropathy, deformity, callus, elevated plantar pressure, poor microcirculation are the main cause of diabetic foot ulceration (Ann, 2011). Hyperglycemia is one of the main causes of diabetic foot problem. Hyperglycemia induces metabolic derangement that directly affects the Schwann cells and nodes of Ranvier (Dyck and Giannini, 1996). It may also affect the structure and function of endoneurial microvessels, and thus altering the blood-nerve barrier and causing nerve hypoxia (Dyck and Giannini, 1996). Peripheral sensory neuropathy disables the protection mechanism that is normally activated when the plantar soft tissue is damaged, so patients with diabetic neuropathy are more prone to unnoticed injury.

The plantar pressure depends on the biomechanical properties of the plantar soft tissues, as well as the foot structure and the footwear (Burnfield et al., 2004). There is an increasing attention in diagnostic ultrasound for musculoskeletal conditions in recent years. Ultrasound devices can be used to assess the underlying soft tissues properties of

the foot. Yet, very few studies have been reported on the biomechanical assessment of the plantar soft tissue and wound tissue in patients with diabetes. Our research collaborator developed an ultrasound indentation system that measures biomechanical properties of the soft tissues. Our findings in Chapter 4 demonstrated that ageing brings a significant impact on the plantar soft tissue stiffness (Kwan et al., 2010). Persistent hyperglycemia may lead to various complications including peripheral neuropathy, which results in pathological changes in plantar soft tissues, which might stiffen its structure and diminish its ability to effectively distribute foot-ground contact loads. Recent findings by our research team also demonstrated that patients with diabetic peripheral neuropathy demonstrated stiffer plantar soft tissue than did the healthy control subjects (Sun et al., 2011), however, no comparisons were made between the patients with diabetic ulcers and the healthy control, hence further research with age matched patients with diabetic ulcers is needed.

Diabetic ulcers frequently occur at pressure sensitive sites, which are commonly found in the heel, big toe and metatarsal heads. Besides, the biomechanical properties of the plantar soft tissues, the wound tissue mechanical properties that partly determined by the thickness and distribution of the extracellular matrix were believed to affect the wound healing (Lee and Moon, 2003). The interaction between fibroblasts and various extracellular matrix analogs that leads to contractile phenomena has been investigated *in vitro* model of wound contraction (Eastwood et al., 1998, Roy et al., 1997, Roy et al., 1999). Under normal circumstances, wound healing involves three overlapping stages including the inflammation, tissue formation and tissue remodeling stages. Mechanical stresses at the wound site are hypothesized to affect the wound healing by regulating the

orientation of the collagen fibers and guide the contractile activity of the original unwounded tissue (Farahani and Kloth, 2008). There are changes in biomechanical properties of wound tissue at different stages of healing. Although the normal reference value of stiffness coefficient at specific sites was not available so far, it is suggested that a decrease in mechanical strength of the wound tissue would lead to reduction in resilience, toughness and maximum extension of tissue (Lee and Moon, 2003). Thus, previous study suggested that stiffness measured from the wound tissues might serve as reference for healing stages (Chao et al., 2010, Chao et al., 2011). However, limited studies have been done to examine the biomechanical properties in patient with diabetic ulceration, and no previous studies investigated the biomechanical properties of wound tissue during different stages of healing. Therefore, the optical coherence tomography (OCT) based air jet indentation system with the validity and reliability demonstrated in previous studies performed by our research team (Chao et al., 2010, Chao et al., 2011, Huang et al., 2009), was used to provide a quantitative and reliable monitoring on the biomechanical tissue property of diabetic ulcer during different healing stages.

Therefore, there were two studies included in this chapter. Study A: aimed to compare the biomechanical properties of human plantar soft tissues, in terms of thickness and stiffness between patients with diabetic ulceration and the healthy control subjects (intact skin). In addition, the relationships of these properties with the tactile sensation in the patients with diabetic ulceration were examined. Study B: A case study was done to investigate the change of wound tissue stiffness in a patient with diabetic ulcer during the healing process – using a non contact method.

5.2 METHODS

5.2.1 Study A

Subjects with diabetic ulceration (DU) were recruited from Kwong Wah Hospital. Their diagnosis of diabetes was confirmed by physicians. Subjects with any foot lesions other than diabetic ulceration, major medical conditions including peripheral vascular disease or stroke, or a history of orthopedic or neurological disorders were excluded. Healthy age-matched subjects with no history of diabetes were recruited from the community by convenience sampling. The purpose of the study, procedures of measurement, and potential risks of the study procedures were explained to all subjects before obtaining their written consent to participate. All subjects were evaluated by the same assessor.

The tactile sensation was detected by the Semmes-Weinstein monofilaments (SWM) at the big toe, first metatarsal head, third metatarsal head, fifth metatarsal head, and the heel. The subjects lied in a supine position during the measurement. A set of 20 monofilaments ratings between 1.65 and a maximum of 6.65 were applied perpendicularly to the skin until a first-order buckling occurred, and the threshold was determined by a subject's verbal response to say 'yes' each time he or she senses the application. Three trials were taken at each measurement site, alternately with one sham application in which no filament is applied (Apelqvist et al., 2000). When the subject was unable to respond correctly in more than one trial, a heavier monofilament was taken. Details of SWM refer to Chapter 3 Section 3.1.1.

The tissue ultrasound palpation system (TUPS) (Research Institute of Innovative Products and Technologies, HKSAR, China) was used to investigate the thickness and

the Young's modulus of the plantar soft tissue (Zheng et al., 1999, Zheng and Mak, 1996). The thickness and indentation depth of the soft tissue layer were determined from the time of flight of the ultrasound echo signal that reflected from the soft tissue-bone interface. The load signal and the ultrasound signal were both digitized, stored in a computer, and shown in real-time. The deflection of the ultrasound echo due to the deformation of soft tissue was determined using a cross-correlation technique. The effective Young's modulus was calculated by the computer program that showed the stiffness or elasticity of the plantar soft tissue. The plantar soft tissues covering the big toe, first metatarsal head, third metatarsal head, fifth metatarsal head, and the heel of the contralateral side of the foot with ulceration were palpated and marked (Zheng et al., 2000). The metatarsal heads were chosen because they are areas of high plantar pressures that are at risk of ulceration (Garcia et al., 2008, Singh et al., 2005). A water-soluble acoustic gel was applied on the testing site to minimize the loss of ultrasound signal due to reflection. The plantar soft tissue was preconditioned by loading and unloading the probe on the testing site for a few times. The probe was then held with minimal force and searched for the best alignment that until the maximum ultrasound echo signal can be obtained. The manual load was increased gradually in a perpendicular manner to the plantar soft tissues, which was repeated for 5 cycles in each trial. The above trial was repeated for two trials at each testing site and the mean was taken for subsequent analyses. Technical details of this instrument please refer to Chapter 3 Section 3.3.1.

Typical ultrasound indentation testing requires direct contact of the rigid transducer to the target tissue, which may damage the delicate wound bed tissue during the

indentation process or there is a potential chance of causing infection due to contamination. Hence, this technique is not suitable for assessing active diabetic foot ulcer in patients. Instead, a non contact air jet indentation system was used to assess the biomechanical properties of the wound tissues in Study B.

5.2.2 Study B

A case study was conducted in one of the subjects with active diabetic ulcer. The change of biomechanical property (i.e. stiffness) of the wound tissue over time was monitored by the optical coherence tomography (OCT) based air jet indentation system in a non-contact manner (Huang et al., 2009). Four cycles of loading and unloading with a total was carried out. Only the loading phase at the second to fourth cycle was utilized to calculate the stiffness coefficient. The wound bed and the peri-ulcer area consisted of different content of micro-structure including fibroblasts that may affect the stiffness of the tissue (Agren et al., 1999), therefore various wound sites including the central portion of the ulcer, and the upper, lower, medial and lateral edges of peri-wound areas were tested. Two trials were conducted and averaged to represent the data for each testing sites. For technical details of this instrument please refer to Chapter 3 Section 3.3.2.

During the optical coherence tomography (OCT) based air jet indentation system measurement, each subject was asked to lie in a supine position with the ankle maintained in a neutral position and the knee in an extended position. The subject was told to relax during the study, and the head was supported with a pillow to maintain a

neutral cervical spine. The lower leg and ankle was supported by a foot stand padded with towels, so that the subject could maintain this posture comfortably throughout the assessment.

Statistical analyses for Study A were performed using the software package IBM® SPSS® Statistics 17.0 (IBM Corporation, Chicago, IL). The differences between groups with regard to the tactile sensation, thickness and stiffness of the plantar soft tissue were calculated by multivariate analysis. The relationship between tactile sensation and the biomechanical properties of the plantar soft tissue was calculated in the patients with diabetic ulceration using the Pearson correlation coefficient. The level of significance was set at $P < 0.05$.

5.3 RESULTS

5.3.1 Study A

Demographic characteristics of subjects are presented in Table 5.1. There was no significant difference in age, gender, body mass index and fasting plasma glucose between groups.

Table 5.1 Demographic characteristics (mean (SD)) for various groups.

	DU (n=9)	Control (n=32)	*P*-value
Age (years)	60.7 (14.4)	58.9 (9.1)	0.657
Gender (M:F)	6:3	9:23	0.053
BMI (kg/m^2)	27.0(5.5)	23.7 (2.0)	0.166
FPG (mmol/L)	6.5 (3.3)	5.6 (0.7)	0.339

DU: Patients with Diabetic Ulceration

BMI: Body Mass Index; FPG: Fasting Plasma Glucose

M: male; F: female

5.3.1.1 Stiffness of plantar soft tissues

The mean stiffness (Young's modulus) of the big toe, first metatarsal head, third metatarsal head, fifth metatarsal head, and the heel were significantly different between groups. The diabetic ulceration group had a significantly stiffer big toe (103.9 vs. 29.6 kPa), first metatarsal head (113.5 vs 47.2 kPa), third metatarsal head (128.7 vs. 44.6 kPa), fifth metatarsal head (73.5 vs 40.1 kPa), or heel (74.3 vs. 51.2 kPa) plantar soft tissue than did the control group (all $P \leqq 0.001$) (Table 5.2).

5.3.1.2 Thickness of plantar soft tissues

The mean thickness of the big toe, first metatarsal head, third metatarsal head and the heel were each significantly different between the two groups (P <0.05) (Table 5.2). Plantar soft tissue thickness at the fifth metatarsal head did not differ between groups (P =0.055)

The plantar soft tissues were significantly thicker at the big toe (8.31 vs. 6.19 mm), the first metatarsal head (11.78 vs 8.54 mm), third metatarsal head (12.72 vs. 9.15 mm) and the heel (22.69 vs. 20.20 mm) of the diabetic ulceration group than that of the control.

Table 5.2 Comparisons of the stiffness and thickness of plantar soft tissues between the diabetic ulceration and control groups (mean (SD)).

	DU	Control	*P*-value
Thickness (mm)			
Big toe	8.31 (1.56)	6.19 (2.48)	0.015*
First MTH	11.78 (1.28)	8.54 (2.52)	0.003*
Third MTH	12.72 (1.69)	9.15 (1.45)	<0.001^{+}
Fifth MTH	10.63 (3.00)	8.15 (3.47)	0.055
Heel	22.69 (3.97)	20.20 (2.80)	0.017*

Stiffness (kPa)			
Big toe	103.9 (38.7)	29.6 (13.5)	0.001^{+}
First MTH	113.5 (31.8)	47.2 (22.3)	$<0.001^{+}$
Third MTH	128.7 (46.9)	44.6 (20.0)	0.001^{+}
Fifth MTH	73.5 (23.0)	40.1 (20.7)	$<0.001^{+}$
Heel	74.3 (20.2)	51.2 (18.2)	0.001^{+}

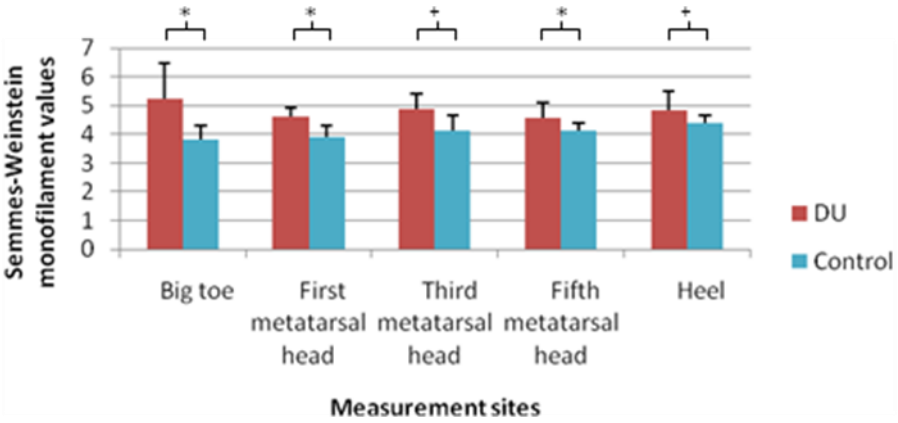
DU: Patients with Diabetic Ulceration

MTH: Metatarsal head

$*P<0.05$; $^{+}P \leqq 0.001$

5.3.1.3 Tactile sensation

A significant difference existed between the two groups for the Semmes-Weinstein monofilament test (P <0.05) (Figure 5.1). The diabetic ulceration group had a significantly higher tactile sensation value than the control group at the big toe, first metatarsal head, third metatarsal head, fifth metatarsal head and the heel (all P <0.05).



DU: Patients with Diabetic Ulceration

Figure 5.1 Comparisons of tactile sensation between patient with diabetic ulceration and healthy controls. A significant difference was found at (a) the big toe, (b) first metatarsal head, (c) third metatarsal head, (d) fifth metatarsal head, and (e) heel (P <0.05 each).

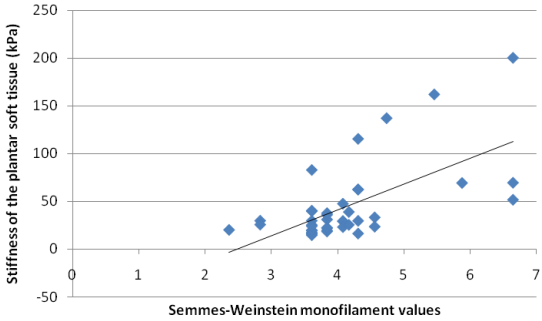
*P <0.05; $^{+}P \leqq 0.001$

5.3.1.4 Correlation between tactile sensation and the stiffness of the plantar soft tissue in patients with diabetic ulceration

The Pearson correlation coefficients for a comparison of tactile sensation with the stiffness of the plantar soft tissue for patients with diabetic ulceration are presented in Figure 5.2. There was a fair to strong positive correlation between tactile sensation and the stiffness of the plantar soft tissues measured at the big toe (r=0.618), first metatarsal

head (r=0.498), third metatarsal head (r=0.484), fifth metatarsal head (r=0.626), and the heel (r=0.363) (all P <0.05).

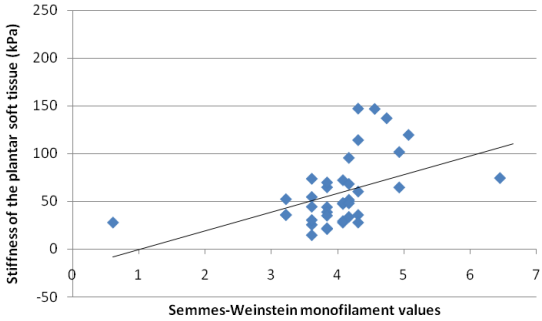
(a)



Big toe

r=0.618, P<0.001^{+}

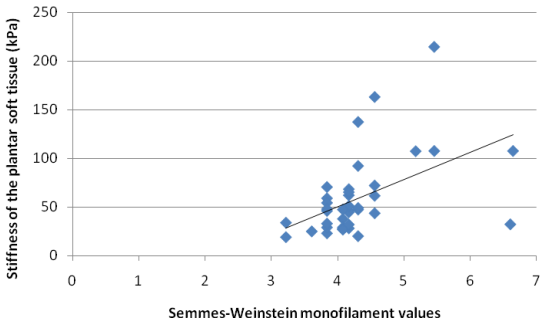
(b)



1st MTH

r=0.498, P=0.001^{+}

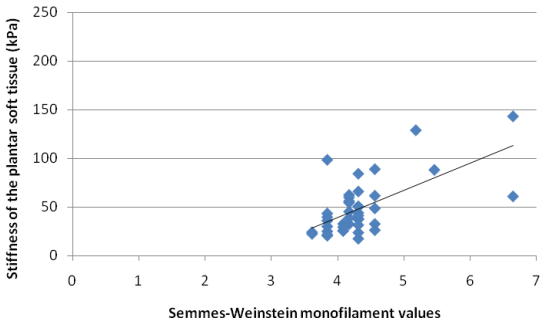
(c)



3rd MTH

r=0.484, P=0.002*

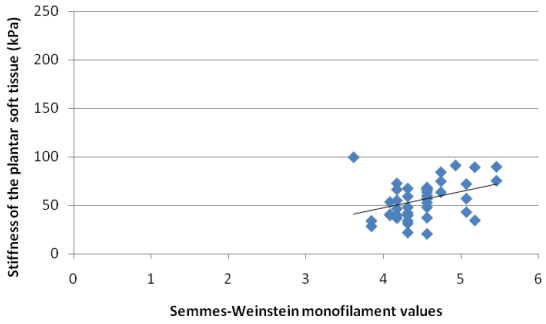
(d)



5th MTH

r=0.626, P<0.001^{+}

(e)



Heel

r=0.363, *P*=0.018*

Figure 5.2 The linear correlation between tactile sensation and the stiffness of plantar soft tissues. A significant correlation was found between tactile sensation and the stiffness at (a) the big toe, (b) first metatarsal head, (c) third metatarsal head, (d) fifth metatarsal head, and (e) heel. * P <0.05; $^{+}P \leqq 0.001$.

