Microcirculation in Wound Healing

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Abstract

The microcirculation has a substantial role in wound healing, as it provides oxygen and proliferative/immunological mediators necessary to the healing process. In this chapter, we discuss the concept of the wound as a temporary added "organ" due to its increased metabolic needs and how the microcirculation functions as a reserve to support healing. We provide a brief overview of the normal healing process, including the factors driving angiogenesis, and address the differences in the microarchitecture of the skin's circulation. While microcirculation is a highly plastic system, we consider aspects that may contribute to dysfunctional perfusion to the skin, such as aging and other pathologies, and their consequences in delayed healing and the development of ulcers. This chapter will provide an in-depth explanation of the structure and physiology of microcirculation in the paradigm of the healing process and address the role of microcirculation in some of the most common chronic and often difficult to heal wounds including, venous, arterial, neuropathic, diabetic and pressure ulcers.

Keywords

Venous ulcers, arterial ulcers, neuropathic ulcers, diabetic ulcers, pressure ulcers, skin aging, wound healing and blood flow, microvasculature and wounds, leg ulcers, venous insufficiency.

Introduction

To heal a skin wound or ulcer that initially has a certain extent requires blood flow adequate to meet the increased demands of the metabolizing wound tissue. In this sense, as and if the wound goes on to heal, it may be compared to a temporarily added "organ". However, if healing is significantly delayed or prevented, a more-or-less permanently added "organ" taxes the localized microcirculatory system with the continued added demand for blood flow. Most of the information that we have regarding the relationship between wounds, their healing and blood flow is derived from measurements and outcomes of wounds that are present on the skin, and of those, most are wounds on the skin of the lower extremities. Blood flow and the microcirculation, in one form or another, plays a role in wounds of all etiologies that include arterial ulcers, venous ulcers and neuropathic ulcers with the latter most notably, but not exclusively, observed in persons with diabetes mellitus (DM).

For arterial ulcers the causative factor for the ulcer and its difficulty of healing is usually, if not always, inadequate nutritional skin microcirculation resulting in tissue ischemia as causation and inadequate microvascular reserve to support the healing of chronic ischemic ulcers. The deficit in microvascular reserve may be attributable to structural or functional issues such as may be present in some patients with DM, but in general the main factor is an overall deficient blood flow associated with the presence of peripheral arterial disease (PAD).

The situation is quite different in the case of venous ulcers in which skin ulceration is generally secondary to chronic venous insufficiency (CVI) that causes pressure induced injury in superficial lower extremity veins followed by skin breakdown and the occurrence of the venous ulcer. Although full details on the microcirculatory involvement are still not completely clarified, plugging of capillaries via activated

leukocytes is at least thought to be involved causing diminished skin nutritional microcirculation. An important non-leg related ulcer is the so-called decubitus or pressure ulcer. Its linkage to blood flow and more directly to deficits in skin and subcutaneous microcirculation, is attributable to non-relieved skin tissue pressure and shear forces that cause microvascular perfusion deficits sufficient to cause skin break down and ulcer development.

In this chapter these wounds and others are considered in detail especially with respect to linkages and roles of blood flow and the microcirculation in either or both wound development and healing. Prior to that, a review of the normal wound healing process is described, and the angiogenesis processes is presented to provide context. Factors considered in the angiogenesis process are the roles of growth factors, tissue oxygen levels, and the extravascular matrix involvement. Thereafter, further details related to the skin microcirculation are presented followed by microcirculatory aspects of wound healing in specific conditions including aging and ulcers classified as venous, arterial, diabetic and pressure ulcers.

Normal Wound Healing Process: Overview of Phases

Following injury, most skin lesions heal rapidly, an ability that is vital to human survival and maintaining the protective barrier that lines the body's surfaces and cavities [1]. Wound healing is an intricate progression of reactions and interactions between cells and mediators, affected by many intrinsic and extrinsic factors [2]. The wound healing process is divided into four overlapping stages, and an interruption during any of the stages can lead to a spectrum of pathologies: From prolonged inflammation and an inability to re-epithelialize the wound causing chronic ulcers; an overgrowth of granulation tissue leading to pyogenic granulomas; to an excessive fibrotic response generating hypertrophic scars and keloids [3]. These four stages—hemostasis, inflammation, proliferation, and remodeling—are

each characterized by distinct molecular, cellular, and physiological events described in brief below: [3,4]

Hemostasis begins after injury to stop blood loss. Local mediators trigger vasoconstriction in vascular smooth muscle cells to reduce blood flow. Platelets and the coagulation cascade form a fibrin clot, which acts as a reservoir for cytokines and growth factors and, in later stages, a fibronectin-rich scaffold for a provisional extracellular matrix (ECM) [5,6]. These platelet-derived mediators and bacterial byproducts act as chemotactic cues to recruit immune cells, initiating inflammation hours after injury [7].

During the inflammatory stage, local vasodilation allows plasma-like fluid to leak into the tissue space. The influx of this fluid transports nutrients, antibodies, and substances such as histamine, serotonin, proteolytic enzymes, kinins, prostaglandins, and cells into the wound bed [8]. The wound is infiltrated by immune cells: First by neutrophils, which kill bacteria and degrade the damaged matrix; then monocytes within 24 hours. These monocytes then become macrophages which phagocytose microbes and remove debris. Macrophages release growth factors and chemokines, such as platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), and Transforming Growth factor-a and -b (TGF-a and TGF-b), which assists in the transition to the proliferation stage [9,10].

In this stage, released chemotactic agents induce cells' to mobilize, proliferate, and differentiate [8]. Stem cells migrate into the wound bed from the basal layer, with a significant contribution from remnants of dermal appendages—such as the residual stumps of hair follicles [5]. These stem cells and keratinocytes proliferate and migrate to achieve re-epithelialization and coverage of the wound [6,8]. Fibroplasia

is the accumulation and proliferation of fibroblasts and their production of collagen and ground matrix proteins forming the granulation tissue. This early ECM includes collagens, proteoglycans, and elastin and fills the wound space acting as scaffolding and contact guidance for migrating cells and supporting angiogenesis [11].

Toward the end of the proliferative stage, the fibroblasts differentiate into actin-rich, contractile myofibroblasts, which pull together wound edges [12]. The final remodeling stage involves converting the dermis from type III to type I collagen and removing cells from earlier stages. This tightly controlled synthesis of new collagen and lysis of old collagen is mainly achieved through the actions of Matrix Metalloproteinases (MMPs) [13]. Even during normal physiological wound healing, the end-product is neither aesthetically nor functionally perfect. The connective tissue scar that reconstitutes the wound consists of poorly reorganized collagen in dense parallel bundles compared to the basketweave configuration of unwounded tissue. Additionally, hair bulbs or sweat glands do not regenerate if the injury is too deep into the dermis, and there are no remnants of these epidermal appendages [5].