5.3.1.5 Correlation between tactile sensation and the thickness of plantar soft tissue in patients with diabetic ulceration

There is a fair positive correlation between tactile sensation and the thickness of the plantar soft tissues at the big toe (r=0.404). However, the correlation between tactile sensation and the thickness of plantar soft tissues at the first metatarsal head, third metatarsal head, fifth metatarsal head and heel was not significant (all *P*>0.05).

5.3.2 Study B: The changes of tissue stiffness in diabetic wound over time

The case study involved a patient with diabetic ulcer on the foot (Male; aged 75 years; body mass index 33.2).

The normalized stiffness coefficient of the central wound bed area decreased 9.56% on day 7 and continued to decrease on day 14. It slightly returned on day 21, and finally increased on day 49 (Table 5.3).

For the peri-ulcer area, the average normalized stiffness coefficient decreased 22% on day 7. However, it showed an increase of 36.66% and 22.83% as compared to the original stiffness coefficient by day 14 and day 21 respectively. Then, it decreased to 89.27% of the baseline stiffness coefficient on day 49 (Table 5.3).

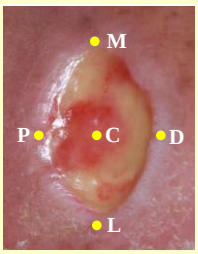
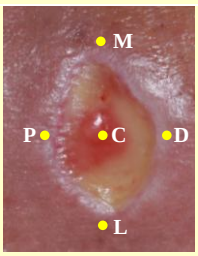
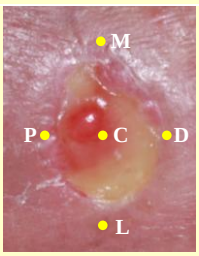
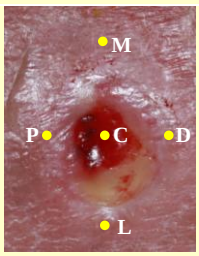
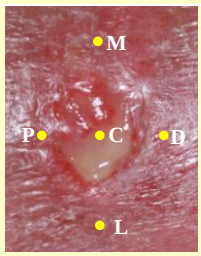
	Baseline	Day 7	Day 14	Day 21	Day 49
					
C	100	90.44(-9.56)	73.74(-26.26)	94.71(-5.29)	117.20(+17.20)
P	100	82.10(-17.90)	160.06(+60.06)	175.12(+75.12)	76.85(-23.15)
D	100	65.53(-34.47)	132.65(+32.65)	84.74(-15.26)	87.15(-12.85)
M	100	100.72(+0.72)	167.40(+67.40)	120.35(+20.35)	123.77(+23.77)
L	100	65.23(-34.77)	86.54(-13.46)	111.11(+11.11)	69.32(-30.68)
A	100	78.40(-21.61)	136.66(+36.66)	122.83(+22.83)	89.27(-10.73)

Table 5.3 The normalized value of the stiffness coefficient across the five time point, taking baseline value as 100 for each individual measurement points.

C: Central wound bed area; P: Proximal peri-ulcer area, D: Distal peri-ulcer area, M: Medial peri-ulcer area, L: Lateral peri-ulcer area; A: An average of the four peri-ulcer areas

The data in brackets are the percentage change as compared to the baseline measurement.

5.4 DISCUSSION

Peripheral neuropathy is one of the common complications of prolonged hyperglycemia. Our findings demonstrated that the tactile sensation was significantly increased in patients with diabetic ulceration as compared to the healthy control subjects. Sensory neuropathy may gradually lead to a loss of protective sensation for pain, pressure, temperature and proprioceptive awareness in the foot. Patients usually are not aware of the problem until biomechanical alteration in the foot occurred (Payne, 1998). Patients with sensory neuropathy showed a decrease in toe loading and an increase in metatarsal head pressures. The sensory attenuation hypothesis was proposed that there is a perception illusion of plantar impact (Robbins et al., 1993), so more weight is borne by the metatarsal in patients with sensory neuropathy. The repetitive impact with walking may moderate foot biomechanics. The plantar soft tissue may stiffened up, especially at

the metatarsal heads for accommodation. Thus, those patients with severe diabetic neuropathy are more prone to ulcer formation by day to day activity (Boulton, 2004).

Our findings demonstrated that the plantar soft tissue thickness was shown to be increased in patients with ulceration by around 12% to 40% as compared to the healthy control. Craig et al (2008) suggested that the thickness of plantar fascia is a predictor of peripheral nerve abnormalities and contributes to the development of neuropathy (Craig et al., 2008). In people with diabetes, hyperglycaemia causes non-enzymatic glycation and was hypothesized to increase mechanical load on the foot resulting in increased thickening of the plantar soft tissues and callus formation. As the loss of sensation in the plantar foot progresses, the patient might exert more pressure on that area involuntarily, which leads to tissue stiffening and thickening (Thomas et al., 2003). This is likely leading to formation of callus, further repetitive load may lead to callus break down and formation of ulceration.

Our findings demonstrated a positive and significant correlation between tactile sensation and the stiffness of the plantar soft tissue (r from 0.363 to 0.626, $P<0.05$). This may partly contribute to the formation of the foot ulcer formation in patients with diabetes. An increase in stiffness of collagen-rich soft tissues in diabetes has been reported in an earlier study (Reihsner and Menzel, 1998). Alterations in collagen structure and function are related to glucose-exposure, including increased fibril diameter, closer packing of fibrils and irregular fibril shapes (Layton and Sastry, 2006). Important findings demonstrated that the cross-linking of adjacent collagen fibrils and nonenzymatic glycosylation of keratin occur, causing substantial stiffening of the

affected tissues, including hyperkeratosis and formation of thickened callus in the plantar skin of people with diabetes (Duffin et al., 2002). Glycosylation has an overall effect of mechanically stiffening the plantar diabetic tissue, whereupon soft tissue that pads the metatarsal heads becomes less elastic and less able to distribute pressure through deformation (Gefen, 2003). Hence the overall cushioning property of the plantar soft tissue, especially at high-pressure sites such as those under the metatarsals is damaged. The micro-tears may develop in ulcer and in severe case, later get infected and ultimately requires lower extremity amputations. This is particularly true for people suffering from peripheral neuropathy, who are prone to developing foot ulcerations.

Study B was the first case study in utilizing the air jet indentation system for monitoring of diabetic wound tissues stiffness in a non-contact way over healing process. Our findings showed that the normalized stiffness coefficient of the central wound bed area decreased at the inflammatory stage. It slightly increased by day 21, and finally increased by day 49. For the peri-ulcer area, the average normalized stiffness coefficient decreased at the inflammatory stages. However, it showed an increase in the intermediate stages. Then, it decreased to 89.27% of the baseline stiffness coefficient at the remodeling stage. The granulation tissue starts forming at the wound region at inflammatory stage, then followed by the collagen deposition for remodelling the wound by replacing the newly synthesized type III collagen with type I collagen. This maturation of the collagen matrix contributes to the mechanical strength of dermal tissue (Lee and Moon, 2003). During the intermediate stages of wound healing, myofibroblast appears in large numbers at the wound site (Grinnell, 1994). The activation of fibroblast into myofibroblast was a vital cellular activity that affected the wound healing. Also, the

myofibroblast is the key cell for remodelling the connective tissue by enhancing the closure of wound with its contractile ability (Alizadeh et al., 2007, Thorey et al., 2004, Desmouliere et al., 2005, Prakash et al., 2007). Concurrent with the differentiation of fibroblasts is an increase in the stiffness of the wound due to extracellular matrix production and remodelling processes (Grinnell, 1994). Witte and Barbul (1997) reviewed that the myofibroblast induces the wound edges to approach for wound contraction (Witte and Barbul, 1997), so the wound closure starts from the peripheral of the wound, and hence the peri-ulcer areas were found to be stiffer at the later stage of healing in our study. After the wound has closed, the density of myofibroblast decreases and the scar tissue becomes sparsely populated by normal dermal fibroblasts as extracellular matrix remodeling slows down (Grinnell, 1994), and thus the peri-ulcer area stiffness was shown to be decreased on day 49 in our study.

Appropriate footwear and early evaluation on the biomechanical properties and tactile sensation is essential to prevent further foot complications for the diabetic population. As the sample size is small in the present study, future large scale study will be needed to confirm the findings of this case study on the changes of biochemical properties of the wound tissues over different stages of wound healing.

5.5 CONCLUSIONS

Our findings demonstrated that the biomechanical properties of the plantar soft tissues of patients with diabetic foot ulcers are significantly thicker and stiffer than healthy controls. The thickness and stiffness of unloaded plantar soft tissues is associated with

an increase in sensory loss. Our case study demonstrated that the stiffness of diabetic wound tissue change at different healing stages. The stiffness of the central wound bed area decreased during the inflammatory stage, and then become stiffer at the later stage of wound healing. The change of stiffness of the wound tissue might reflect the change in collagen content and degree of growth, remodeling and function of the cells.

CHAPTER 6

EFFECTIVENESS OF PULSED ELECTROMAGNETIC FIELDS ON PROMOTING HEALING OF DIABETIC FOOT ULCER – A RANDOMIZED CONTROLLED TRIAL

This Chapter was published as

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and received

'Dr. Robert W. Hobson II Award for Best DLS Abstract Poster Presentation 2nd Place.'

Refer to Appendix VII for published conference abstract.

We performed a systematic review to examining the evidence of commonly used electrophysical therapies for enhancing wound healing (see Appendix V). Pulsed electromagnetic therapy (PEMF) is a potential treatment that can be used to promote healing of diabetic ulcer. Previous study showed that PEMF can promote nerve regeneration and microcirculation in patients with diabetes. In this chapter, we will report the findings of a randomized controlled trial that examined the effects of pulsed electromagnetic field on promotion of diabetic wound healing in terms of restoring normal biomechanical properties of the wound tissues; facilitating microcirculation, and reducing wound size.

6.1 INTRODUCTION

The incidence of diabetes mellitus is increasing globally (World Health Organization, 2011). Non healing diabetic ulcers are the primary cause of non-traumatic amputations (Trautner et al., 1996). Diabetes impairs nearly all stages of wound healing from early homeostasis to matrix deposition and angiogenesis (Frank et al., 1995). The endothelial dysfunction and impaired nerve axon reflex activities in patients with diabetes results in poor healing of the wound. Particularly, diabetes causes an alteration in blood flow that is crucial for tissues recovery (Lioupis, 2005). The mal-distribution of blood flow between nutritive capillaries and arteriovenous shunts flow is a crucial factor leading to the development of diabetic peripheral neuropathy and ulceration. Therefore, to restore the microvascular function is important in preventing non- healing wound in patients with diabetes.

Diabetes may lead to an alteration in blood flow especially decrease the blood flow in the nutritive capillaries. The fibroblast growth factor and vascular endothelial growth factor that are critical to blood vessel formation and wound recovery are also found deficient (Bitar and Labbad, 1996, Brown et al., 1997). In Chapter 5, we showed that there are changes in biomechanical properties of wound tissue at different stages of healing. The wound tissue stiffness of the peri-ulcer area decreased at the inflammatory stage, increased at the proliferative stage and decreased at the remodeling stage. And at the central wound bed, there was decrease in inflammatory stage in wound tissue stiffness, and it became stiffer at the later stage. The ideal wound healing is to restore wounded tissue back to its structural and functional level before wounding. The central and peri-wound area were shown to be stiffer when compared to the healthy tissues, and was suggestive that the stiffness of soft tissue is site specific (Chao et al., 2011). It was suggested that the restoration of an optimal mechanical strength of the wound tissue at its respective stages of healing is essential and the stiffness measured from the soft tissues can serve as reference for wound repair (Chao et al., 2010, Chao et al., 2011).

For clinical studies, PEMF was shown to be an effective treatment in promoting nerve regeneration (Musaev et al., 2003) and improving microcirculation (Webb et al., 2003) in patients with diabetes, which is a major risk factor for developing diabetic ulceration. Previous study showed that PEMF is a safe and effective therapy for managing neuropathic pain for patients with peripheral neuropathy, in reducing vibration perception threshold and number of insensitive points tested by Semmes-Weinstein monofilament testing (Bosi et al., 2005). Moreover, the PEMF was shown to have a positive impact on pain (Wrobel et al., 2008). The PEMF could also enhance the

transcutaneous partial pressure of oxygen measurements and Doppler flow in patients with diabetes without complications (Webb et al., 2003). Hence PEMF might be a potential modality to restore microcirculatory dysfunction and biomechanical properties changes associated with diabetic ulcers.

Based on the findings of animal studies, PEMF has been shown to accelerate wound closure, promote angiogenesis, and prevent tissue necrosis in diabetic mice (Callaghan et al., 2008). Research evidences have shown that PEMF can enhance the healing of venous leg ulcers (Ravaghi et al., 2006).

Therefore, PEMF is a potential treatment that can be used to promote healing of diabetic ulcer with the comorbidities of neuropathy and /or tissue ischemia. We hypothesize that PEMF could enhance the healing of diabetic ulcer in human. The present study examined the effectiveness of electromagnetic therapy for promoting the healing of diabetic foot ulcers in terms of restoring normal biomechanical properties of the wound tissues; facilitating microcirculation, and reducing wound size.

6.2 METHODS

6.2.1 Study Design

This prospective, randomized, double blinded controlled study was designed to examine the effectiveness of pulsed electromagnetic field (PEMF) in the management of diabetic ulcers, as compared to a sham device.

6.2.2 Subjects

Thirteen subjects (7 for experiment group and 6 for sham group) were recruited from Kwong Wah Hospital. Each potential subjects of this study was screened by a registered podiatrist, and their ankle brachial index (ABI) should fall between 0.9 and 1.2 to reassure that arterial insufficiency was not causing or contributing to the non-healing wound (Brem et al., 2004).

People diagnosed with Type 2 diabetes with unsatisfactory healing of ulcer(s) in the preceding 4 weeks were recruited from a local hospital. Written consent was obtained from the subjects. Only Wagner grade 1 or 2 ulcers on the foot without exposure of bone, muscle, ligaments or tendons were considered. Exclusion criteria were ulcers secondary to non-diabetic etiology, poorly-controlled diabetes, pregnancy, and people wearing a pacemaker, malignancy, or history of radiotherapy. Subjects who were taking medications that might affect their circulation, sensation and motor functioning, and subjects with any history of alcoholism were also excluded. In order to recruit a more homogeneous sample, subjects who had clinical signs of infection or were taking antibiotics at the time of enrollment were also excluded. The patients were informed of the daily treatment protocol and follow-up schedule in order to increase their compliance of completing the whole study.

6.2.3 Sample Size Estimation

Sample size was estimated by the software PASS. Sixty subjects (30 with diabetic ulcer and 30 sham group) will be needed with alpha level of 0.05 and power equals to 0.8. With about 30% drop out rate, at least eighty subjects (40 for experiment group and 40 for sham group) would be recruited.

6.2.4 Standard of Care Treatment

The subjects were recommended to stay with the same medication and diet habit throughout the study period. Standard foot care education using consistent procedures was provided to each patient at the time of enrollment.

6.2.5 Treatment protocol

Subjects were randomly allocated to either the active PEMF or control (sham) group using a computer-generated randomization table. During the treatment period, subjects lied in a supine position comfortably with knee fully extended and supported with a foot stand. The foot stand provided support to the lower leg and ankle so that subjects could maintain a neutral ankle position throughout the treatment. The PEMF System (model XKC-600W, manufactured by Magnetopulse International, Australia) was used. The frequency of PEMF was delivered at 12Hz, with magnetic flux density of 12G. Each treatment session lasted for 1 hour, which repeated for 14 sessions within three weeks. Subjects remained in the study until the wound healed or 14 sessions were completed,

whichever came first. The sham PEMF group received treatment by using the same PEMF unit, but the output was controlled by a switch box (either channel A or B). The internal circuit of the applicator was disconnected by choosing one of the channels of the switch box. When the sham channel was selected, the light of the control panel and the timer was turned on just in the same way as if the active channel was selected, but no pulsed electromagnetic field was delivered to the wound bed. Subjects did not have any special sensation no matter if they received active or sham PEMF. Subjects and investigators were blinded from the group allocation, only the technician who made the switch box knew which channel delivered active PEMF. After the data analyses were done, the investigator was informed that channel B was the active PEMF channel. Details of the PEMF unit please refer to Chapter 3 Section 3.5. The subjects received individual treatment to prevent discussion among them.

6.2.6 Outcome Measures

Assessments of the healing of ulcers were done at the baseline, after fourteen treatment session, and one-month follow-up (Figure. 6.1). Outcome measures included microcirculation, biomechanical properties of the wound bed and wound closure assessment.

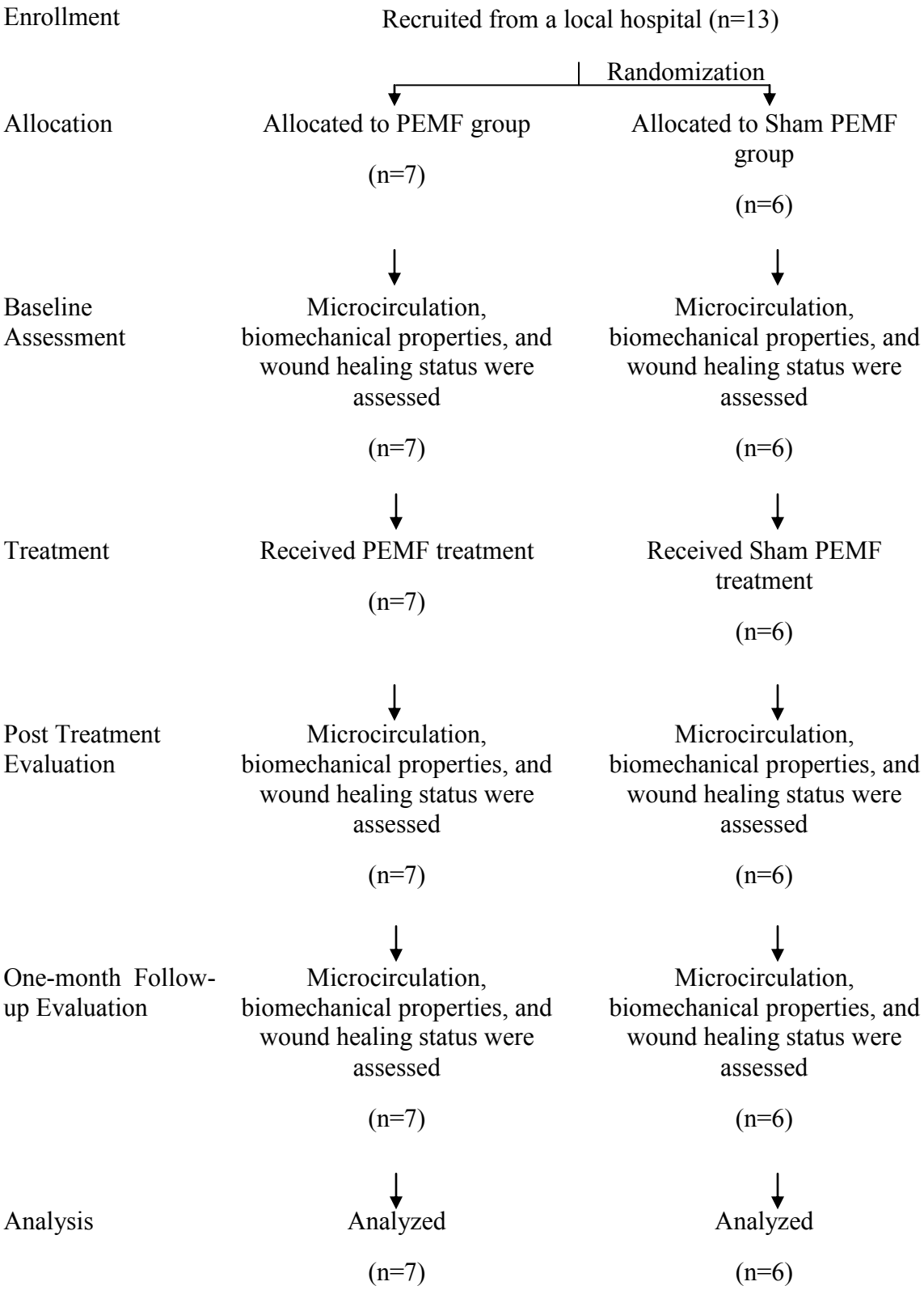


Figure 6.1 Flow diagram for subjects participated in the randomized controlled trial

6.2.6.1 Microcirculation

Video capillaroscopy is commonly used to investigate the blood velocity of the nutritive capillaries in a direct manner (Abbink et al., 2001). The microcirculation of the nutritive capillaries over the big toe nail fold was examined by video capillaroscopy system (CapiScope, Version 3.33.0.0, KK Technology, Devon, England). The diameter and the blood flow velocity of the region were identified. The mean capillary blood velocity of the capillaries was calculated and reported. Technical details of this instrument please refer to Chapter 3 Section 3.2.1.

In contrast, laser Doppler flowmetry examines the compartment flow occurring in underlying structures including arterioles, venules and arteriovenous anatomises. The Laser Doppler Flowmetry (DRT 4, Moor Instruments Ltd, Devon, England) was used to assess the skin blood flow (flux) of the microcirculation at the pulp and the nail fold of the big toe. Details of this instrument refer to Chapter 3 Section 3.2.2.

6.2.6.2 Biomechanical Properties of Wound Tissue

The stiffness of the diabetic wound tissue was assessed by the optical coherence tomography (OCT) based air jet indentation system (Chao et al., 2011). The central portion of the ulcer, and the proximal, distal, medial and lateral edges of peri-wound areas were tested. The stiffness coefficient k (N/mm), that is the slope of the force-deformation relationship, was then calculated from each trial, and it was used to represent the biomechanical properties of the wound tissue. Two trials were conducted

and averaged to represent the data for each testing sites. As the stiffness of wound tissue is site specified (Chao et al., 2011), data were normalized to 100% of the baseline wound tissue stiffness for comparisons between subjects. Technical details of this instrument please refer to Chapter 3 Section 3.3.2.

6.2.6.3 Evaluation of wound healing

The wound cavity was filled with a wound ultrasound transmission gel (SonoView^{TM} Wound Mapping Gel, Hudson Diagnostic Imaging, Elmwood Part, New Jersey) and then covered with a sterile disposable acrylic adhesive film dressing (Opsite^{TM} Flexigrid^{TM}, Smith & Nephew, Hull, UK). A small individual package of ultrasound transmission gel was used to fill the wound cavity and to eliminate any dead space or air pockets. An ultrasound biomicroscopy (Vevo 770 High Resolution Imaging System, VisualSonics, Canada) utilizes a linear array transducer with a frequency of 55MHz. Longitudinal and transverse scans were obtained by placing the ultrasound probe in either direction. The wound depth was calculated using the software provided by the ultrasound system. For technical details of the ultrasound biomicroscopy unit please refer to Chapter 3 Section 3.4.1.

The size and colour of the most prominent ulcer for each subject was captured with a high resolution digital camera (10.0 Megapixel). The distance of the camera was fixed with a stand and the light source from the environment was kept constant. The image of the ulcers together with the standard square of 3cm^2 was taken by the digital camera,

and the size of the ulcers by tracing the boundaries with VeV wound measurement system (Vista Medical Ltd, Canada). Details of the wound measurement system refer to Chapter 3 Section 3.4.2.

6.2.7 Data Analysis

IBM® SPSS® Statistics 17.0 (IBM Corporation, Chicago, IL) was used for analysis. Descriptive characteristic between the treatment group and the sham group was analyzed by independent *t*-test, and gender was analyzed by Chi-square test between groups. Repeated measures ANOVA was performed to investigate the change in outcome measures over time (within-group), and between groups, followed by Bonferroni post-hoc test. 'Intention to treat' analysis was used for any subjects who drop out of the study. Statistical significance was set at $p<0.05$ for all tests.

6.3 RESULTS

6.3.1 Descriptive Statistics

A total of 13 subjects completed the study, with no drop out. Demographic characteristics and baseline variables of our subjects are presented in Table 6.1, and no statistical difference was found between the PEMF group and the sham PEMF group.

Table 6.1 Demographic characteristics (mean (SD)) for PEMF group and sham PEMF group

	PEMF group n = 7	Sham PEMF group n = 6	*P-value*
Gender (M/F)	5/1	5/2	.563
Age (Years)	61.5 (15.2)	63.8 (9.2)	.757
Body mass index (kg/m²)	26.6 (4.6)	27.3 (4.9)	.846
Duration of diabetes (Years)	9.3 (7.9)	13.8 (6.5)	.287
Duration of ulcers (Years)	1.8 (1.9)	1.3 (1.8)	.629
Grading of ulcers located at different sites#	Fourth toe (1) Metatarsal head (1) Metatarsal head (2) Metatarsal head (2) Plantar foot (1) Plantar foot (2) Dorsal foot (2)	Big toe (1) Big toe (2) Second toe (2) Fifth toe (1) Metatarsal head (1) Metatarsal head (2)	-

Each wound was graded by Wagner Grading system.

6.3.2 Microcirculation

Repeated measures ANOVA demonstrated a significant group-vs-time interaction in capillary diameters (P=0.011). At the post treatment evaluation, a 14% increase in capillary diameters (from 9.81±2.48 μm to 11.19±3.58 μm) (P= 0.928) was found in the PEMF group as compared to an 18% decrease (from 11.02±1.49 μm to 8.97±1.88 μm) (P=0.368) in sham PEMF group was found (Figure 6.2a). The between-group difference in capillary diameters did not reach significance (P=0.200).

At the one-month follow-up, a significant between-group difference was found in the capillary diameters (P= 0.038). The capillary diameters continued to increase 10% (from 11.19±3.58 μm to 12.32±2.72 μm) (P=0.070) in the PEMF group, whereas there was an 8% decrease in capillary diameters for the sham PEMF group (from 8.97±1.88 μm to 8.27±3.12 μm) (P=1.000) as compared to the baseline measurement although both group did not reach statistical significance (Figure 6.2a).

In contrast, we found a 28% increase in capillary blood velocity (from 57.40±18.88 μm/s to 73.37±22.20 μm/s) (P=0.450) in the PEMF group, but a negligible change (from 50.36±32.85 μm/s to 49.43±17.58 μm/s) (P= 1.000) in sham PEMF group (Figure 6.2b) at the post treatment evaluation, a marginal non-significant difference in the capillary blood velocity was found between the groups (P=0.057).

As compared to the post treatment evaluation, there was significant increase in capillary blood velocity (from 73.37±22.20 μm/s to 103.29±30.25 μm/s) (P= 0.006) observed in

the PEMF group, but only a 6% increase (from 49.43±17.58 μm/s to 52.38±5.94 μm/s) (*P*= 1.000) in the sham PEMF group (Figure 6.2b). A significant difference was found in the capillary blood velocity between groups at the one-month follow-up (*P*= 0.004).

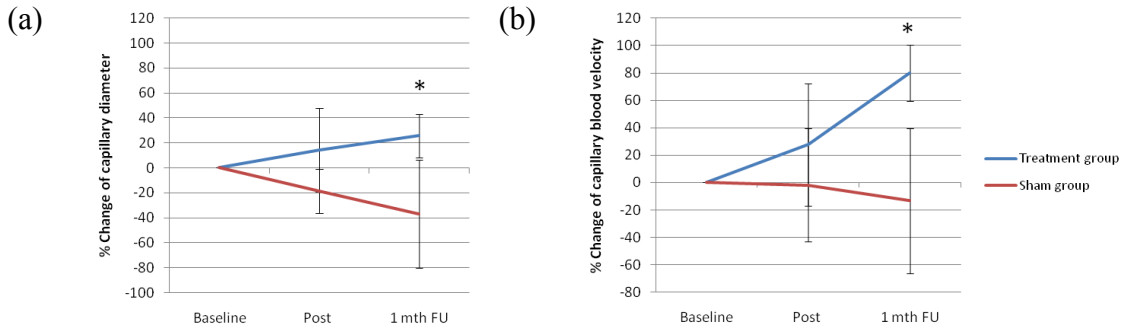


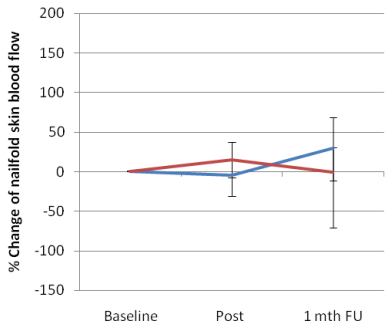
Figure 6.2 The mean percentage change in (a) capillary diameter and (b) capillary blood velocity measured by the video capillaroscopy. *$P<0.05$.

The skin blood flow (flux) of the PEMF group measured at the nailfold decreased from 52.20±31.47 perfusion unit to 49.63±21.42 perfusion unit at the post treatment evaluation, and increased to 67.43±26.63 perfusion unit at one-month follow-up (*P*=0.154). For the sham PEMF group, the skin blood flow (flux) measured at the nailfold slightly increased from 41.78±20.86 perfusion unit to 47.95±24.61 perfusion unit at post treatment evaluation, then returned to 41.42±44.07 perfusion unit at the one-month follow-up (*P*=0.814). No significant difference was found between the two groups at the post treatment evaluation (*P*=0.898) and the one-month follow-up (*P*=0.216) (Figure 6.3a).

Repeated measures ANOVA demonstrated a significant group-vs-time interaction in skin blood flow (flux) measured at the pulp of big toe (*P*=0.011). At the post treatment evaluation, there was a 48% increase (from 126.13±80.66 perfusion unit to 186.89±91.09 perfusion unit) in skin blood flow (flux) measured at the pulp of big toe in the PEMF group (*P*=0.486). In contrast, there was a 16% decrease (from 186.93±142.24 perfusion unit to 151.46±62.68 perfusion unit) in skin blood flow (flux) measured at the pulp of big toe (Figure 6.3b) for the sham PEMF group (*P*=1.000). However, no significant difference was found between the two groups at the pulp of big toe at post treatment evaluation (*P*=0.440).

At the one-month follow-up, the mean percentage change of pulp of big toe skin blood flow was significantly different between two groups (*P*=0.048). The skin blood flow (flux) measured at the pulp of big toe in the PEMF group continued to increase for 22% (from 186.89±91.09 perfusion unit to 228.84±193.79 perfusion unit) (*P*=0.443). For the sham PEMF group, a 44% decrease (from 151.46±62.68 perfusion unit to 84.92±58.34 perfusion unit) in skin blood flow (flux) was observed at the pulp of big toe (Figure 6.3b) (*P*=0.117).

(a)



(b)

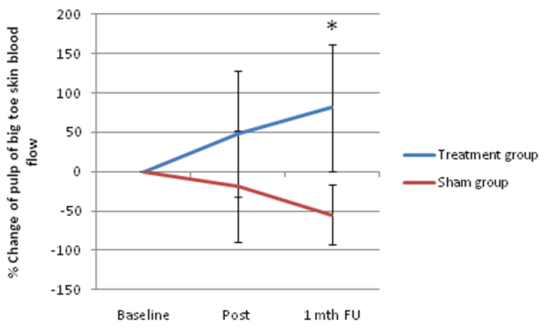


Figure 6.3 The mean percentage change in skin blood flow (flux) at the (a) nailfold and (b) pulp of big toe measured by laser Doppler flowmetry. *P<0.05.

6.3.3 Biomechanical Properties of Wound Tissue

The average stiffness coefficient of the peri-ulcer area of the PEMF group decreased from 0.39±0.17 N/mm to 0.36±0.08 N/mm at the post treatment evaluation, and maintained at 0.39±0.09 N/mm at the one-month follow-up (P= 0.450). For the central wound bed, the average stiffness coefficient decreased from 0.36±0.18N/mm to 0.34±0.18 N/mm at the post treatment evaluation, and further decreased to 0.27±0.04 N/mm at the one-month follow-up (P= 0.540).

After normalization, the peri-ulcer area of the PEMF group decreased 6% at the post treatment evaluation, and returned to 102% at the one-month follow-up (P= 0.609). For the central wound bed, a negligible change was found in the normalized average stiffness coefficient at the post treatment evaluation, and further decreased to 85% at the one-month follow-up (P= 0.668) (Figure 6.4).

The sham PEMF group showed similar trend at the post treatment evaluation, the average stiffness coefficient of the peri-ulcer area decreased from 0.42±0.18 N/mm to 0.30±0.20 N/mm, then it increased to 0.38±0.18 N/mm at the one-month follow-up (P= 0.407). For the central wound bed, the average stiffness coefficient decreased from 0.41±0.20 N/mm to 0.25±0.16 N/mm (P= 0.394), but increased to 0.37±0.21 N/mm at the one-month follow-up (P= 0.239).