Angiogenesis and Microcirculation in Wound Healing

Angiogenesis occurs throughout all four overlapping stages of wound healing and is initiated immediately after tissue injury. New capillaries sprout from pre-existing vessels and penetrate the wound bed forming a microvasculature network visible in the wound bed 3-5 days after injury [14]. Granulation tissue that forms during the proliferative phase acts as a matrix for proliferating blood vessels, migrating fibroblasts, and new collagen [15,16]. These proliferating capillaries are vital to tissue regeneration by providing O_2 and micronutrients and removing catabolic waste [14,17]. Angiogenesis is regulated by complex growth factor-receptor, cell-cell, and cell-matrix reactions in an orderly cascade of molecular and cellular events [18].

Growth Factors in Angiogenesis

Regulation of vascular growth is a balance between pro-angiogenic, and anti-angiogenic factors present throughout the body. When these angiogenic stimulators and inhibitors are at a physiological balance, vascular growth is suppressed [19]. Anti-angiogenic factors include angiostatin, tissue inhibitors of matrix metalloproteinase-2 (TIMP-2), thrombospondin-1 (TSP-1), endostatin, platelet factor-4 (PF-4), and interferon a/b/g. These inhibitory factors circulate in the bloodstream at low levels and are stored in the ECM surrounding the blood vessels [18].

Immediately following injury, pro-angiogenic stimuli are released into the wound bed, including thrombin, fibrinogen fragments, thymosin-b_4, and growth factors. This initiates angiogenesis and shifts the balance towards vascular growth [20-22]. Tissue damage releases basic fibroblast growth factor (FGF-2) from previously intact cells [23]. FGF-2 and acidic fibroblast growth factor (FGF-1) are synthesized by inflammatory cells and dermal fibroblasts involved in wound healing. FGF-1 and -2 are released from the ECM and bind to the high-affinity protein family of transmembrane tyrosine kinases FGF receptor-1 (FGFR-1), expressed on endothelial cells of different origins, and FGFR-2, expressed under some circumstances [24].

Whereas FGF-1 and -2 that are released from endothelial cells act in an autocrine manner, both mechanisms modulate cell proliferation, migration, protease production, receptor expression, and gap-junction communication [24,25]. The highly expressed $\alpha\upsilon\beta 3$ integrin on endothelial cells during angiogenesis and its interaction with FGF-2 augments the cell's mitogenic activity and cell adhesion to ECM, thereby allowing sustenance for neovascularization [26].
Platelets and inflammatory cells in the bloodstream also store growth factors. During bleeding and hemostasis, thrombin in the wound upregulates vascular endothelial growth factor (VEGF) [27]. Platelets release platelet-derived growth

factor (PDGF), transforming growth factor (TGF-a and TGF-b), VEGF, FGF-2, platelet-derived endothelial growth factor, and angiopoietin-1 (Ang-1), which then stimulates endothelial proliferation and migration [28-30]. Also, macrophages provide a source of cytokines to amplify the angiogenesis cascade and stimulate fibroplasia and neovascularization [14]. Macrophages and monocytes release PDGF, VEGF, Ang-1, TGF-a, FGF-2, IL-8, and TNF-a during the inflammatory phase. PDFG, VEGF, and FGF-2 act with a synergistic ability to vascularize tissues [31].

Role of Hypoxia

Hypoxia is the driving force for vascular proliferation. The hypoxic gradient between injured and healthy tissue promotes the gene expression of HIF-1a and triggers VEGF production [31,32]. Expression of COX-2 during the inflammatory stage also leads to the production of VEGF and other promotors of angiogenesis. VEGF that is present in wound tissue and exudate, increases vascular permeability and transwall hydraulic conductivity [32,33]. The leakage of fibrinogen and fibronectin through these fenestrations is essential to constructing the provisional ECM [34]. Hypoxia also triggers endothelial cell production of nitric oxide (NO), promoting vasodilation and improving local blood flow [35].

Extracellular Matrix Degradation and Vascular Stabilization

When angiogenic growth factors bind to cell surface receptors of pre-existing venules (parent vessels), this growth factor-receptor-binding activates signaling pathways within these endothelial cells. Activated endothelial cells release proteolytic enzymes, such as thrombin-exposed endothelial cells releasing release gelatinase A (MMP-2), which dissolves the basement membrane of the parent vessels [36]. Endothelial cells sprout outward through the basement membrane and migrate into the wound bed facilitated by the actions of integrins (avb3, avb5, and avb1). The tissue matrix is dissolved ahead of the sprouting vessel by the MMPs [18].

While these proteases break down damaged tissue matrix, pro-angiogenic stimulators are released, such as fragment E, yielded from fibrin's cleavage, which stimulates angiogenesis directly and enhances VEGF and FGF-2 [37]. As capillary sprouts develop, they digest endothelial cells and infiltrate the ECM after penetrating the vascular basement membrane and continue to branch and form networks [38]. These vascular sprouts form tubular channels that connect to vascular loops, which then differentiate into afferent (arterial) and efferent (venous) limbs [18].

Vascular stability is controlled by Ang-1 and tyrosine kinase with immunoglobulin-like and EGF-like domains 2 (Tie-2) in the wound bed. Ang-1 binding to its receptor, Tie-2, on activated endothelial cells produces PDGF and recruits mural cells, smooth muscle cells and pericytes [39-41]. Stabilizing the vascular architecture allows for blood flow to begin in the mature vessel [18]. At the terminal stages of healing, growth factor levels decline in the wound, and angiogenesis is suppressed [42]. Pericytes secrete an inhibitory form of activated TGF-b that impedes vascular proliferation [41,43]. Endostatin, a cleavage product of collagen XVIII, is present surrounding the vascular basement membrane and inhibits wound vascularity [44].

Microcirculatory Aspects of Wound Healing in Skin

Skin circulation has two primary functions: Regulating body temperature and delivering nutrients and oxygen for the skin cell metabolism [45]. The microvasculature architecture is not homogeneous, as differences depend on anatomical and topographic characteristics specific to the skin area [46]. These microcirculatory areas have been classified according to four features: 1) presence/absence of arteriovenous anastomoses (AVAs); 2) structure and location of end arteriole blocking devices; 3) structure of valve-containing venules; and 4) microvessel tissue relationships [47]. Most skin has nutritive perfusion provided through capillaries. However, specific sites also have arterioles and venules that

directly communicate with each other. These AVA provide low resistance pathways from arteriole to vein through which blood flow is modulated to meet thermal demands [48,49]. Some anatomical regions, such as the sole and dorsum of the foot, fingertips, and nail folds do not contain these AVAs [50].