The peri-ulcer area of the sham PEMF group decreased 24% at the post treatment evaluation after normalization, and returned to 111% at the one-month follow-up (*P*= 0.363). For the central wound bed, 30% decrease was found in the normalized average stiffness coefficient at the post treatment evaluation, and further returned to 106% at the one-month follow-up (*P*= 0.296) (Figure 6.4).

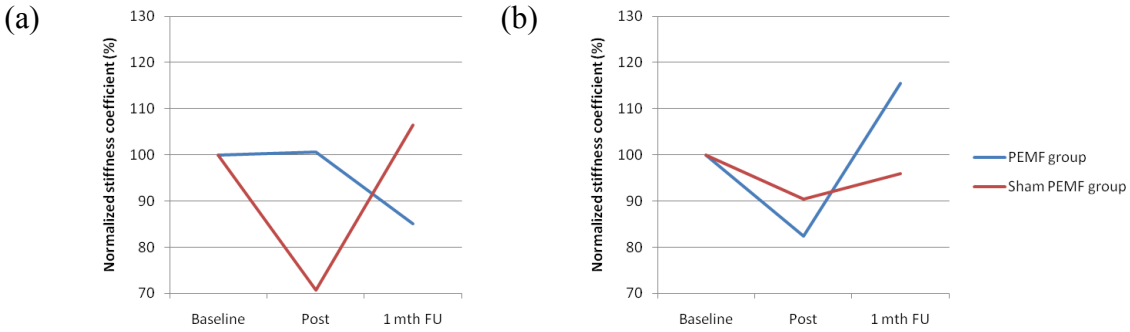


Figure 6.4 Normalized mean stiffness coefficient of the wound tissues at (a) central wound bed and (b) peri-ulcer area among PEMF and sham PEMF groups.

6.3.4 Wound Assessment

Over time, the wound surface area decreased in both PEMF group and sham PEMF group although it did not reach a significant difference between the groups (*P*=0.215), with a 18% decrease (from 3.15±5.23 cm^2 to in 2.58±4.27 cm^2) (*P*= 0.822) in the PEMF group and a 4% decrease (from 0.28±0.27 cm^2 to in 0.27±0.28 cm^2) (*P*= 1.000) in the sham PEMF group. At the one-month follow-up, the average wound size of the PEMF group decreased from 2.58±4.27 cm^2 to 1.68±2.79 cm^2 (*P*= 0.568) whereas the wound

size of the sham PEMF group decreased from 0.27±0.28 cm^2 to 0.07±0.12 cm^2 (*P*= 0.406) (Figure 6.5).

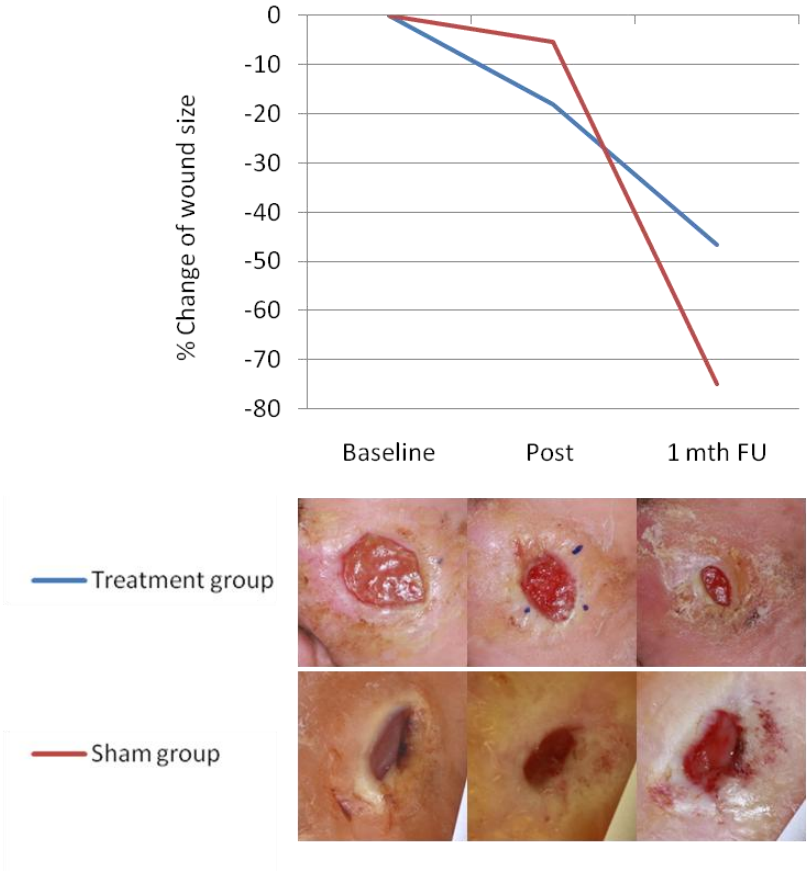


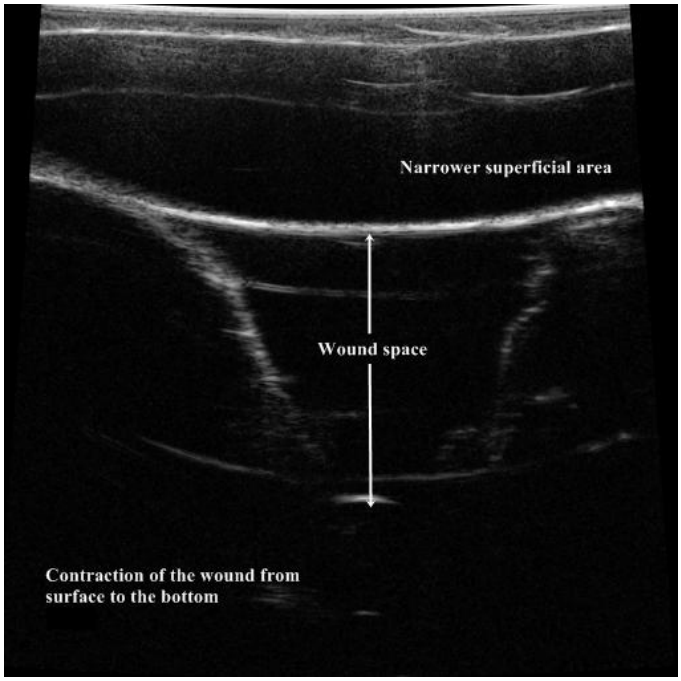
Figure 6.5 Mean percentage change in wound size in the PEMF group and sham PEMF group. Typical wound image were taken from one subject each from the PEMF group and sham PEMF group.

The wound depth can be more clearly defined in the ultrasound scan than in the photographs. The wound depth decreased significantly in the PEMF group (*P*= 0.021).

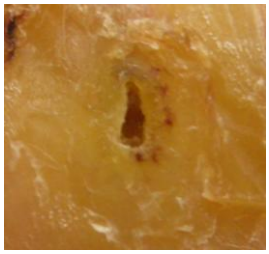
Post hoc analyses showed that the wound depth significantly decreased to 1.47±1.23 mm at one-month follow-up evaluation as compared with baseline (P= 0.028). However it did not reach statistical significance after 14 treatment sessions in the PEMF group (from 2.91±0.95 mm to 2.08±1.09 mm) (P= 0.454). For the sham PEMF group, the wound depth decreased from 1.18±0.92 mm to 0.76±1.00 mm at the post treatment evaluation, and further decreased to 0.47±0.53 mm at the one-month follow-up (P= 0.157).

The ultrasound biomicroscopy images demonstrate acoustically distinguishable structures throughout the depth of each wound. From a typical image taken from a patient with diabetic ulcers in the PEMF group, the wound space can clearly be seen on the baseline evaluation (Figure 6.6 Ia). The increase echogenicity at the post-treatment evaluation showed an increase in the deposition of collagen fibres as the granulation tissue is replaced by the scar tissue. The surface area of the wound varies between subjects as the splinting action is lost after eschar dehiscence or necrotic tissue removal (Figure 6.6 IIa). At the one-month follow-up, the epidermis has regenerated sufficiently for wound closure (Figure 6.6 IIIa).

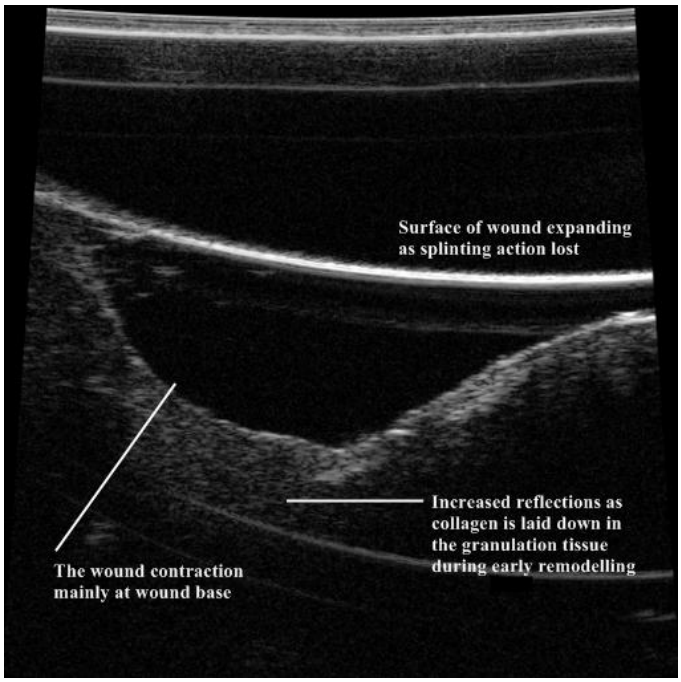
I(a)


Narrower superficial area
Wound space
Contraction of the wound from surface to the bottom

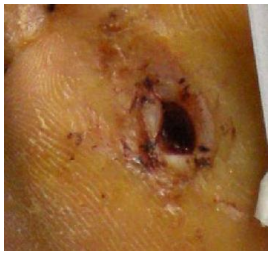
(b)

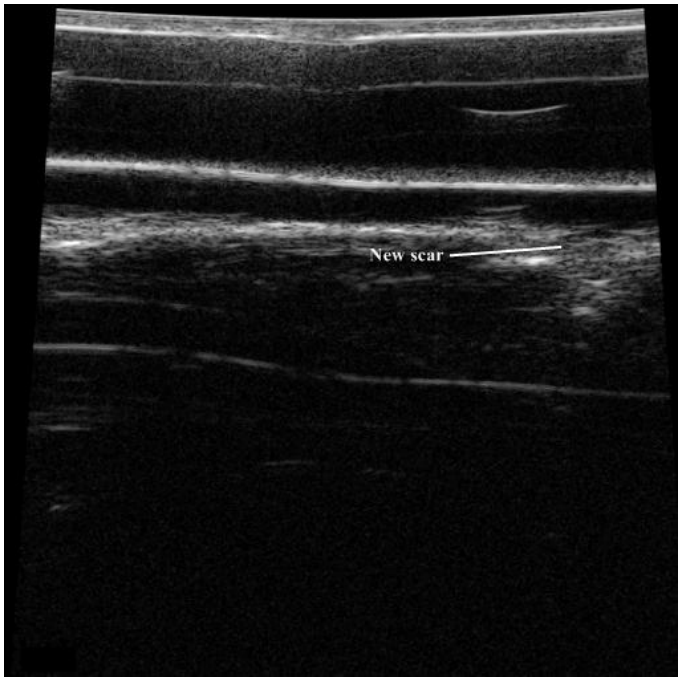


II(a)


Surface of wound expanding as splinting action lost
Increased reflections as collagen is laid down in the granulation tissue during early remodelling
The wound contraction mainly at wound base

(b)



III(a) 

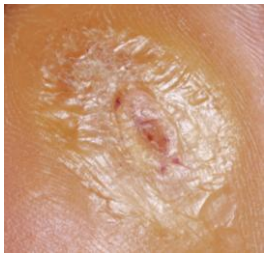
(b) 

Figure 6.6 (a) Ultrasound biomicroscopy image taken from a typical diabetic patient in the PEMF group and (b) Photographs of the wound surface taken on baseline evaluation (I), post-treatment evaluation (II), and the one-month follow-up (III).

6.3.5 Power analysis

For a post hoc power analysis, it was calculated that to reach significance differences in capillary diameter (power = 0.63); capillary blood velocity (power = 0.95); pulp of big toe skin blood flow (power = 0.99); nailfold skin blood flow (power = 0.18); biomechanical properties of peri-ulcer area (power = 0.15); biomechanical properties of central wound (power = 0.29); wound depth (power = 0.91) and wound area (power = 0.98), with the variance and differences observed between groups in the present investigation, we should include at least 60 subjects in each group.

6.4 DISCUSSION AND CONCLUSIONS

This is the first randomized, double-blinded controlled trial to investigate the effects of PEMF on diabetic foot ulcer healing. From our systematic review, a previous study that used PEMF in treating diabetic ulcers were methodological weak or treating the stump wound rather than foot ulcer which is having a different etiology (Isakov et al., 1996). The present study included a sham control group, and standardized the vascular status of the patient population, by enrolling patients with ankle brachial index that was between 0.9 and 1.2 (Brem et al., 2004), as well as controlling the ulcer severity by selecting patients with ulcers of either Wagner grade one or two that were free from infection. The current report comprises on comprehensive assessment on diabetic ulcer healing, including the microcirculation, biomechanical properties of the ulcer tissue, as well as clinical monitoring in wound healing status. Previous studies mainly focused on wound size measurement that was found inconsistent and not reliable, thus we incorporate the ultrasound biomicroscopy for measuring the wound depth for a better monitoring on wound healing status. This is the also the first trial describing the wound tissue stiffness as a quantitative outcome measure that might be an essential component in wound healing to prevent ulcer recurrence.

Our findings demonstrated that PEMF treatment can elicit vasodilation and increase the blood flow particularly in the nutritive capillaries. In contrast, the subjects in the sham group showed a decrease capillary diameters and blood flow during the study period. These pathological changes of nutritive capillaries in the subjects of the sham group might be caused by the arteriovenous shunting, which is typically found in patients with

diabetes (Flynn and Tooke, 1995, Veves et al., 1998). It was suggested that PEMF may modify the control in intracellular concentration of calcium ions, and modulate blood flow via calcium ion channels (Okano et al., 2005b). Another proposed mechanism is the generation of free radical nitric oxide which is recognized as an endogenous modulator of microvascular permeability (McNeely et al., 1995, Okano et al., 2005a). Previous studies showed that persistent dilation of arterioles can lead to angiogenesis (Yuan et al., 1990). Also, the increase in microcirculation through the soft tissue might affect the inhibition of inflammation and accelerate the cell proliferation (Callaghan et al., 2008), thus might partly attributed in promoting wound healing. As the microcirculation continued to improve in the one-month follow-up period, this indicates that PEMF produces carry over effects beyond the treatment period.

Our study showed that both the PEMF and sham PEMF group had the same decreasing trend in peri-ulcer tissue stiffness at the post treatment session but an increase at the one-month follow-up. This followed a similar trend as the case study reported in Chapter 5. The present study demonstrated that the PEMF group had a larger degree changes as compared to the sham PEMF group, but no significant between-group difference was found. The ability of a healing wound to resist rupture when stressed depends on both tensile strength and elasticity (Forrest, 1983). In the early stage of healing, both central wound bed area and peri-ulcer area showed decrease tissue stiffness, this allows considerable elasticity with the connective tissue fibers existing as a loose network. At the intermediate stage of healing, the increase in mechanical stress not only control the contractile activity of the granulation tissues and myofibroblast differentiation (Hinz et al., 2001), but also guide the collagen and affect the extent of

scarring (Farahani and Kloth, 2008). A previous study reported that pulsed electromagnetic field can modulate cell proliferation and angiogenesis (Callaghan et al., 2008). In particular, PEMF showed an increase in cell proliferation induction on dermal fibroblast and keratinocyte epithelial cells (Huo et al., 2010). The fibroblast growth provides traction force on extracellular matrix fibrils and brings collagen fibrils closer to each other to enhance wound closure (Lee and Moon, 2003). Future study with a large sample size should be done to confirm if PEMF was a potential tool in promoting ulcer healing in terms of regulating wound tissue biomechanical properties.

The surface area and wound depth reduced in both treatment and sham group over time in our study, also the ulcer continued to heal after the cessation of treatment. The rate of wound closure was not significantly different between the PEMF and sham PEMF groups. However, the quality of ulcer healing varied in the two groups. It could be explained by the effects of PEMF on the underlying hemodynamic problem of diabetic foot, which must be corrected to prevent the high recurrence of diabetic ulcers. Photographing the wound was a common method used in clinical settings to monitor wound healing, however the splinting action of the scab interfered with the wound surface area (Dyson et al., 2003). In combination with the use of ultrasound biomicroscopy image, we could also monitor the granulation tissue contraction that provides better understanding in the changes of morphology during the wound healing. However, due to the small sample size, our results did not reach statistical significance. Also, the ulcers of patients recruited were present at different site on the feet, the thickness of plantar soft tissue and the microcirculation distribution at the different sites

might affect the treatment outcome. Further study with a larger sample size will be needed to confirm the present findings.

As a conclusion, PEMF appears to produce a positive influence on improving the microcirculation, biomechanical properties of wound tissue, as well as rate of wound closure in patients with diabetes.

CHAPTER 7

CONCLUSIONS

Peripheral neuropathy and peripheral arterial disease are the common causes of diabetic foot ulcers (Kumar et al., 1994), which may lead to infection, gangrene or even lower limb amputations. Ageing is another additional factor that results in delay in wound healing. Recent research findings suggest that the change in microcirculation and biomechanical properties of plantar soft tissue is a potential risk factor for developing foot ulcer (Cavanagh et al., 1993, Bevans, 1992). In addition, the biomechanical changes on the wound tissue can reflect the healing stages of diabetic ulcer.

In Study I, we demonstrated that the biomechanical properties of plantar soft tissues appear to change with age in healthy individuals. The stiffness of unloaded plantar soft tissues significantly increased with age at the big toe, first metatarsal head, third metatarsal head, fifth metatarsal head and the heel; and there is a trend of increasing thickness of the plantar soft tissue with advancing age. There is also a strong positive correlation between age and the stiffness at all measurement sites. The altered biomechanical properties in plantar soft tissues may be responsible for the higher incidence of foot problems in elderly individuals. This may partly explain why elderly people tend to have difficulties in controlling their posture, and are thus prone to injurious falls (Boyd and Stevens, 2009). Future study in evaluating the ageing effect on the biomechanical properties in weight bearing position may be warranted.

In Study II, we examined the biomechanical properties of plantar soft tissue in patients with diabetic ulcers, as compared to age-matched subjects. Also, we explored the relationship between the biomechanical properties of plantar soft tissues and tactile sensation. Specifically, we conducted a case study that used optical coherence

tomography (OCT) based air jet indentation system in investigating the wound tissues biomechanical properties in a patient with diabetic foot ulcer over healing stages in a non-contact manner. Our findings demonstrated that the biomechanical properties of plantar soft tissues of the patients with diabetic foot ulcers are significantly thicker and stiffer than the healthy controls. Also, there was a positive and significant correlation between tactile sensation and the stiffness of the plantar soft tissue. This may partly contribute to the formation of the foot ulcer in patients with diabetes particularly those with sensation loss. The case study demonstrated that the stiffness of diabetic wound tissue change at different healing stages. The normalized stiffness coefficient of the central wound bed area decreased at the inflammatory stage, and this considerable elasticity when the connective tissue fibers existing as a loose network. It slightly increased in the intermediate stage, and become stiffer for the later stage in collagen deposition. The change of stiffness of the wound tissue might reflect the degree of growth, remodeling and function of the cells, and hence the biomechanical properties of wound tissues measured by the non contact air jet indentation system could act as a potential outcome measure for diabetic wound healing.

In Study III, this thesis conducted a systematic review to examine the evidence of the commonly used electrophysical modalities in promoting wound healing and found that very few randomized controlled trials on electrophysical modalities such as ultrasound, lasers, and electromagnetic therapy. Among these modalities, pulsed electromagnetic field (PEMF) is a modality that can be delivered in a non contact manner that prevents the chance of infection. In the last part of our study, a randomized controlled trial was conducted and demonstrated that pulsed electromagnetic therapy appear to be a useful

adjunct treatment for diabetic foot ulcers. With 60-min daily PEMF treatment delivered at 12Hz and 12Gauss, the PEMF group shown a significant improvement in microcirculation as compared to the sham PEMF group, and this might be important to prevent ulcer recurrence. The wound tissue was found to be more extensible in the PEMF group. There was slight decrease in the normalized average stiffness coefficient in the central wound bed at the post treatment evaluation, and further decreased to 85% at the one-month follow-up at the PEMF group, while there was a 30% in the normalized average stiffness coefficient at the post treatment evaluation, and further returned to 106% at the one-month follow-up of the sham PEMF group. Also, the PEMF group had an average of 18% decreased in wound size as compared to a 10% decrease in the sham PEMF group, however the group difference did not reach statistical significance. As the sample size of this study is small, a larger scale randomized controlled study is needed to confirm the effectiveness of PEMF on promoting healing of diabetic ulcer.

APPENDICES

APPENDIX I

醫院管理局
HOSPITAL AUTHORITY

Quality Patient-Centred Care Through Teamwork

2 October, 2009

Dr YIP Siu Leung
Resident,
Department of Orthopaedics & Traumatology
Kwong Wah Hospital

Dear Dr. YIP,

KWC-CREC Reference: KW/EX/09-087

Effectiveness of pulsed electromagnetic fields on the healing of diabetic foot ulcer – a randomized controlled trial

The Kowloon West Cluster Clinical Research Ethics Committee (KWC-CREC) is authorized by the Cluster Chief Executive to review and monitor clinical research. It serves to ensure that research complies with the Declaration of Helsinki, ICH GCP Guidelines, local regulations and HA policy. It has the authority to approve, require modifications in (to secure approval), or disapprove research. This Committee has power to terminate / suspend a research at any time if there is evidence to indicate that the above principles and requirements have been violated.

KWC-CREC has approved your research application on 16 September, 2009 by expedited review process, and reached the following decision on the documents submitted as shown below. You are required to adhere to the attached conditions.

Study site(s)	**Kwong Wah Hospital**
Document(s) approved	I. Clinical Research Ethics Review Application Form (revised on 26 August, 2009) II. Research Protocol dated 16 September, 2009 III. Research Project Informed Consent Form – Chinese Version (revised on 10 September, 2009) IV. Research Project Informed Consent Form –English Version (revised on 10 September, 2009)
Document(s) reviewed	I. CV of Principal Investigator II. Investigator's Conflict of Interest Declaration Form of the Principal Investigator
Conditions	1. Do not deviate from, or make changes to the study protocol without prior written REC approval, except when it is necessary to eliminate immediate hazards to research subjects or when the change involves only logistical or administrative issues. 2. Apply a clinical trial certificate from Department of Health if indicated. 3. Report the followings to KWC-CREC* : (i) study protocol or consent document changes, (ii) serious adverse event, (iii) study progress (iv) new information that may be relevant to a subject's willingness to continue participation in the study. 4. Report first study progress to KWC-CREC at **12-monthly intervals** until study closure. [*Forms are available from KWC-CREC intranet webpage]

Please quote the CREC Reference **(KW/EX/09-087)** in all your future correspondence with the KWC-CREC, including submission of progress reports and requesting for amendments to the research protocol.

If you have any inquiry, please feel free to contact Ms Catherine CHENG, Secretary of the KWC-CREC, at 2990 3749. Thank you for your attention.

Yours sincerely,

(Dr TSAO Yen-chow)
Chairperson
Clinical Research Ethics Committee
Kowloon West Cluster

c.c. HCE, KWH

Secretariat of Clinical Research Ethics Committee, Kowloon West Cluster
Room 133, Block J, Princess Margaret Hospital, Lai Chi Kok, Kowloon, Hong Kong Tel (852) 2990 3749 Fax (852) 2990 1039

APPENDIX II

Research Project Informed Consent Form

Research title: Effectiveness of Pulsed Electromagnetic Fields (PEMF) on the healing of diabetic foot ulcer – a randomized controlled trial

You are invited to participate in a research. Before you decide, it is important that you understand why the research is done and how you will be involved. Please read the information carefully and discuss it with friends, relatives and your family doctor if you wish. Ask if there is anything unclear or if you wish to obtain more information. Take time to decide whether you wish to participate in the research.

The purposes of this study are (i) To examine the effectiveness of pulsed electromagnetic field energy (PEMF) in promoting healing of diabetic ulcer and (ii) To monitor the healing process of diabetic ulcer, and investigate the correlation between morphological changes in human skin tissue, biomechanical properties (i.e. stiffness and thickness) in the plantar tissues, microcirculation as measured by blood perfusion, blood flow velocity and diameter of capillary and wound size at various stages of healing.

Eighty people aged above eighteen diagnosed with Type II diabetes, with unsatisfactory healing of ulcer(s) in the preceding 4 weeks will be recruited from Kwong Wah Hospital. It is up to you to decide whether to participate or not. If you decide to participate, you will keep this information sheet and sign a consent form. You will be free to withdraw at any time and without giving a reason. This will not affect the standard of care you receive.

Sometimes we do not know which treatment is the best. Therefore, we need to make comparisons. Subjects recruited will be selected by chance and divided into groups. Subjects in each group will have either pulsed electromagnetic field (PEMF) or sham treatment. Neither you nor the doctor knows which treatment group you are in. The PEMF system will be used. Each treatment session will last for 1 hour, which will be repeated for 14 daily sessions in three weeks. Assessment will be carried out on the 1st day before treatment, and 14th session and one-month post treatment for follow up.

During this course of research, you are strongly advised to stay with your present lifestyle and medication. All the measurements methods are absolutely safe with no known side-effects. Subjects will not be exposed to any painful stimulation during assessment. Subject's personal information and data acquired from this study is strictly confidential and will not be disclosed to people who are not related to this study.

We hope the treatment may help you. However, it cannot be guaranteed. The information we get form this research may help us treat future patients with the diabetic ulcer better.

If you would like more information about this study, please contact Dr. Siu-leung Yip at tel. no. 3517 or Dr Gladys Cheing at telephone 2766 6738 and you will be given a signed copy of consent form.

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 Siu-leung Yip at telephone 3517 or Dr Gladys Cheing at telephone 2766 6738 for any questions about this study. I know I will be given a signed copy of this consent form.

Signature (subject):_____ Date: _____

Signature (witness):_____ Date: _____

Signature (researcher):_____ Date: _____

APPENDIX III

科研同意書

科研題目：脈衝電磁療對治療糖尿病足部潰瘍之臨床研究

閣下被邀請參加是項研究。在閣下決定參加前，必須清楚明白是項研究之目的及閣下如何協助。請仔細閱讀以下內容，亦可以與閣下的朋友、親屬或家庭醫生商討。如需要更多詳細資料，請歡迎提問。請詳細考慮是否參與是項研究。

本研究之目的包括: (一) 測試脈衝電磁療對糖尿病足部潰瘍的臨床效用； (二) 驗查糖尿病足部潰瘍傷口的軟組織變化。

凡年滿十八歲被醫生確診為二型糖尿病患者，而傷口四個星期以上未癒合的，將被邀請參加是項研究。 閣下可自行決定參加是項研究與否。如閣下同意參加，請保存這份同意書。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。

暫時科學上未有一種被確認為最好治療糖尿病足部潰瘍的方法，所以我們需要作出比較。參加者，約八十位，會被隨機分派到兩個組別，分別接受脈衝電磁療或對照電磁療刺激進行治療，為期三個星期 (共十四課) 的治療。並於治療前及第十四次治療後、療程完成後一個月以噴氣式光學印壓方法檢測系統、高頻超聲波、激光多普勒儀等技術來測試參加者的傷口組織形態變化、足部血液微循環、皮膚溫度等變化。

療程期間，病人應盡量保持個人生活及藥物使用不變。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。

雖然我們未得絕對肯定治療成效，但我們希望能夠對閣下情況給予幫助。研究完畢後，若我們發現脈衝電磁療對糖尿病足部潰瘍有臨床效用，對照組病人有權接受一個療程的脈衝電磁療。此研究為脈衝電磁療對糖尿病人患足部潰瘍的應用，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。

若本人對此研究有何疑問，可致電研究負責人葉紹亮醫生〈電話：9550　　〉或鄭荔英博士 〈電話：2766 6738〉查詢。本人亦明白，參與此研究課題需要本人簽署一份同意書。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，可致電研究負責人葉紹亮醫生〈電話：9550　　〉或鄭荔英博士 〈電話：2766 6738〉查詢。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：_____

APPENDIX IV

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The effect of aging on the biomechanical properties of plantar soft tissues

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ABSTRACT

Background: Foot problems are common in elderly people and stiffened plantar soft tissues may lead to ulceration in people with Diabetes Mellitus. It is unclear how the biomechanical properties of plantar soft tissues change with advancing age. Therefore, this study examined the age-related differences in the biomechanical properties of plantar soft tissues.
Methods: Sixty healthy volunteers without foot problems, aged from 41 to 83 years, were examined using tissue ultrasound palpation system. The thickness and stiffness of the plantar soft tissues under the big toe, first metatarsal head, third metatarsal head, fifth metatarsal head, and heel were measured. The load–deformation curve of the plantar soft tissues was plotted. The correlation between age and biomechanical properties was examined and comparisons were made between four age groups.
Findings: The mean stiffness of the plantar soft tissues at big toe, first metatarsal head, third metatarsal head, fifth metatarsal head, and the heel significantly increased with age ($P<0.001$). The plantar soft tissues at the heel were the thickest ($P<0.001$), with the plantar soft tissue tending to be increasingly thicker with age, although there was no statistical significance. Strong positive correlations between age and stiffness of the plantar soft tissues were found at the big toe ($r=0.608$), first metatarsal head ($r=0.549$), third metatarsal head ($r=0.657$), fifth metatarsal head ($r=0.633$), and heel ($r=0.584$) (all $P<0.001$).
Interpretation: The loss of compliance in the plantar soft tissues may be one of the factors responsible for the higher incidence of foot problems in elderly individuals.

1. Introduction

The heel and metatarsal head regions of the human foot are specially designed to provide cushioning and shock absorption to the underlying bone during all weight bearing tasks (Cavanagh, 1999; Scott et al., 2007). Menz and Lord (1999) stated that with advancing age, the structure of the foot changes. There is a reduction in the range of motion, tactile sensation, and strength of the foot, and a higher prevalence of foot deformities. The load-carrying ability under the plantar foot becomes impaired (Gefen, 2003). These structural and functional changes may contribute to foot pain.

Foot problems are extremely prevalent in the aging population (Menz and Lord, 1999). The stiffened plantar soft tissues due to aging under the metatarsal heads may impair the reaction of the tissues to different impact velocities, eventually developing into metatarsalgia in the elderly (Hsu et al., 2005). The altered mechanical properties of the heel pad also leads to such shock-induced injuries as heel pain and Achilles tendinitis (Hsu et al., 1998). Various orthopedic foot deformities and metatarsal problems may lead to inactivity, and subsequently to weakness and falls (Menz and Lord, 1999). The soft tissue stiffening under the metatarsal heads may account for the common location of foot ulcers reported in people with diabetes (Gefen, 2003).

Ultrasonography has been used to evaluate the thickness of the plantar soft tissues under the metatarsal heads (Wang et al., 1999). Zheng et al. (1999) developed an ultrasonic indentation probe to examine the total thickness and stiffness (elastic modulus) of the plantar soft tissues. The ultrasound technique was employed to determine the change in the thickness of the soft tissue layer in the indented site of the tested body part. The measurements of thickness and stiffness would reflect the geometric effects and the mechanical properties of the soft tissue (Zheng et al., 2000), which would subsequently influence the adaptability of the plantar soft tissues during weight bearing situations. Ledoux et al. (2004) examined the damping properties of the plantar soft tissue at the primary regions, including the heel, the five metatarsal heads, and the big toe. However, they used fresh frozen cadaveric tissue rather than live specimens, in which the loading–unloading properties might have been altered. Most other biomechanical tests of the plantar soft tissue have focused on the heel region (Hsu et al., 1998; Ozdemir et al., 2004). It is unclear how aging would influence the change in the properties of the plantar soft tissue at various testing sites of the foot.

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Understanding the influence of aging on the biomechanics of plantar soft tissue is of great importance for designing footwear and preventing injuries. It is hypothesized that the biomechanical properties of human plantar soft tissues can be altered with aging, by an increase in thickness and stiffness. The present study compared the biomechanical properties of human plantar soft tissues, in terms of thickness and stiffness, among four age groups. In addition, the relationship of these properties with aging was examined.

2. Methods

A total of 60 healthy volunteers (46 females and14 males) were recruited from the community by convenience sampling. The subjects were divided into four age groups: 40–49, 50–59, 60–69, and >70 years. Subjects with any foot lesions, major medical conditions including diabetes, peripheral vascular disease or stroke, or a history of orthopedic or neurological disorders were excluded. The purpose of study, procedures of measurement, and risks associated with the study were explained to all of the subjects before their written informed consent to participate in this study was obtained.