The anatomical arrangement of the cutaneous microcirculation is organized in two horizontal plexuses: A deep (lower) plexus containing arterioles and venules starting at the dermal-subcutaneous junction, and a superficial (upper) plexus within the papillary dermis located 1-1.5 mm below the skin surface. These two plexuses are structurally different and connected through paired ascending arterioles and descending venules [51]. Autonomic nerves regulate perfusion in the deep plexus, primarily allowing AVA to serve a thermoregulatory function. AVAs act as regulatory capillary flow devices, have a much greater vasodilatory response to heat, and are also affected by emotions [51-54]. At the dermal-subcutaneous junction, collecting veins, the presence of bi-cusped valves prevent retrograde blood flow [51]. The superficial plexus is predominantly composed of smaller diameter (10-20 mm) post-capillary venules that contribute to the capillary loops of the dermal papillae. Arterial capillaries rise to form the dermal papillary loops representing the nutritive component of the skin microcirculation. These capillaries are sites of inflammatory cell emigration, histamine-induced vascular permeability, and deposition of immune complexes in vasculitis [51].

A primary function of skin papillary capillaries is to deliver O_2 and nutrients to maintain and promote epithelial cell proliferation [55]. Pericytes are effector cells involved in regulating some nutritive capillary blood flow via activation of their contractile proteins (actin and myosin) [56,57]. Perfusion in papillary capillaries is regulated locally based on metabolic needs [54]. Pericytes constrict or dilate in response to PCO_2, pH, and local humoral factors (endothelin and NO) [58]. The O_2

delivery capacity depends on the product of microvascular blood flow and oxygen extraction. Papillary oxygen extraction is high compared to other tissues, limiting the need for a high blood flow [45].

Following skin injury or wounding, the skin healing process is associated with an increase in the local metabolic rate that facilitates cellular proliferation and production of the extra cellular matrix. During the repair process, a critical function of this regulatory system is to maintain O_2 delivery for the expansion of stem cells at the epidermal basement membrane. The O_2 is delivered via non-innervated nutritive papillary skin capillaries from the superficial plexus [59]. Increased blood perfusion in the deep thermoregulatory plexus occurs between 30 to 60 minutes after trauma, but papillary capillary nutritive perfusion does not appear to change [60]. As long as the O_2 supply is sufficient, pericytes regulating papillary nutritive perfusion remain contracted, narrowing the diameter of nutritive capillaries. However, beyond about an hour, pericytes experience signals of increased metabolic need, and in response to the acidic extracellular pH and elevated PCO_2, will relax, thereby promoting increased capillary network blood perfusion [56-58]. Blood flow is thus increased in the superficial plexus via ascending arterioles from the deep plexus facilitating increased nutritive perfusion.

Angiogenesis is triggered by multiple cellular signals that occur early in the wound healing process and continues throughout. The plasticity of the microcirculatory system permits a quick remodeling process that can occur in hours to a few days [61,62]. After about 24 hours, papillary nutritive capillaries are observed to be elongated and dilated, and the superficial vascular plexuses are more visible [62]. As a consequence, increase capillary surface area allows for a commensurate increase in diffusion capacity that aids the repair process and corresponds to the rise in the tissue's metabolic rate for stem cell production of new epidermal cells [62].

During this process, the density of papillary capillaries and red cell velocities in capillaries near the injury site (2 mm distant) are increased [60]. The repair zone, based on the Krogh model and the maximal O_2 diffusion distance, extends up to one mm from the injury site [63]. The increased metabolic and O_2 needs of this zone is met by an increased number of O_2-carrying red cells passing through the area with reduced diffusion distances. Beyond two mm from the injury site, oxygen may be supplied by erythrocytes in a mechanism analogous to upstream flow-mediated dilation that increases flow to and within the sites with increased needs [64].

Microcirculatory Aspects of Skin Wound Healing in Aging

The aging of the skin is influenced by intrinsic factors related to simple chronological aging effects that are independent of environmental insults and also by extrinsic factors that are related to the cumulative insults of environmental exposure, such as UV radiation [65]. The net effect of the these combined aging processes is a diminished ability to maintain homeostasis leading to a progressive loss of function of the skin barrier and increased vulnerability to environmental insults [66]. At the dermal-epidermal junction, the rete ridges are flattened with senescence. Thus, in this skin area, where the papillary dermis maintains contact with the epidermis, flattening the dermal-epidermal junction results in decreased surface contact between these two layers and gives the appearance of atrophy [67,68]. In addition to the decline in epidermal and dermal thickness and composition, most resident cells are reduced in number and there is an associated diminished skin microcirculation [68,69]. The skin microcirculation shows impaired regulation, aspects of inflammatory response changes, decreased numbers of progenitor cells, and declines in circulatory mediators [69]. These age-associated changes impede the microvasculature's ability to thermoregulate and respond to injury, thereby

increasing the chance of tissue hypoperfusion that negatively impacts wounds from reaching the angiogenic stage of repair [69,70].

The literature presents conflicting data, with most showing an age-related decrease in angiogenesis, [71,72] and a few showing an increase [73]. Possible mechanisms of impaired angiogenesis are attributed to impaired endothelial cell function and reduced VEGF expression [74]. In animal models, delayed wound capillary ingrowth has been partially attributed to reduced angiogenic factors FGF, VEGF, and TGF-b [72,74]. Additionally, aged endothelial cells secrete less nitric oxide, likely decreasing vasodilation potential [74] and diminishing microvascular reserve.

During wound healing, the ECM of older adults demonstrates prolonged inflammation, increased MMP and elastase expression, reduced TGF-b, and a weakened cellular response [4]. The atrophic skin of the older adult cannot adapt quickly to the mechanical demands of an injury causing the healing response to be prolonged and blunted with amplified inflammation and differences in signal transduction resulting in inferior ECM production [70,75]. Accordingly, re-epithelialization, collagen synthesis, and angiogenesis exhibit an age-related delay [72]. Dermal lymphatic drainage has been shown to be decreased in aging skin causing wounds to be less able to clear pathogens and have diminished wound contraction potential [76].

During the proliferative phase of wound healing, older individuals exhibit a 50% increase in time for keratinocytes to migrate from the basal layer to the surface compared to younger people [77]. The number and size of dermal fibroblasts decreases in advanced age [78], and they have a reduce response to growth factors and capacity to replicate [78,79]. To assemble a new collagen framework, the ECM must first be degraded by MMP during remodeling. Angiogenesis also requires MMP activity to allow for the invasion of endothelial cells [80]. Older adults have been

shown to have higher expression of proteases and higher activity levels, and these proteases, particularly MMP2, are elevated in older postmenopausal women. Thus, estrogen replacement therapy can stimulate the migration and proliferation of keratinocytes and the elaboration of the matrix [81]. Lastly, in photoaged skin, the MMPs activated by UV radiation produce disorganized collagen fibrils, causing an accumulation of abnormal elastin-containing materials [65]. Due to comorbidities associated with older individuals, including vascular disease, venous insufficiency, unrelieved pressure, or DM, this population is more susceptible to chronic wounds.