The tissue ultrasound palpation system (TUPS) (Research Institute of Innovative Products and Technologies, HKSAR, China) with a pen-size probe was used to investigate the thickness and the Young's modulus of the plantar soft tissue (Zheng and Mak, 1996; Zheng et al., 1999). A 5 MHz and 9 mm ultrasound transducer was installed at the tip of the probe, and served as the indenter. A 10-N compressive load cell was connected in a series with the ultrasound transducer to record the corresponding force response. The thickness and indentation depth of the soft tissue layer were determined from the time of flight of the ultrasound echo signal that reflected from the soft tissue–bone interface. The load signal and the ultrasound signal were both digitized and collected by a computer, and shown in real-time with a program. The deflection of the ultrasound echo due to the deformation of soft tissue was determined using a cross-correlation technique. The effective Young's modulus was calculated by the computer program that showed the stiffness or elasticity of the plantar soft tissue.

Each subject was asked to lie in a supine position with the ankle maintained in a neutral position and the knee in a straight position. The subject was told to relax during the entire study, and the head was supported with a pillow to maintain a neutral cervical spine. The lower leg and ankle was supported by a foot stand padded with towels, so that the subject could maintain this posture throughout the assessment. The plantar soft tissues covering the big toe, first metatarsal head, third metatarsal head, fifth metatarsal head, and the heel of the right foot were palpated and marked (Zheng et al., 2000). The metatarsal heads were chosen because they have been recognized as areas of high plantar pressures and at risk of ulceration (Garcia et al., 2008; Singh et al., 2005). A water-soluble acoustic gel was applied on the testing site to minimize the reflective loss of ultrasound. The plantar soft tissue was preconditioned by loading and unloading the probe on the testing site for a few times. The probe was then held with minimal force in a suitable alignment to obtain the maximum ultrasound echo signal. The load was then gradually applied manually and perpendicularly to the plantar soft tissues, repeated for 5 cycles in each trial. The direction of indentation was carefully monitored by the real-time visual feedback of the indentation response displayed on the computer monitor, which allowed maintaining the maximum ultrasound echo signal throughout the test. The maximum indentation depth was kept to within 10% of the initial thickness (Zheng et al., 2000). The above trial was repeated for two trials at each testing site and the mean was taken for subsequent analysis.

Statistical analyses were performed using the software package SPSS version 17.0. The differences between the age groups with regard to the thickness and stiffness of the plantar soft tissue were calculated by a one-way analysis of variance (ANOVA) test. Significant findings were followed by Bonferroni post hoc analyses to examine group differences, and independent *t*-test to investigate the gender effect. The relationship between age and the thickness or stiffness of the plantar soft tissue was calculated using the Pearson correlation coefficient. The level of significance was set at $P<0.05$.

3. Results

Descriptive statistics on the height, weight, and body mass index of different age groups are presented in Table 1. No statistical difference in any of these demographic characteristics was found among the groups.

3.1. Stiffness of plantar soft tissues

The mean stiffness (Young's modulus) of the big toe, first metatarsal head, third metatarsal head, fifth metatarsal head, and the heel were significant between the four age groups (all $P<0.001$) (Table 2).

The Bonferroni post hoc analyses indicated that the over 71 years age group had a significantly stiffer big toe, first metatarsal head, third metatarsal head, fifth metatarsal head, or heel plantar soft tissue than did the group aged 51–60 years or the group aged 41–50 years (all $P<0.01$).

The plantar soft tissues of the big toe and fifth metatarsal head of the 61–70 years age group were significantly stiffer than those of the 51–60 years age group and the 41–50 years age group, and the tissues of the first metatarsal head and the third metatarsal head were significantly stiffer than those of the group aged 51–60 years (all $P<0.05$).

3.2. Thickness of plantar soft tissues

For each age group, the plantar soft tissue at the heel region was significantly thicker than that at the big toe and the metatarsal heads ($P<0.001$). The plantar soft tissues at the big toe, the fifth metatarsal head, and the heel tended to be thicker with increasing age, from the thinnest for the group aged 41–50 years to the thickest for the over-71 years age group. However, this difference did not reach the level of significance (Table 2).

3.3. Correlation between age and the stiffness of the plantar soft tissue

The Pearson correlation coefficients for a comparison of age with the stiffness of the plantar soft tissue are presented in Fig. 1. There is a strong positive correlation between age and the stiffness of the plantar soft tissues at the big toe ($r=0.608$), first metatarsal head ($r=0.549$), third metatarsal head ($r=0.657$), fifth metatarsal head ($r=0.633$), and heel ($r=0.584$) (all $P<0.001$).

Table 1
Demographic characteristics (mean (SD)) for various age group.

	Age group			
	41–50 ($n=7$)	51–60 ($n=19$)	61–70 ($n=17$)	>71 ($n=17$)
Age (years)	45.1 (3.3)	56.4 (2.4)	66.6 (2.8)	74.3 (3.3)
Gender (male:female)	0:7	3:16	4:13	7:10
Height (cm)	157.3 (6.7)	158.4 (6.4)	154.9 (7.6)	155.9 (8.7)
Weight (kg)	54.9 (5.8)	61.1 (9.5)	57.0 (6.6)	58.1 (9.2)
Body Mass Index (kg/m^2)	22.2 (2.2)	24.3 (3.1)	23.8 (2.5)	23.9 (3.4)

Table 2
Comparisons of the stiffness and thickness of the plantar soft tissues among different age groups (mean(SD)).

	41–50	51–60	61–70	>71	*F*-value	*P*-value
Stiffness (kPa)						
Big toe	21.7(6.6)	32.8(18.4)	62.0(34.5)	77.8 (40.9)	9.723	<0.001*
First metatarsal head	36.8(11.6)	53.6 (36.6)	94.9 (54.6)	107.3(53.8)	6.756	0.001*
Third metatarsal head	31.7(13.0)	50.0(28.5)	87.3 (48.7)	122.9 (51.9)	12.719	<0.001*
Fifth metatarsal head	29.8 (15.1)	41.6(19.3)	79.2 (46.1)	93.8 (45.2)	9.495	<0.001*
Heel	32.4(11.9)	55.0(16.42)	70.7(22.1)	76.7(21.8)	10.854	<0.001*
Thickness (mm)						
Big toe	5.37(0.86)	7.18(2.96)	6.29(1.55)	6.74(1.25)	1.631	0.193
First metatarsal head	9.19(2.77)	8.90(2.73)	9.35(2.27)	8.75(2.29)	0.130	0.942
Third metatarsal head	9.97(0.94)	9.14(1.56)	9.52(1.77)	8.73(1.85)	1.046	0.380
Fifth metatarsal head	6.39(1.86)	8.84(4.00)	8.39(2.51)	8.62(2.04)	1.294	0.285
Heel	20.88(1.82)	20.77(3.37)	22.66(3.83)	22.49(3.49)	1.125	0.347

*$P \leq 0.001$.

4. Discussion

As skin ages, the collagen and elastic fibers in the cell matrix become less soluble and more fragile. As a result, the elasticity of the skin diminishes and its ability to resist an external shearing force is reduced (Edelstein, 1992). Our findings show that the stiffness of the plantar soft tissue under the metatarsal heads and heel increases linearly with age. The stiffened soft tissue may reduce the adaptability of the tissue to respond to sudden or repetitive stress, which may lead to foot problems in elderly people (Hsu et al., 2005). Degeneration related to repetitive microtrauma causes a gradual loss of collagen in the fat pad of the heel, a decrease in water content and elastic fibrous tissue, and thus a decrease in the elasticity of the heel's fat pad (Ozdemir et al., 2004). Elderly people tend to lose propulsive force during the late stance phrase of walking (Edelstein, 1992). The inelastic and weakened soft tissue may then contribute to the instability of the foot in stance phrase of walking, making it less capable of achieving postural control in weight bearing positions.

Increased stiffening of collagen-rich soft tissue has been found in patients with diabetes (Reihsner and Menzel, 1998). The soft tissues become less elastic and less able to distribute pressure through deformation. Thus, the cushioning property of the plantar soft tissue is impaired, especially at high-pressure sites such as the metatarsal heads (Gefen, 2003). With the effect of aging, the micro-tears that are caused by repetitive shear stress during walking are expected to increase for patients with diabetes. This is particularly true for people suffering from peripheral neuropathy, who are prone to developing foot ulcerations. However, there was uneven gender distributions in most of the sub-groups including the group aged 41–50 years, the group aged 51–60 years and the group aged 61–70 years, subsequent analysis of gender effect on plantar soft tissue stiffness was performed only in the group whose age was over 71 years old. Although the female subjects tended to have stiffer plantar soft tissue, significant difference in the stiffness of plantar tissue was found between male and female only in the heel region ($P<0.05$), but not in other measuring sites (all $P>0.05$).

The thickness of soft tissue is an important predictor of peak plantar pressure, whereas high pressure during walking could lead to skin breakdown or other injuries (Mueller et al., 2003). Although the thickness of the plantar soft tissue was not significantly different between the four age groups in this study, nevertheless there was a trend of thicker plantar soft tissue in the older groups. There are many factors behind the change in the total thickness of the plantar tissue of the elderly, including choice of footwear, systemic diseases, and gender (Menz and Lord, 1999). As the male and female ratio was significantly different in most age sub-groups, the gender effect on plantar soft tissue thickness was not able to be examined in the present study. This may explain why our result is not statistically significant. An increase in the distribution of body fat may be the cause of the increased thickness of the plantar soft tissues, and may lead to increased pressure in the sealed fibrous compartment, resulting in the stiffer soft tissues noted in the older subjects (Hsu et al., 2005).

The plantar soft tissues at the heel were the thickest, compared with those at the big toe and the metatarsal heads. The functional thickness of the cushioning layer under the big toe and the metatarsal heads is supposed to be thinner than the values we obtained, as we measured the total thickness of all soft tissue layers, including the flexor tendon, which is not responsible for shock absorption. The peak plantar pressure sustained more energy distributed at the forefoot region than at the heel in both walking and running (Wang et al., 1999). Thus, the metatarsal head region is more susceptible to injuries and prone to ulceration than the other sites of the foot.

We postulate that an increase in the overall thickness of the plantar soft tissue may lead to a decrease in tactile sensation and hinder afferent sensory feedback. Together with changes in the stiffness of the plantar soft tissue and motor function, this may partly explain why elderly people tend to have difficulties controlling their posture, and are thus prone to injurious falls (Boyd and Stevens, 2009). However, since no sensory tests for plantar soft tissues were performed in our study, further investigation should be done to confirm this hypothesis.

The use of orthotics and supportive devices to relieve contact pressure is likely to re-distribute the contact pressures and reduce the stresses under the metatarsal heads. Clinicians may consider using ultrasound technology to examine the biomechanical properties of the plantar soft tissues, and enhancing their clinical prognosis and prescription of footwear for those who are aging or suffering from diabetes. Since the function and mobility of the plantar fat pads are not well known and our study emphasized the vertical compression of plantar soft tissues in a non-weight bearing position, the shear displacements of the underlying soft tissues were not studied. Also, our findings might not be fully transferrable to the measurement performed in a standing position, which is a weight bearing position with the force of gravity presented. A future study can be conducted to evaluate the relationship between the altered tissue properties and the development of forefoot ulcers for patients with diabetes in weight bearing position.

5. Conclusions

Our findings demonstrated that the biomechanical properties of plantar soft tissues appear to change with age in healthy individuals. The stiffness of unloaded plantar soft tissues significantly increased with age. Moreover, there is a trend of increasing thickness of the plantar soft tissue with advancing age. The altered biomechanical properties in plantar soft tissues may be responsible for the higher incidence of foot problems in elderly individuals. Therefore, adequate orthotics and supportive devices are suggested for aging people to prevent foot problems.

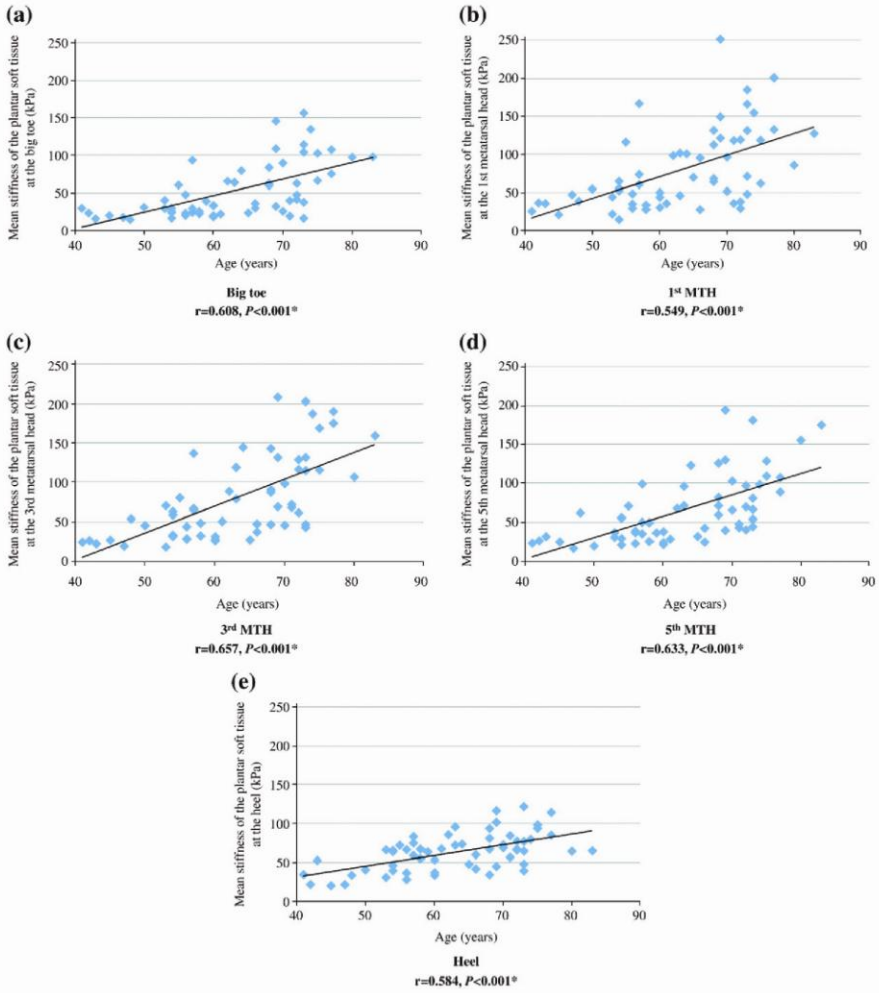

Fig. 1. The linear correlation between age and the stiffness of plantar soft tissues. *$P<0.001$, a significant correlation was found between age and stiffness at (a) the big toe, (b) first metatarsal head, (c) third metatarsal head, (d) fifth metatarsal head, and (e) heel.

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APPENDIX V

ELECTROPHYSICAL THERPAY FOR MANAGING DIABETIC ULCERS: A SYSTEMATIC REVIEW

INTRODUCTION

More than 220 million people worldwide have diabetes, and the World Health Organization (WHO) projects that deaths from diabetes will double between 2005 and 2030 [1]. The lifetime risk for foot ulcers in people with diabetes could be as high as 25% [2]. Diabetic ulcer may lead to persistent non-healing ulcers, ending with amputation or even death [3]. Over 70% of non-traumatic lower limb amputations were performed in patients with diabetes that caused high morbidity and mortality [4]. The economical burden is enormous, and the implied cost of managing diabetic foot problems costs £252 million per year in the United Kingdom [2].

Peripheral neuropathy and ischemia are the major causes of foot ulceration in people with diabetes. Neuropathy results in a loss of sensation and impaired microvascular circulation [5]. Despite the application of various types of dressings, debridement, offloading or the performance of revascularization surgery [5,6], a significant proportion of diabetic ulcers do not heal with these traditional treatment approaches. Physical modality is an alternative approach to promoting the healing of chronic wounds; however, the research evidence is unclear on its use as an adjunct therapy or alone in the absence of other proven therapies in promoting the healing of diabetic ulcers.

Electrophysical therapy is one of the fundamental elements in the daily practice of physiotherapy, which includes a variety of treatments ranging from electrical stimulation to the use of sound waves (ultrasound) and light (laser) to electromagnetic energy [7,8]. It has been used to promote tissue repair, and has been found to enhance fibroblast activity [9] and angiogenesis [10]. However, the findings of these studies were inconclusive, and the clinical use on diabetic wound healing is still under investigation. Many of the conclusions from these published works were in vitro [11-14], from animal studies [15.16] or on wounds caused by venous insufficiency and pressure sores [17-24]. Whether sufficient proof has been found in human diabetic ulcers is still unknown.

Thus far, there have been no published reviews or meta-analyses evaluating the efficacy of electrophysical modalities in the treatment of diabetic ulcers. The aim of the current systematic review is to critically appraise published clinical trials designed to assess the efficacy of electrophysical modalities on the management of diabetic ulcers; and, if appropriate, to identify the most effective electrophysical modalities for managing diabetic ulcers.