The sequelae of these conditions whether venous leg ulcers, diabetic foot ulcers, arterial ulcers, or pressure ulcers, disproportionately afflict older adults [70]. Since blood flow adequacy is an important determinant of wound healing efficiency, it is useful to further consider the potential impacts of age-related changes in skin blood flow. Early work using the hairless mouse skin model demonstrated age-related changes based on laser Doppler perfusion measurements [82]. In humans, early measurements of skin blood flow in deltoid skin using ^{133}Xenon washout methods in 65 men aged 20 to 70 pointed to a 40% skin blood flow reduction over this age range potentially impacting the skin's ability to adapt to stressors [83]. Laser Doppler methods have also been used to demonstrate reduced microvascular vasodilatory reserve in older vs. younger persons. For example, using direct skin heating as a stimulus, increases in forearm skin blood perfusion were less in men aged 60-79 years than in men between the ages of 20 to 39 years.[84] A similar reduction in heat-induced hyperemia was observed in the foot dorsum of elder men vs. younger men that was attributed to a reduction in vascularity of the capillary network. [85] A similar conclusion was reported based on laser Doppler measurements of heat-induced hyperemia in a small group of young and older men [86]. Further work by Inoue and co-workers compared responses of passive heating of young (20-25 years) vs. older (64-76 years) men and showed a reduced heat-

induced skin perfusion increase in skin of the back, chest, forearm and thigh subsequent to passive heating [87]. Further evidence for an age-related reduction in the maximum achievable skin blood flow was provided by a sustained one-hour heating protocol that used venous occlusion plethysmography[88].

Given the importance of adequate blood flow increases during the wound healing process, it is likely that the age-related decline in vasodilatory reserve is relevant to slowed or blunted wound healing in some patients. Contrastingly, in at least one study, the post-ischemic forearm skin hyperemic response was not found to be different based on a three-minute occlusion-release protocol in a small group of 10 young and 10 older subjects [89]. More recently, an age-related differential response in finger pad blood perfusion of the third finger was observed during simulated Braille reading with the index finger [90]. During this procedure finger pad perfusion decreased in both young (20-24 years) and older (64-70 years) subjects but a significantly greater reduction was observed in the younger persons. Since it had been established that the finger blood perfusion is subject to neurovascular control [91-94] it is likely the age-related features are at least in part attributable to such neurovascular differences. An example of the neurovascular assessment using the response to a deep and rapid inspiratory gasp is illustrated in **figure 1**. However, the decreased skin blood flow and pulse pressure that accompanies the inspiratory gasp reflex (figure 1), has not been evaluated for possible age-related differences.

However, the potential role of age-related changes in neurovascular functioning as a contributor to delayed or absent healing has been suggested with possible linkages to sensory nerve function [95]. These workers demonstrated a reduced axon reflex vasodilation to be present in 15 elderly patients who were afflicted with chronic venous ulcers, thereby potentially implicating age-related changes in skin C-fiber function impacting skin blood perfusion. Although a major function of these skin

sensory nerves is pain signal transmission, they also release substances that impact microvessel vasodilatory states thereby affecting blood flow and, in that way, may impact wound healing.

A potential role of sensory afferent fiber function deficit with increasing age is consistent with the age-related trend in their laser Doppler perfusion response to capsaicin challenge [96]. In this study of a group of eight men between the ages of 65-80, the vasodilatory response of forearm skin blood perfusion to capsaicin was significantly less than observed in men either 40-55 years or men 18-30 years. When sensory nerve related vasodilation was blocked using a local anesthetic, the age related difference in heat-induced vasodilation between young vs. older men was reported to be lost [97]. This further supports a role for an age-related sensory nerve changes that may impact wound healing via limitations on maximal vasodilation. However, direct linkages have yet to be confirmed experimentally. The use of such localized heating in combination with assessments of skin blood perfusion responses is useful to evaluate microvascular blood flow reserve and its potential limitations. Figure 2 illustrates a typical response to local skin heating as influenced by the presence of significant peripheral arterial disease in only the left leg of a patient. The much-reduced microvascular reserve has a negative impact on wound healing.

Pressure Ulcers

Major Features and Blood Flow Involvement

The Centers for Medicare and Medicaid Services (CMS) has referred to pressure ulcers as describing a chronic skin lesion mainly caused by excess pressure over bony prominences that occlude blood flow [98]. Although such ulcers tend to develop in elderly and also in persons with spinal cord injury [99], they can occur in anyone subject to sufficiently large and unrelieved pressure. Interfaces between the body surface at points of contact at bony prominences such as the trochanter, heel, malleolus, and sacral region are particularly vulnerable to the development of

pressure ulcers. Use of thermal scanning of the sacral region to predict sacral ulcer development in hospital admitted patients failed to be sufficient selective [100].

However, measurements of sacral skin blood perfusion via laser Doppler imaging reveal a high level of normal skin blood perfusion in resting healthy persons compared to other nearby and distant skin sites [101]. Deprivation of this blood flow during sustained supine lying is likely one factor in pressure ulcer development. Indeed, laser Doppler imaging of acquired sacral ulcers demonstrate increased blood perfusion at the center of the ulcer [102]. Measurements of skin blood perfusion and its response to local heating in peri-ulcer regions have demonstrated elevated pre-heat values and augmented hyperemia in comparison to normal skin but these skin regions have a significantly reduced oxygen as determined by transcutaneous oxygen measurements [103]. The important potential role of altered microvascular perfusion as a main source of the genesis of pressure ulcers has been studied by determining the blood flow deficits during tissue loading and subsequent unloading on the foot heel, a common site for pressure ulcer development [104-110]. A normal hyperemic response to heel loading on a support surface and its release is shown in Figure 3 demonstrating that even rather short durations of compression-related ischemia are followed by significant hyperemia in normal tissue.

Tissue injury and risk factors

Studies of the details of pressure ulcers demonstrate injuries to skin and underlying tissue due to sustained external pressure and shear forces thought to be attributable to internal tissue deformation [111,112]. This distortion or deformation is caused by compression and/or shear between the skeleton and support surface (e.g. bed or chair) that leads to localized acute damage associated with the tissue ischemia, and potentially subsequent tissue necrosis [111,113,114]. Therefore, this type of injury is commonly found in immobile patients on the skin and soft tissue that as was

previously noted occurs most frequently at bony areas of the body, such as the occiput, trochanters, sacrum, malleoli, and heel [111]. Other common locations include ischial, patella, and pretibial ulcers [115,116].

Generalized Risk Factors

It has been reported that pressure ulcers are a significant disease burden worldwide and the highest disability index compared to other dermatological conditions [1]. As already noted, predisposing factors include loss of movement, loss of sensation, and failure of reactive hyperemia [117]. The development of pressure ulcers depends on an individual's health status and tissue tolerance. Age, comorbidities (such as Type 2 DM), and nutritional status are significant contributing factors [118] with two-thirds of pressure ulcers occurring in those aged 60-80 years [119].