METHODS

Data Sources and Searches

A search was conducted to identify relevant published studies. The databases that were searched were MEDLINE, CINAHL, EMBASE, and the Cochrane Central Register of Controlled Trials (CENTRAL) of the Cochrane Library. The databases were searched from their inception until November 2011. The search was restricted to articles

published in English. Keywords and Medical Subject Headings (MeSH) that were identified included “lower extremity,” “foot,” “foot ulcer,” “wound healing,” “diabetes mellitus,” “ diabetes complications,” “diabetic foot,” “physical therapy modalities,” “electric stimulation therapy,” “electromagnetic(s) fields,” “laser(s),” “direct current,” “phototherapy,” and “ultrasound.” A manual search of bibliographic references of relevant articles and existing reviews was also conducted to identify studies not captured in the electronic database search. The results of the searches were compared, and duplicates were removed.

Study Selection

Published studies that reported on the efficacy of electrophysical modalities in treating diabetic ulcers were eligible for inclusion. The inclusion criteria were as follows:

1. The study design was a randomized controlled trial (RCT).
2. The participants described as having diabetic ulcers, at any age, and in any care setting.
3. Any electrophysical modalities were performed compared with sham treatment, conventional treatment, or other electrophysical modalities.

The exclusion criteria were as follows:

1. No inclusion criteria were described.
2. Participants had a clinical diagnosis of wound other than diabetic origin.

Data Extraction and Quality Assessment

The literature search was conducted by two authors (RK and SV) independently of each other. Articles were screened according to title and selected after the abstract was read. The full text of publications satisfying the inclusion criteria was obtained for review, as well as for further assessment when the abstract was unclear.

The same two reviewers (RK, SV) independently graded each study using a modified Jadad scale [25]. The modified Jadad scale that was used is an eight-item scale designed to assess randomization, blinding, withdrawals/dropouts, inclusion/exclusion criteria, adverse effects, and statistical analyses. The score for each article can range from 0 (lowest quality) to 8 (highest quality). Scores of 4 to 8 represent good to excellent (high quality). It has been widely used because it has the advantages of being simple, short, reliable, and valid. In addition to the items listed in the modified Jadad scale, the following data on aspects of quality were extracted:

1. Evidence that a calculation of sample size was applied before the commencement of the trial;
2. Allocation concealment
3. Use of intention to treat analysis

Disagreements between the two researchers were resolved by consensus or through discussion with the third author (GC).

Details of the studies were extracted and summarized using a data extraction sheet. Attempts were made to obtain any missing data by contacting the authors of the studies. Data from studies published in duplicate were included only once. The data collection form consisted of items of demographic data (author, year published, country of study), data on the participants (sample size, age), electrophysical intervention, control

intervention, duration of the intervention, follow-up time, outcome measures, summary of the results, and adverse effects.

Primary outcomes

Objective measures of healing were investigated, including the healing rate of diabetic ulcers; the time to complete healing; and the proportion of ulcers healed within the trial period.

Data Synthesis and Analysis

To assess the outcome of each study, the effect size was calculated. A meta-analysis was performed for only three studies on electrical stimulation [26-28] because of the heterogeneity of the treatment modalities and outcome measures used in the included trials. Effect size *r* was calculated using Review Manager (RevMan) (Version 5.0. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2008) if means and standard deviations were available.

RESULTS

Search results

Using the pre-defined keywords and MeSH, a total of 2,812 publications pertaining to electrophysical therapy for diabetic ulcers were found. Seventeen articles were excluded due to duplication. After reading the titles and abstracts, 2,766 publications were excluded, leaving 29 for a full-paper evaluation. Of these 29 articles, 21 were excluded because of the study design of non RCTs (n=11), and non-diabetic ulcers (n=10). Finally,

8 papers that specifically examined the effect of electrophysical therapy on diabetic ulcer were critically appraised [26-33]. Figure 1 illustrates the trial selection process.

Characteristics of studies

Table 1 presents descriptive information on each of the studies that was reviewed. The trials were conducted between 1992 and 2011 in the U.S., Canada, Brazil and Iran. Six out of eight studies randomly assigned participants into two groups, with an intervention group receiving electrophysical therapy and a control group receiving sham treatment [27,30-33], conservative treatment [29]. Two studies assigned participants into three [28] and four groups [26] with different intervention approaches.

Overall, there were four trials on electrical stimulation [26,28-30], one trial on ultrasound [31], one trial on electromagnetic fields [29], and two trials on phototherapy [32,33]. The majority of them (5/8; 62.5%) were published after 2000. The mean age of the participants in the experimental groups was 59.8 years and 60.3 years in the control groups. Sample sizes in the experimental groups ranged from 7 to 32 (mean 23.65), and from 7 to 32 in the control groups (mean 17.24). The total sample size was 323 (n=186 for the experimental groups, and n=137 for the control groups) with 366 ulcers being treated.

The outcomes measured in the studies varied. The “percentage of wounds healed during the study period” was measured by eight studies, “healing time” by two studies; “blood flow” by one study; “subject compliance” by one study; “treatment time” by one study;

"ulcer granulation rate" by one study; "number of patients with complete healing in each group' by one study; and "difference between estimated and actual healing time" by one study.

Methodological characteristics

Studies were graded into two categories of quality: (i) high-quality studies with a modified Jadad scale score of 4 and above; and (ii) other studies with a modified Jadad scale score of 3 and below. After the review, there were six high-quality trials, namely two on electrical stimulation, one on ultrasound, one on electromagnetic fields, and two on phototherapy [27,29,30,32,33]. The summary of methodological quality is presented in Table 2.

Only five trials had detailed explanation of how randomization was carried out and provided an adequate report on the assignment [27,29-31,33]. Five trials were double blinded to treatment allocation [27,30-33] and one trial was single blinded, as the subject blinding [29] was not stated. Five studies clearly explained the reasons for withdrawals and drop-outs, and reported drop-out rates ranging from 10% to 59% [27,29-31,33]. Moreover, only two studies indicated the number of withdrawals and the reasons for the withdrawals and drop-outs, and followed the intention-to-treat principle [27,31].

Efficacy of electrophysical therapy

Electrical stimulation

The four electrical stimulation trials used different types of protocol. Two trials compared electrical stimulation with sham treatment [27,30]. One trial compared electrical stimulation with an infrared heat lamp or in a warm room [28]. The other trial compared two different protocols of electrical stimulation, with the control group receiving either very low levels of current or no electrical stimulation [26].

Lunderberg and co-worker (1992) and Peters and collaborators (2001) investigated the treatment effect of electrical stimulation, but the authors did not provide information on calculations of sample size, or on allocation concealment [27,30].

The waveform used in the four trials included symmetrical biphasic, monophasic, or square-wave pulse [26-28,30]. The healing rate was the main outcome for all of the four trials. Other outcomes included skin blood flow, compliance with the use of devices, proportion of wounds healed, and time until the wounds healed. Three out of four trials found significant between-group differences in healing rates, with the experimental groups in all of these studies shown to be more favorable than the control groups [26-28]. The exception was the trial reported by Peters and collaborators, which showed no significant between-group differences in healing rates [30].

As the standard deviation was not provided in one of the original articles [30], the results in only three of the trials [26-28] was integrated by meta-analysis, by comparing healing rates (Figure 2).

The mean differences of the ulcer healing rate in favor of electrical stimulation in these studies were 20.0 (95% confidence interval [CI]=13.71-26.29), 74.4 (95% CI=61.98-86.62), and 9.70 (95% CI=8.02-11.38) between people receiving electrical stimulation and people receiving a sham or control treatment. The pooled estimate of the treatment effects of electrical stimulation compared to sham electrical stimulation or a control treatment was statistically significant with a mean difference of 33.94 (95% CI=7.99-59.88). However, as a high-quality study [23] was excluded from the meta-analysis and a beneficial effect was not demonstrated in this study, this exclusion may have some influence on the final results.

Ultrasound

The study on ultrasound was a high-quality trial by modified Jadad scale. The researchers compared the efficacy of a 40 KHz ultrasound device with a sham device that delivered a saline mist without producing ultrasound for managing diabetic ulcers [31].

Both the experimental group and sham group showed improvement from pre-treatment to post-treatment in the proportion of wounds healed, with the healing of the treatment group (40.7%) differing significantly from that of the control group (14.3%) ($p=0.0366$, Fisher's exact test). In addition, the experimental group healed significantly more quickly (mean time to heal 9.12(0.58) weeks) than did the sham group (mean time to heal 11.74(0.22) weeks) (log rank $p<0.0144$).

Ennis et al. (2005) reported that ultrasound therapy produced a significant improvement in the proportion of wounds healed and time required to heal; however, they did not provide any details on the calculation of their sample size [31].

Phototherapy

Minatel et al. (2009) performed a study that examined the efficacy of phototherapy, with a combination of 660 and 890nm lights, whereas Kaviani et al. (2011) investigated the effect of low-level laser therapy in promoting the healing of chronic diabetic ulcers as compared to a sham group [32,33].

Minatel and colleagues reported a significant improvement in ulcer granulation and healing rates. The mean ulcer granulation and healing rates were significantly higher for the experimental group than the sham group throughout the course of the treatment ($p<0.02$). After the whole course of treatment, 58.3% of the experimental group with ulcers was completely healed and three-fourths achieved 90-100% healing. By contrast, in the sham group with ulcers, only one ulcer healed was demonstrated to have healed completely with none of them attained more than 90% healing. The author concluded that the combination of 660 and 890nm lights promotes tissue granulation and the rapid healing of diabetic ulcers. However, their study did not provide details on the randomization method, calculation of sample size, allocation concealment, intention to treat analysis, or the number of participants who started and finished the study [32].

The study done by Kaviani and colleagues also reported a significant improvement in number of patients with complete healing and the mean reduction in ulcer size. By week

4, the reduction in ulcer size in the treatment group is significantly greater than the control group (p=0.046). By week 20, all patients in the treatment group had complete healing while three patients in the control group experienced complete healing. The author concluded that laser could be a safe adjunct therapy to patients with diabetic foot ulcers [33].

Electromagnetic fields

Isakov et al. (1996) examined the efficacy of using electromagnetic stimulation to manage stump wounds for diabetic amputees as compared with a conservative treatment [29]. There was no significant mean difference between the estimated and actual healing times, and no significant improvement in the mean healing time with the experimental groups (healed in 27.8(11.8) days and control groups (healed in 25.9(9.9) days. No significant improvement was found in the mean healing time.

Adverse effects

None of the trials conducted on electrical stimulation or electromagnetic fields reported any adverse events. For the ultrasound study conducted by Ennis and co-worker, a total of one hundred and ninety-three adverse events were reported in all study groups, including the development of cellulite, the development of additional wounds on the index foot, the draining of wounds, and erythema [31]. With 83% of the events not related to the device, the remaining possibly device-related events included pain, erythema, enlargement of ulcers, ulcer infections, and the development of additional

blisters, edema and others. The therapeutic outcomes and adverse events associated with these trials are presented in Table 2. No adverse event was found in the studies on phototherapy [32,33].

DISCUSSION

The use of electrophysical therapy, including electrical stimulation, ultrasound, laser, and electromagnetic energy for healing wounds, has drawn attention from physical therapists, and is commonly performed in clinical settings. This systematic review is the first to investigate the efficacy of electrophysical modalities in treating diabetic ulcers.

Electrical stimulation has been used to treat diabetic ulcers that have not responded to standard wound treatments [26-28,30]. The studies by Lunderberg and co-workers [27] and Peter and collaborators [30] investigated the effect of electrical stimulation on diabetic ulcers and trial quality was high assessed by the modified Jadad scale. However, in the study by Baker et al., the control group refers to subjects either receiving no stimulation or low-level stimulation, whereas we cannot rule out the effect of low-level stimulation [26]. One concern with the studies was that the author did not provide information on the calculation of sample size, or allocation concealment. Similarly, the studies performed by Baker et al. and Petrofsky et al. did not report details on how randomization and blinding was done, withdrawals and adverse effects, calculation of sample size, allocation concealment, and the intention to treat analysis [26,28]. The results of our meta-analysis support the efficacy of electrical stimulation on the healing

of diabetic ulcers in human subjects [26-28]. However, the exclusion of a high-quality study [30] that did not demonstrate a beneficial effect may have affected the final results of our review.

Minatel and colleagues examined the effect of lasers and reported a significant improvement in ulcer granulation and healing rates, but the study did not provide details on the randomization method, calculation of sample size, allocation concealment, the intention to treat analysis, or the number of participants who started and finished the study [32]. Kaviani et al. also reported the significant effect of lasers in mean size reduction; however even they reported the withdrawal and dropouts rate, the intention to treat analysis was not reported [33]. It has been considered a non-invasive treatment with no reported side effects. However, there is still little agreement on protocol on wound healing.

Therapeutic ultrasound has been widely used to manage many musculoskeletal conditions in clinical settings, but with conflicting results. The clinical use of ultrasound to promote wound healing is still under investigation. The possible mechanism of ultrasound in promoting tissue repair is likely due to its mechanical effect, with micromassage changing the permeability of membranes and stimulating the proliferation of fibroblasts [13]. Ennis et al. (2006) reported significant improvements in the proportion of wounds healed and the time required to heal with ultrasound treatment, and the effects were also considered strong by a modified Jadad scale; however, no details were provided on the calculation of sample size [31]. Interpretation of the results

should be careful since this is an essential element directly related to the reduction of bias.

The study conducted by Isakov et al. (1996) that demonstrated the efficacy of electromagnetic therapy was considered to be methodologically strong [29]. Although no significant improvement was found in the mean healing time, the pathology and prognosis of the diabetic stump wound may be different from that of chronic foot ulceration. Also, the use of electromagnetic therapy does not elicit any complications from direct contact with the electrodes that are adopted by other electrophysical modalities. Indeed, electromagnetic therapy can be applied in the presence of casts or wound dressings, with a low risk of infection. As there is only one study on electromagnetic therapy included in this review, further research is warranted before dismissing any beneficial effects from this therapy.

A number of animal studies have reported that electrical stimulations [34], lasers [35], ultrasound [36], and electromagnetic therapy [37] enhance tissue repair in diabetic ulcers. Conversely, other animal studies have shown no significant differences in healing between treated diabetic ulcers and control wounds [38]. Research on electrophysical therapy has mostly been done on animal wounds consisting of surgically excised skin. These experimental wounds excluded common problems associated with delays in healing such as ischemia, infection, necrotic debris, or sinus formation. Therefore, these animal wound models may not be ideal for studying the effect of electrophysical therapy on human diabetic ulcer healing [39].

Classification of the diabetic ulcers is important in describing the lesions that we treat, in order to monitor the progress of the treatment and the prognosis, as well as to ensure that the study population is homogeneous. In the most widely accepted and universally used grading systems, the Wagner-Meggitt system [40] and the University of Texas Diabetic Wound Classification System [41-42], the stages of diabetic ulcers are separately identified and classified according to the presence of ischemia, infection, or both. Among the reviewed studies, only Ennis et al. (2005) and Kaviani et al. (2011) indicated that subjects with Wagner grade 1 or 2 wounds were recruited, while Peters et al. (2001) recruited subjects with grade 1A-2A wounds under the University of Texas Diabetic Wound Classification System [30,31,33]. None of the studies evaluated the stage of the wounds by the end of the treatment, although this might be an important outcome measure in monitoring the progress of diabetic ulcers.

The choice of treatment parameters and the dosage of various electrophysical therapies for managing various human wounds are still uncertain. Many existing studies did not provide complete details on the characteristics of the treatment, including the staging of diabetic ulcers, how diabetic ulcers are diagnosed, or the condition of wounds with different dressings. Different nursing regimens are known to influence the rate of healing [44]; however, only one study conducted on ultrasound had controlled for the nursing regimens during the study period [31].

The present review demonstrates methodological shortcomings in RCTs on the efficacy of electrophysical therapy in healing diabetic ulcers. It is remarkable that none of the studies provided information about calculations of sample size and allocation

concealment. That small sample sizes could lead to low statistical power and allocation concealment has been shown to be associated with exaggerated treatment effects [44,45]. Since withdrawals and dropouts are essential elements directly related to the reduction of bias, the characteristics of the participants leaving the study should be examined in detail in future RCTs.

Interpretations of studies with poor methodological quality must be regarded with caution when applied clinically. There is a clear need for well-designed RCTs examining electrophysical therapy for diabetic ulcers. Trials should be clinically meaningful and adequately powered. Furthermore, investigators should consider the findings of this systematic review when designing future studies and attempt to overcome the limitations of the studies presented, by using true randomization, blinded assessment, allocation concealment, intention to treat analysis, and by paying attention to withdrawals and dropouts. In addition, the procedures for diagnosing diabetic wounds, and the stage of the wounds, should be described. Based on the positive effects of electrical stimulation, lasers and ultrasound treatment, high-quality trials with larger sample sizes are warranted in these areas.

Limitations

In this review, the search was restricted to English publications, which may have resulted in a language bias [46].