Normally as tissue distortion occurs and causes ischemia, CNS signals of discomfort and pain stimulate protective movements to relieve the pressure. However, Immobility or a loss of sensation may prevent signals from being communicated to restore circulation to the area, resulting in the development of a pressure ulcer [120]. Even in healthy patients, ischemic responses occur with positions such as supine, lateral lying, and high sitting, corresponding to pressures between skin and support surface from 30-90 mm Hg [121]. Thus, underlying factors in individual anatomy (tissue structure and geometry) and physiology (microcirculation and nutrition factors) are generalized factors involved [118].

Tissue Damage Causation

Prolonged external pressure of sufficient magnitude on skin occludes capillary perfusion resulting in ischemic soft tissue injury, skin breakdown, ulcer development and possible necrosis depending on the amount of pressure and its duration of action. Clinical experience in nursing home facilities and in hospital settings shows that despite proper positional changing, pressure ulcers still occur [122]. Two

different damage mechanisms, both of which depend on tissue ischemia, have been put forward to account for the pathogenesis of pressure ulcers. The first is ischemic damage due to interruption of perfusion to tissue that causes direct cell damage [112]. The other is ischemic damage caused by decreased nutrients and oxygen and increased toxic metabolites in the tissue. It has been reported that this latter process occurs at relatively low internal tissue strains and can take several hours to develop [123]. Contrastingly, direct cell damage would be caused by higher pressures and greater internal strains and can happen within tens of minutes. The concept is that the development of a pressure ulcer can be associated with both the magnitude and duration of tissue deformation – high deformation for short durations and low deformation for long periods [112] with the type of injury dependent on the characteristic of the tissue loading pattern [124]. Continuous interface pressure monitoring of patients with spinal cord injuries are consistent with this concept [125]

Tissue Pressure and Distortion as a Cause of Ischemia

It has been reported that tissues can sustain pressure of around 30–32 mm Hg for a short duration, but pressures exceeding this will cause occlusion of the microcirculation [113,114]. However, these values are in part based on what was assumed to be an average arterial-end capillary intravascular pressure and thus larger values would cause sufficient compression to cause capillary occlusion. However, this concept does not consider the tissue structure and its properties that lay between the pressurized skin surface and the underlying vessels to be compressed. Further, such values do not intrinsically include the fact that skin surface shear and underlying tissue strain may be equally or more important in affecting the underlying tissue and vessels therein [126].

The manner in which surface forces are transmitted to and through underlying tissue to impact the microcirculation is complex and in very much need of new

research efforts. The impact of support surface dynamic pressure patterns on underlying microcirculatory parameters are also of interest [127]. There is also the possibility that the focus on "compressing" capillaries has hidden the potential involvement of the lymphatic vessels that have a much lower internal pressure. It would appear that sustained occlusion of the underlying lymphatic vessels should be considered as a potential contributor to some forms of pressure ulcer development. Some data supports this concept [128] and points to the variability of thresholds among patients [129]. Despite the caveats of this paragraph, much work in related areas has been done and concepts put forward that help clarify the factors involved as they relate to the involvement of microvascular perfusion.

For example, the potential involvement of venous vessels, that have a low intravascular pressure, and possible effects on the subpapillary thermoregulatory A/V shunts has been described [130]. Also, the potential role of tissue creep that allows soft tissues to accommodate an external load thereby reducing the surface external stress but in so doing, increasing internal tissue strain [131]. Such strain and associated tissue distortion, if large enough, decreases blood flow via a variety of possible mechanisms [132]. Distortion related vascular kinking can compromise tissue blood perfusion causing ischemia and possibly necrosis within hours [117]. Further, such internal shearing forces tend to distort and occlude blood flow more easily due to bending or kinking vessels rather than direct compression [133].

Other Issues and Considerations

Differences in local tissue structure and microvasculature lead to differential abilities to withstand distortion forces and hence different involvements of the localized microcirculation. For example, foot soles have a thin, soft tissue covering blood vessels that are adapted for weight bearing and foot soles rarely develop pressure ulcers. The sacrum and ischial tuberosities do not have such well-adapted blood

vessels. Even though these regions have a thicker layer of soft tissue, they can develop pressure ischemia even under light compression.

Depending on the pressure application duration, its relief is normally associated with a reactive hyperemia allowing rapid restoration of O_2 and waste product removal in previously ischemic tissue. A failure of the reactive hyperemic cycle or a major deficit in it, will prevent the tissues from recovering from the pressure-induced ischemia and, eventual permanent damage. In addition, following periods of ischemia, cellular injury resulting from reperfusion may occur when blood flow is restored. Nutrient and oxygen-deprived tissue may reduce metabolism to preserve function. When perfusion is restored, high levels of cytotoxic reactive oxygen species may be produced that exceed the capacity of the free radical scavenging mechanism. [122] It is likely that ischemia-reperfusion injury, in addition to ischemic necrosis, contributes to the formation of some pressure ulcers [122,134].

In addition to hyperemic responses to prior ischemia, some blood vessels exhibit pressure-induced-vasodilation (PIV) in which a pressure applied to skin causes cutaneous microvessels to vasodilate thereby potentially mitigating ischemic effects [135]. However, once a threshold level is exceeded, the PIV response doesn’t compensate for compression pressures that would normally be experienced. At these higher pressures (as low as 60 mmHg), complete microvascular occlusion has been observed [129]. Similar pressures were shown to cause local ischemia and inflammation and accumulation of metabolites due to anaerobic metabolism [136-138]. Additionally, persons with an already compromised microcirculation are at greater risk of a pressure ulcer and once it occurs may require a longer recovery period [139].

Venous Ulcers

Incidence, location, and Factors

About 80% of lower extremity wounds are venous ulcers with nearly 95% of them located in the gaiter area, somewhere between ankle and knee, but often closer to the malleolus than knee. An example of a venous ulcer located in the gaiter area is shown in **Figure 4**. This figure demonstrates some of its common features such as being a shallow ulcer with irregular margins often with surrounding hyperpigmentation. The predilection for the gaiter area may be related to reported differences in the veno-arterial vasoconstrictor response evaluated by assessing the skin perfusion reduction from supine to standing [140].

Factors involved in venous ulcer development include venous reflux and venous hypertension due to incompetence of deep and communicating vein valves and thrombosis of deep vein segments [141]. Also, CVI is often present and associated with venous drainage obstruction and increased venous pressure and reflux due to arteriovenous fistulas. Previous leg injuries, deep vein thrombosis, phlebitis, and older age are among the risk factors for the development of venous leg ulcers [142,143]. Approximately 1.5% of Americans have venous ulcers with a female to male ratio of near 1.6 to 1. About 20% of those developing venous ulcers do so prior to age 40 and about 40% of patients that develop venous ulcers have a history of deep vein thrombosis and have a diagnosis of CVI. Although the evolution of skin ulcers from venous hypertension is not fully understood; contributory factors include inflammatory processes, intercellular and vascular adhesion molecule upregulation, protein rich edema, leukocyte trapping, oxygen deprivation, and microcirculatory deficits [144].