The heterogeneity in sampling and treatments among studies, as well as the limited number of studies, small sample sizes and poor methodological quality of many of the studies, limits the overall conclusion made on the efficacy of electrophysical therapy on managing diabetic ulcers. This highlights the importance of further research.

Conclusions

There is still a need for additional evidence to support the use of electrophysical therapy for promoting the healing of diabetic ulcers because current studies are few and tend to have small sample sizes. The three studies on electrical stimulation included in the meta-analysis are either underpowered or methodologically weak, and only a few studies were conducted on other electrophysical modalities such as ultrasound, lasers, and electromagnetic therapy. Nevertheless, the positive effects of electrical stimulation, phototherapy, and ultrasound interventions are sufficient to encourage high-quality RCTs with larger sample sizes in these areas.

Table 1. Outcomes of Electrophysical Modalities for Treating Diabetic Ulcers

Reference	***Quality score***	***Study design (participants)***	***Intervention***	***Duration / follow up***	***Outcome measure***	***Main results***	***Adverse effects***
Baker et al., 1997, U.S.	3	RCT (n=80 patients) E(A): 21 (16 M, 5 F) MA: 58(2)yr (40-82) E(B): 20 (11 M, 9 F) MA: 50(2)yr (33-64) E(C): 19 (14 M, 5 F)	E(A): Electrical stimulation, phase duration 100μs, frequency 50pps, symmetric biphasic E(B): Electrical stimulation, phase duration 300 μs, frequency 50pps, square-wave pulse E(C): Electrical stimulation, 4mA,	Until wound healed / NM	1. Healing rate	1. Significant increase in healing rate for E(A) by 60% compared with C; E(B) did not increase compared with C.	NM

		MA: 51(2)yr (30-64) C: 20 (14 M, 6 F) MA: 52(2)yr (30-66)	phase duration 10 μs, frequency 1pps C: No stimulation				
Ennis et al., 2005, U.S. and Canada	8	RCT, double-blinded (n=55 patients) E: 27 (13 M, 14 F) MA: 56(11)yr C: 28 (19 M, 9 F) MA:54(12)yr	E: MIST^{TM} therapy system, 40KHz with distal displacement of 65 microns. 1.25 W/cm^2 x 4 minutes C: sham device with equivalent volume, flow rate and pressure of saline mist to wound bed	12 weeks / NM	1. Proportion of wounds healed 2. Time required to heal	1. Significantly higher in E group than in C group. E: 40.7% vs. C: 14.3% (p=0.0366). 2. Significantly faster in E group	193 reported cases in total study population, development of cellulite, development of additional wounds on the index

						(9.12(0.58) weeks) than in the sham group (11.74(0.22) weeks) ($p<0.0144$).	foot, pain, wound drainage, and erythema.
Isakov et al., 1996, *country not reported	6.5	RCT, single-blinded (n=18 patients) E: 8 MA: 66.7(5.3)yr (59-74) C: 10 MA: 66.0(7.6)yr	E: Electromagnetic stimulator, pulsed rectangular form, 200V x 50Hz x 30% duty cycle x 2 hours daily. With conservative treatment. C: Conservative treatment	Until wound healed / NM	1. Mean total magnetic field treatment time 2. Mean healing time 3. Mean differences between	1. 44.3±19.6 hours 2. No significant difference. E: 27.8(11.8) days vs. C: 25.9(9.9) days 3. No significant difference. E: -2.8(16.4) days vs. C: -	NM

		(58-79)			estimated and actual healing time	9.8(11.9) days	
Lundeberg et al., 1992, *country not reported	7	RCT, double-blinded (n=64 patients) E: 32 (13 M, 18 F) MA: 66(7.9) yr C: 32 (13 M, 18 F) MA: 67.5(8.6) yr	E: Electrical nerve stimulation unit, square-wave pulses, pulse width 1msec, 20 minutes twice daily C: Sample unit without output	12 weeks / NM	1. Cumulative percentage of healed ulcers against time	1. Significant differences ($p<0.05$) in ulcer area and healed ulcers in E group than C group.	NM
Peters et	7	RCT, double-	E: Micro-Z^{TM}, 5.5 x	Every	1. Proportion of	1. E: 65% vs. C:	NM

al., 2001, U.S.		blinded (n=40 patients) E: 20 (16 M, 4 F) MA: 59.9(7.0) yr C: 20 (19 M, 2 F) *as stated in the original article MA: 54.4(12.4) yr	6cm electric stimulation device, to Dacron-mesh silver nylon stocking ($0.6\Omega cm^{-2}$). 50V with 80 twin peak monophasic pulses per second x 10 minutes, followed by 10 minutes of 8 pulses per second of current. At night for 8 hours. C: Electric stimulation units that looked and acted	week until 12 weeks / NM	wounds that healed during the study period 2. Compliance with use of device 3. Rate of wounds heal 4. Time until wounds healed	35% (p=0.058) 2. No significant difference. 3. No significant difference. E: 86.2% vs. C: 71.4% 4. No significant difference E: 6.8(3.4) weeks vs. C: 6.9(2.8) weeks.	

			identically to an active unit except not delivering any current.				
Petrofsky et al., 2007, U.S.	3	RCT (n=29 patients) E(A): 10 MA: 64.7(13.2) yr E(B): 9 MA: 62(7.7) yr C: 10 MA: 63(7.6) yr	E(A):Electrical stimulator, biphasic, 30Hz, 20mA, 2x2cm carbonized rubber electrode; with heater of 32(2)℃ E(B): Electrical stimulator, biphasic, 30Hz, 20mA, 2x2cm carbonized rubber electrode; with local heat by thermocouple	4 weeks	1. Healing rates 2. Skin blood flow	1. E(A): 74.5(23.4) % vs E(B): 55.3(31.1) % 2. Blood flow doubled on the edge of the wound and largest increase in E(A) group.	NM

			C: Only wound treatment				
Minatel et al., 2009, Brazil	6	RCT, double-blinded (n=14 patients with 23 ulcers) E:7 patients with 10 ulcers MA: 63yr (47-77) C: 7 patients	E: Dynatron Solaris 705® phototherapy research device, 32x890nm and 4 x 660nm superluminous diodes in 5cm^2 cluster. Each spot size treated for 30 seconds	Until the ulcers healed fully or a maximum of 90 days / NM	1. Ulcer granulation rate 2. Ulcer healing rate	1, 2. At each of 15, 30, 45, 60, 75, and 90 days, mean granulation and healing rates were significantly higher for E	None

		with 13 ulcers MA: 66yr (50-87)	C: sham applicator with same device			group than for C group (p <0.02)	
Kaviani et al., 2011, Iran	8	RCT, double-blinded (n=23 patients) E:13 patients MA: 60.2yr (9) C: 10 patients MA: 59.4yr (3.7)	E: laser device (BLT, 685nm, 50mW) with a fluence of 10J/cm2 of a power density 50mW/cm2. 6 times/week, at least 2 successive weeks and then every other day up to complete healing C: sham irradiation	Until complete healing / 20 weeks	1. Number of patients with complete healing in each group 2. Mean reduction in ulcer size	1. E: 8 of 13 ulcers (66.6%) healed. C: 3 of 9 ulcers (38.4%) healed. (Log rank test p=0.470) 2. E: 47.5(9)% vs. C: 29.4(7.6)% (p=0.125)	None

RCT – randomized controlled trial, E – experimental group, C – control group, MA – mean age, NM – not mentioned

Table 2. Summary of Methodological Quality

Parameters	**Baker et al.**	**Ennis et al.**	**Isakov et al.**	**Lundeberg et al.**	**Peters et al.**	**Petrofsky et al.**	**Minatel et al.**	**Kaviani et al.**
Randomized	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Randomization appropriate	Not described	Yes	Yes	Yes	Yes	Not described	Not described	Yes
Blinding	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
Blinding procedure	Not described	Double-blinded	Single-blinded	Double-blinded	Double-blinded	Not described	Double-blinded	Double-blinded
Withdrawals and dropouts	No	Yes	Yes	Yes	Yes	No	No	Yes
Inclusion or exclusion criteria	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Adverse events reported	No	Yes	No	No	No	No	Yes	Yes
Method of statistical analysis	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Sample size calculation	No	No	No	No	No	No	No	No
Allocation concealment	No	No	No	No	No	No	No	No
Intention to treat analysis	No	Yes	No	Yes	No	No	No	No

Figure 1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement flow diagram of the literature search

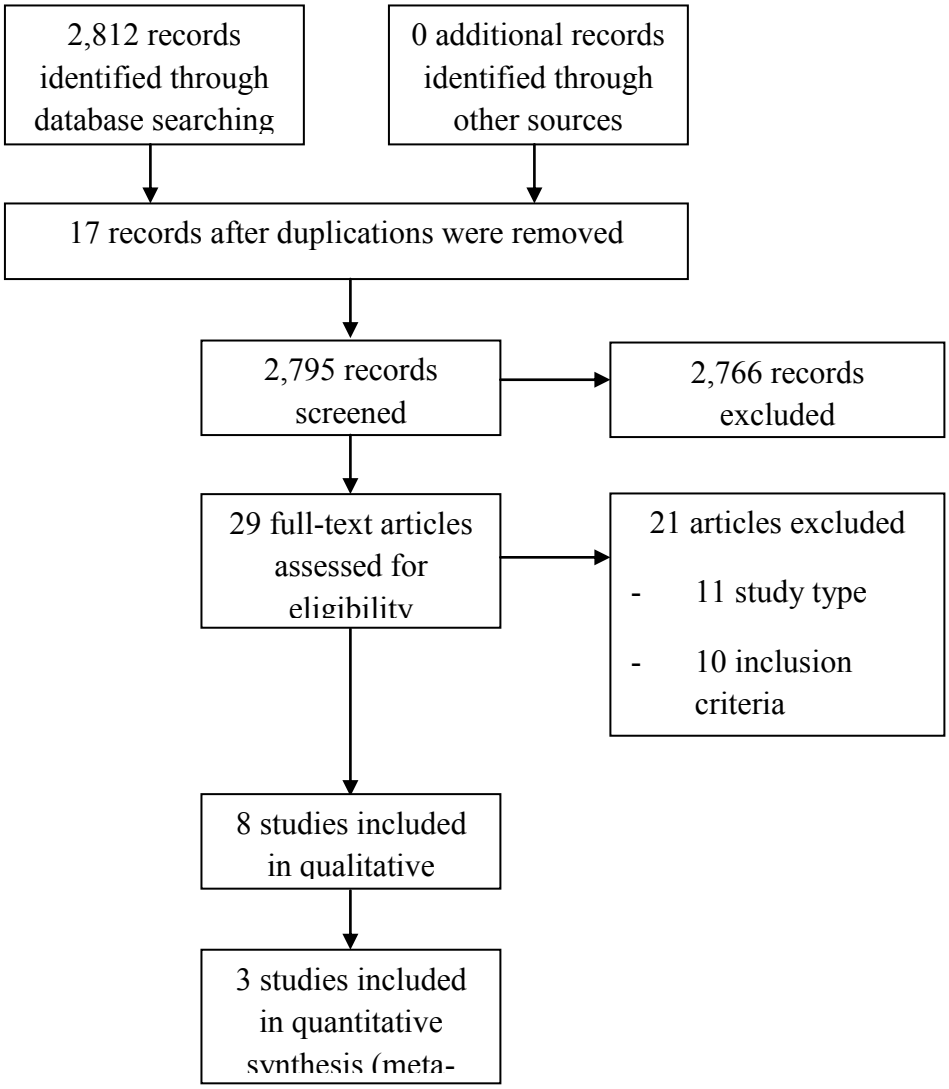
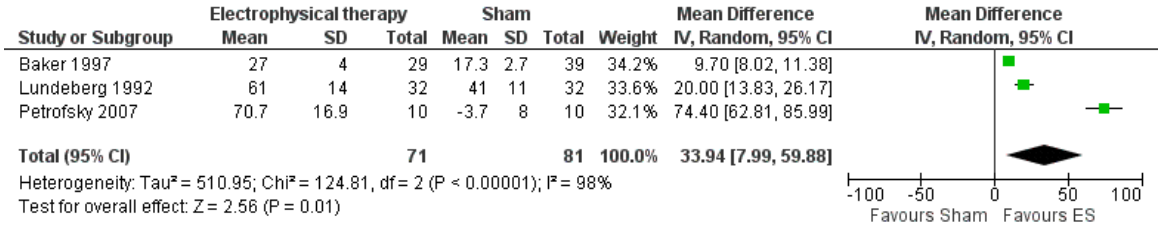


Figure 2 The ulcer healing rate of the electrical stimulation versus sham electrical stimulation therapy or control group



APPENDIX VI

THE EFFECT OF AGING ON THE BIOMECHANICAL PROPERTIES OF PLANTAR SOFT TISSUES

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THE EFFECT OF AGING ON THE BIOMECHANICAL PROPERTIES OF PLANTAR SOFT TISSUES

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Purpose: We aimed to compare the biomechanical properties of human plantar soft tissues among four age groups, and to examine the relationship of these properties with aging.
Relevance: The influence of aging on the biomechanics of plantar soft tissue should be considered when designing footwear for older people to prevent injuries.
Participants: 60 healthy volunteers (46 female and14 male) were recruited from the community by convenience sampling. The subjects were divided into four age groups: 40-49, 50-59, 60-69, and >70 years. Subjects with any foot lesions, major medical conditions including diabetes, peripheral vascular disease or stroke, or a history of orthopedic or neurological disorders were excluded.
Methods: Subjects were tested in a supine position. The thickness and stiffness (Young's modulus) of the plantar soft tissues under the big toe, first metatarsal head, third metatarsal head, fifth metatarsal head, and heel were measured by tissue ultrasound palpation system. The load-deformation curve of the plantar soft tissues was plotted.
Analysis: The group difference in thickness and stiffness of the plantar soft tissue were calculated by a one-way analysis of variance (ANOVA) test. Significant findings were followed by Bonferroni post hoc analyses to examine group differences. The relationship between age and the thickness or stiffness of the plantar soft tissue was calculated using the Pearson correlation coefficient.
Results: The stiffness of the plantar soft tissues at the big toe, first metatarsal head, third metatarsal head, fifth metatarsal head, and the heel significantly increased with age ($P<0.001$). The plantar soft tissues at the heel were the thickest ($P<0.001$), with the plantar soft tissue tend to be increasingly thicker with age, although it did not reach statistical significance. Strong positive correlations between age and stiffness of the plantar soft tissues were found at the big toe ($r=0.608$), first metatarsal head ($r=0.549$), third metatarsal head ($r=0.657$), fifth metatarsal head ($r=0.633$), and heel ($r=0.584$) (all $P<0.001$).
Conclusions: The biomechanical properties of plantar soft tissues appear to change with age in healthy individuals. The stiffness of unloaded plantar soft tissues significantly increased with age. Moreover, there is a trend of increasing thickness of the plantar soft tissue with advancing age. The altered biomechanical properties in plantar soft tissues may contribute to the higher incidence of foot problems in elderly individuals.
Implications: The loss of compliance in the plantar soft tissues may be one of the factors responsible for the higher incidence of foot problems in elderly individuals. Adequate orthotics and supportive devices are recommended for elderly people to prevent foot problems.
Key-words: 1. Biomechanics 2. Aging 3. Ultrasound Indentation
Funding acknowledgements: The project was supported by the General Research Fund of the Hong Kong SAR (PolyU 5128/08E).
Ethics approval: Ethical approval was obtained from the Hong Kong Polytechnic University.

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APPENDIX VII

EFFECTIVENESS OF PULSED ELECTROMAGNETIC FIELDS IN PROMOTING HEALING OF DIABETIC FOOT ULCER

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Purpose:

A randomized controlled trial was conducted to examine the effectiveness of pulsed electromagnetic therapy (PEMF) in promoting the healing of diabetic foot ulcers.

Methods:

Thirteen subjects diagnosed with Type II diabetes with unsatisfactory healing of ulcer were randomly allocated to receive either 60 minutes of active PEMF (12 Hz, 12 Gauss) or sham PEMF for 14 sessions. Assessment on microcirculation, biomechanical properties of wound tissue and wound healing stages were performed at the baseline, last treatment session, and one-month follow-up.

Results:

After 14 sessions of PEMF, there was a 28% increase in nutritive capillary blood velocity and about 14% increase in capillary diameter of the foot. A significant between-group difference was found in the nutritive capillary blood velocity (P= 0.004) and capillary diameters (P= 0.038) at the one-month follow up. The PEMF group demonstrated a slight decrease in the normalized average stiffness coefficient in the central wound bed at the post treatment evaluation, which further decreased to 85% at the one-month follow-up. Also, the PEMF group had an 18% decrease in the wound size (P= 0.021) as compared to a 10% decrease in the sham PEMF group.

Conclusions:

Our preliminary findings showed that PEMF produced a trend in improving microcirculation, biomechanical properties of the wound tissue and healing status for diabetic foot ulcers. A larger scale of clinical trial is needed to confirm our present findings.

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