Microvascular Involvement

Although the precise pathophysiology of venous ulcer development is unclear, various theories have been described [145], and early reviews have considered multiple factors as contributory [146]. For example, the "white cell trapping" theory describes a release of free radicals that result in tissue death as a consequence of venous hypertension [147]. And as noted, microcirculatory deficits due to increased activation of platelets, monocytes and neutrophils leading to microvascular aggregation and microvascular entrapment of neutrophils has been reported [144].

Microvascular Linkages to CVI

It has been proposed that the link to CVI may be that the microvasculature can no longer fully regulate cutaneous blood flow in skin areas severely affected by the CVI [148]. Accordingly, the venous congestion leads to capillary and post-capillary hypertension during ambulation that is manifest in the skin as edema, hyperpigmentation, induration, white atrophy, and skin ulcers [149]. Earlier work by this group described a lack of cutaneous microvascular reserve that prevents blood flow from matching sudden changes in tissue demand due to orthostasis [150]. Since cutaneous microangiopathy appears to precede the development of trophic skin alteration in the presence of chronic venous congestion, and there appears to be a close correlation between the extent of the clinical manifestation and severity of microangiopathy [148,150], it is likely that cutaneous microcirculation forms the link between venous hemodynamics and congestive dermatoses.

In mild CVI, morphological changes in the capillaries, such as moderate dilatations and increased tortuosity, can be seen via capillary microscopy. The higher the ambulatory venous hypertension, the more enlarged and tortuous the capillaries [151]. The increased diameters of pericapillary spaces create a halo effect, that indicates increased transcapillary filtration, that varies in size along the outer border

of the dermal papillae, thereby creating a cobblestone appearance [149,152]. In severe CVI, many capillaries are occluded by white blood cells and hemorrhagic disturbances, leading to decreased capillary density [153]. In areas of white atrophy, there is an avascular field in which there are few or no capillaries [154].

Compression therapy is the mainstay of treatment for CVI, which has been shown to improve most subjective symptoms in patients. Clinically effective compression therapy reverses the pathogenic processes of CVI on the microcirculation and interstitial architecture by improving nutritive blood flow in congested areas [155-157]. One mechanism by which compression therapy improves the situation is by recruiting previously under perfused capillaries thereby increasing capillary density and nutritive blood flow and by promoting angiogenesis. Improvement in nutritive perfusion generally accompanies improved healing of venous ulcers. In the first two weeks, changes in the capillary density can be seen to have prognostic significance. Increased capillary density suggests accelerated healing within approximately six weeks of therapy, while a lack of angiogenesis is related to delayed healing [149].

Normal and Abnormal Calf Pump Function

Normally the venous calf muscle pump helps propel blood in leg veins against gravity toward the heart [141], with each calf muscle contraction compressing the deep veins forcing blood forward with function venous valves preventing backflow into superficial veins. The pressure gradient in the deep veins and the presence of functional valves in the communicating veins, allows blood from superficial veins to flow normally [158]. However, with dysfunction or failure of the valves each contraction of the leg muscles propagates an associated pressure peak into the superficial venous system. The ambulatory venous hypertension is propagated into the nutritive capillaries, and these high-pressure peaks eventually deteriorate the microcirculation of the skin – initially through capillary dilatation, followed by gradual rarefaction [149].

These events and the blood flow dynamics that occur are schematically illustrated in Figure 5 in which the normally low-pressure superficial veins become exposed to the high pressures induced by the reverse flow pathways associated with incompetent valves. Concomitant with the tissue injury are inflammatory processes, increased vascular permeability and edema and or lymphedema. In Figure 6 two examples of venous ulcers are shown in which skin blood perfusion assessed via laser Doppler methods is being measured in the peri-ulcer region of two patients. The initial blood perfusion measurement is made at a skin temperature of 35°C and then locally heated to 44°C with responses as shown in Figure 7. The responses shown in figure 7 demonstrate a common finding for ulcers of venous origin; an elevated peri-ulcer basal resting flow with little if any microvascular reserve when stimulated with heat as shown in part B, but with normal responses in healthy control skin as shown in part A. It has been reported that in patients in which the microvascular reserve is relatively maintained healing of venous ulcers is expedited [159].

The wound and the peri-wound Skin

Within the wound bed of venous ulcers, the capillary density is reported to be heterogeneous [160,161]. Almost no capillaries are seen in areas without granulation tissue. These areas have poor nutritive microcirculation contributing to the formation of venous ulcers but moderate subpapillary blood flow. This moderate subpapillary perfusion may likely facilitate future wound healing and the construction of granulation tissue. [141]

As venous ulcers heal, they form granulation tissue seen grossly as erythematous areas within the ulcers [130], as may be visualized in figure 4. In these areas, a few capillary sprouts are found embedded in small edematous pockets [160-162]. This stage of healing is characterized by a distinct increase in subpapillary perfusion and increased appearance of visible capillaries. Nutritive perfusion in or adjacent to venous ulcers is lower than in normal skin areas although is reported to be higher

than in ulcer areas without granulation tissue [141]. Thus, subpapillary perfusion is crucial for thermoregulation and also for wound healing at this stage [130]. Capillary density is improved but still low compared to non-compromised tissue. Additionally, these capillaries appear long and dilated, unlike the typical glomerular-like morphology in patients with chronic hypertension [130,162,163].

At the scarring stage, the microvascular pattern of skin differs from that in previously intact periulcer skin [162,163], and has glomerular-shaped capillaries characteristic of that in CVI [130]. The nutritive capillary density is relatively high and similar to that seen in the early stages of venous ulcers prior to the ulcer developing granulation tissue [130,162,163]. The capillary density was considered to be moderate (20-30 capillaries/mm^2) in comparison to healthy skin [164] although it is unclear if this capillary density is due to neoangiogenesis or contraction of the tissues. In healed peri-ulcer skin, there is high nutritive and moderate subpapillary blood perfusion that appears close in value to normal skin [165].

Arterial Ulcers

Arterial ulcers represent about 5% of all leg ulcers and are commonly located on the leg or foot and represent about 10-20% of all non-healing lower extremity ulcers [166]. The most prevalent predisposing condition for the development of an arterial ulcer is advanced peripheral arterial disease (PAD) present within arteries that supply the lower leg and foot [167]. Skin breakdown and ulceration is thus attributable to inadequate microvascular perfusion causing ischemic and hypoxic effects on skin and subcutaneous tissue [168]. These ulcers will often occur in areas subject to pressure or trauma such as at the malleolus or posterior heel or the toes leading to necrosis and the need for amputation as illustrated in **Figure 8**. Arterial ulcers are difficult to heal in the absence of an adequate restoration of blood flow, which itself may be more difficult to accomplish due to the presence of comorbid

conditions [169]. In patients such as illustrated in figure 8, assessments of the response to local peri-ulcer heating that is used to measure microvascular perfusion reserve, is similar to that illustrated for the left foot dorsum in figure 2 or in more extreme cases, with even less or an absent perfusion increase. The presence of this microcirculatory deficit is accompanied by abnormally low levels of tissue oxygen levels as assessed by transcutaneous oxygen tension measurements [170-172]. Patients with PAD who also have DM and a foot ulcer are at greater risk of amputation [173]. However, that risk depends on the revascularization approach used [174] and on the extent of the PAD [175]. An epidemiological study reported that about 0.5% of the Korean population had both PAD and a diabetic foot ulcer [176].

Wounds in the Diabetic Patient

Persons with DM are generally at increased risk of developing skin ulcerations in part related to the presence of neuropathy, ischemia, and poor glycemic control [177], although the development of DM foot ulcers has been reported to be less with improved glucose control that also improves microcirculation [178]. The higher likelihood of PAD and the presence of microvascular deficits in DM [179] increase the chances of ischemia, tissue to breakdown, and ulcer formation. In diabetic patients with PAD, the nature and distribution of leg arterial stenoses causing the ischemia is varied [180] but even without the presence of significant PAD, foot Ulcers in diabetic patients are often more difficult to heal for reasons that include reduced blood flow [181,182], wound oxygen deficits [183], and infection. Although wound healing in patients with DM tends to be inferior in the presence of reduced microcirculation [184], the relationship between microvascular assessments and wound healing is not always easy to predict [185].

In some patients with DM, it takes less local pressure to reduce skin blood flow in regions of bony prominence thereby laying the groundwork for ulcerations at these sites. When sensory neuropathy is present, normal pressure/pain signals are diminished or absent, thereby removing warning of developing tissue injury. Most of these types of ulcers develop on the foot, with plantar ulcers often associated with neuropathy. An example of such an ulcer is shown in **Figure 9**. In these cases, elimination of elevated foot pressures combined with standard wound care are indicated. Statistics suggest that about 15-25% of persons with DM will get a foot ulcer [186] with an annual incidence rate of 2- 4% [187] Diabetic-related non-healing ulcers account for 140 000 extremity amputations per year in the US [188] and an annual amputation incidence rate between 0.5-- 0.8% (number of amputations per patient-year).

In terms of mechanisms and processes, DM causes structural and functional metabolic alterations to the arteriolar and microcirculatory systems, especially in the lower extremities of poorly controlled blood sugar patients [189-191]. In the capillary circulation, lumen size is reduced, and the vessels have increased stiffness [192,193]. The increased stiffness is due to a thickened basement membrane and arteriolar hyalinosis, as well as glycosylation and formation of nonenzymatic advanced glycosylation end products (AGEs); [194] thus, limiting the vessel's ability to vasodilate and eventually autoregulate [195]. Simultaneous measurements of transcutaneous oxygen, laser Doppler perfusion, cardiac output, and leg blood flow in 60 patients with and 60 patients without DM led to the conclusion that there was a direct linkage between a diabetic-related deficit in tissue oxygen and a sub-maximal microvascular vasodilatory reserve with little dependence on other circulatory factors[196]. Other work indicated elevated levels of skin tissue water although apparently not significantly related to HbA1c values in patients with DM [197,198]. Such changes likely impact wounds when present in persons with DM.

Indeed, diabetic complications have been associated with impaired vasodilatory capacity. Nitric oxide (NO) dependent smooth muscle vasodilation has been shown to be abnormal in patients with diabetic foot ulcers, and endothelium-dependent and -independent vasodilation is abnormal in diabetic neuropathy [199]. However, in early work it has been reported that within the arteriolar circulation, blood flow may be normal or even increased [200]. It was posited that these characteristic changes are caused by endothelial injury attributable to increased microvascular pressure and shear force in leg microcirculation and leads to an injury response and proliferation of extravascular matrix proteins [201,202]. The thickened membranes impair leukocyte trans-wall emigration thereby increasing the susceptibility of the diabetic foot to infection and additionally reduces the needed hyperemic response to injury [203,204]. As nutritive perfusion of the skin plays a role in ulcer development and wound healing, these changes are a major concern in diabetic patients who have microvascular deficits [199].

As discussed, PAD and peripheral neuropathy are both major causes of a patient developing a diabetic ulcer and contribute to poor healing outcomes [205,206]. However, in many patients with microvascular deficits there is no corresponding clinical evidence of lower extremity macrovascular disease. However, dysfunctional vessels of the microvasculature and impairment of the nerve-axon reflex may be concomitant abnormalities contributing to the neuro-ischemic diabetic limb – leading to ulceration and impaired wound healing [194,207]. It has been shown that revascularization significantly improves, but does not completely resolve, these impairments [208]. However, microangiopathy as a direct cause of ulcer development has not been fully confirmed and in fact one study did not find a relationship between microvascular abnormalities and wound healing [199].

Conclusion

Proper wound healing is inseparable from a functional microcirculation, as adequate localized blood flow and metabolic waste removal are necessary to meet increased demands of healing tissue. Normal wound healing consists of four overlapping phases: Hemostasis, inflammation, proliferation, and remodeling, and microcirculation participation is to varying degrees present at each phase. Throughout these phases, angiogenesis occurs to supply nutrients and O_2 to wounded tissues, with microcirculation delivering growth factors and other needed substrates. Skin has nutritive blood perfusion (via the superficial plexus) regulated by metabolic needs with some areas supplied by arteriovenous anastomoses (the deep plexus) largely under autonomic control. Following wounding, the latter vasodilate to provide increased microvascular blood flow to support the wound healing process including increased nutritive perfusion to support stem cell expansion and production of new epidermal cells. Angiogenesis occurs rapidly thereby increasing microcirculatory potential to meet the needs of healing wound.

Aging affects skin's composition and its microcirculation. Age-associated changes include decreased blood flow, impaired ability to adapt to stressors (including thermoregulation and vasodilatory reserve), prolonged inflammation, decreased progenitor cells and mediators, increased migration time for keratinocytes, and increased matrix metalloproteinase activity. All these factors can contribute to delayed and dysfunctional wound healing. Derangements of the microcirculation can also contribute to the development of wounds, including arterial, venous, pressure, and diabetic (neuropathic) ulcers. In these pathologies, microvascular perfusion is impeded in some way: Peripheral artery disease causes occlusion and ischemia in arterial ulcers; venous congestion, which may contribute to ischemic and inflammatory conditions in venous ulcers; external pressure causes direct damage and ischemia in pressure ulcers; metabolic and structural alterations impair

vasodilatory capacity and contributing to possible infectious processes in diabetic ulcers. In summary, wounds can be viewed as an added “organ” that taxes the microcirculatory system with continued demand for blood flow. These wounds exemplify the effects of dysfunctional microcirculation when the increased needs cannot be met, not only impeding healing but taxing the system to such a degree that the skin structure cannot be maintained.

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Figures and Legends

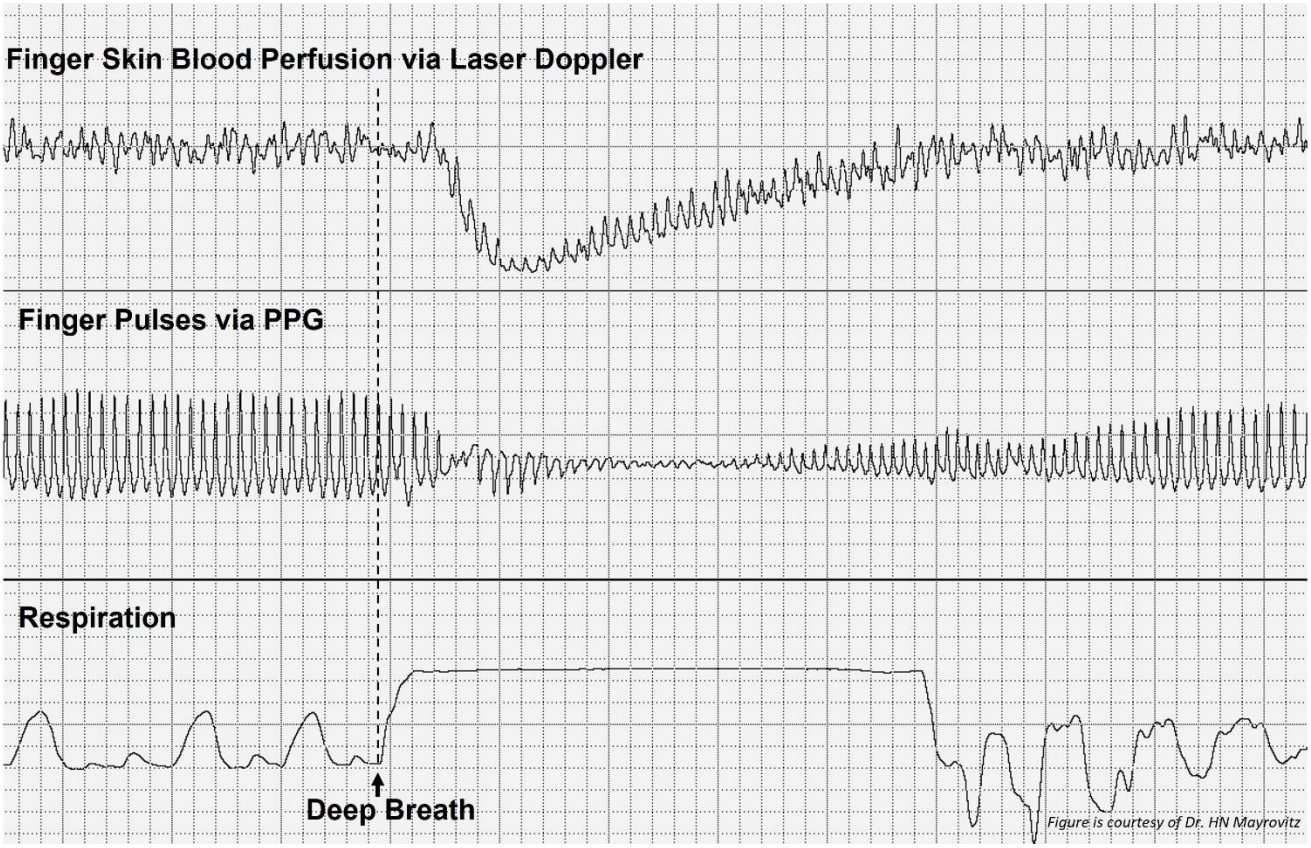

Figure 1. Illustrating the response to an inspiratory gasp held for 25 seconds.

The finger pad skin blood perfusion (upper panel) falls dramatically during a rapid inspiratory gasp but during the breath holding is observed to recover. A similar change is noted in the pulse plethysmographs recording (middle panel) of the index finger pressure pulses of the same hand but with a longer time to recover. Upward movement on the respiration tracing indicates inspiration. Horizontal scale is 1 sec / div. Figure is provided as a courtesy of Dr. HN Mayrovitz Return to text.

Figure 2

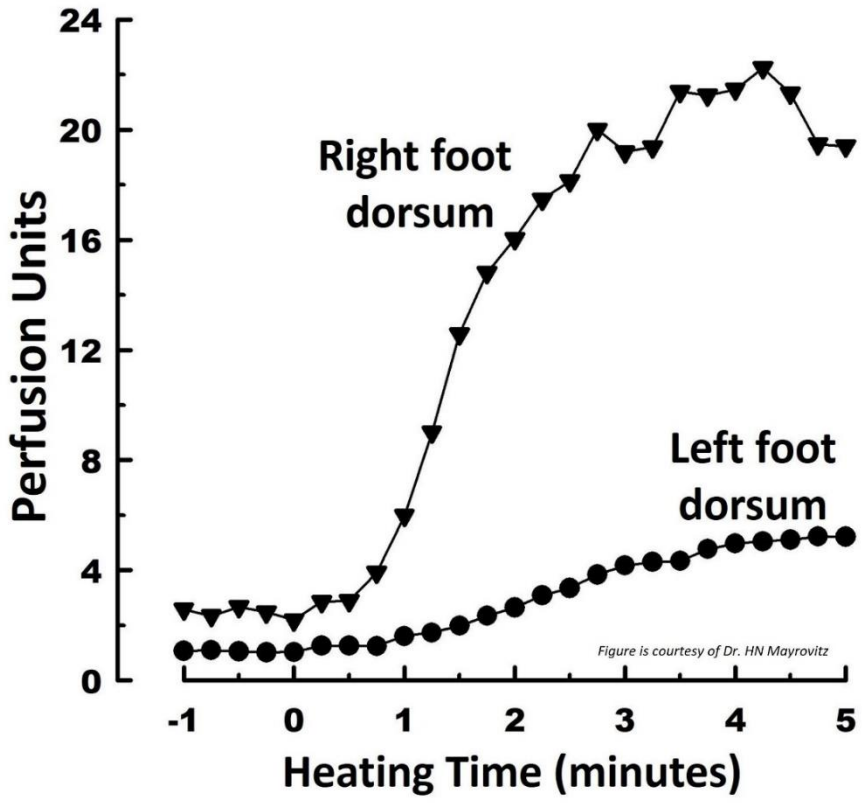

Figure 2. Illustrating normal and reduced microvascular response to local heating. Skin blood perfusion was measured via laser Doppler on foot dorsum of a patient with hemodynamically significant peripheral arterial disease in the left leg only. Heating was local to a skin temperature of 42° C and maintained. The significantly reduced microvascular perfusion reserve is evident by the differences between the right and left dorsum responses. This would have a negative impact on the healing of a metabolically active wound if present. Figure is provided as a courtesy of Dr. HN Mayrovitz

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Figure 3

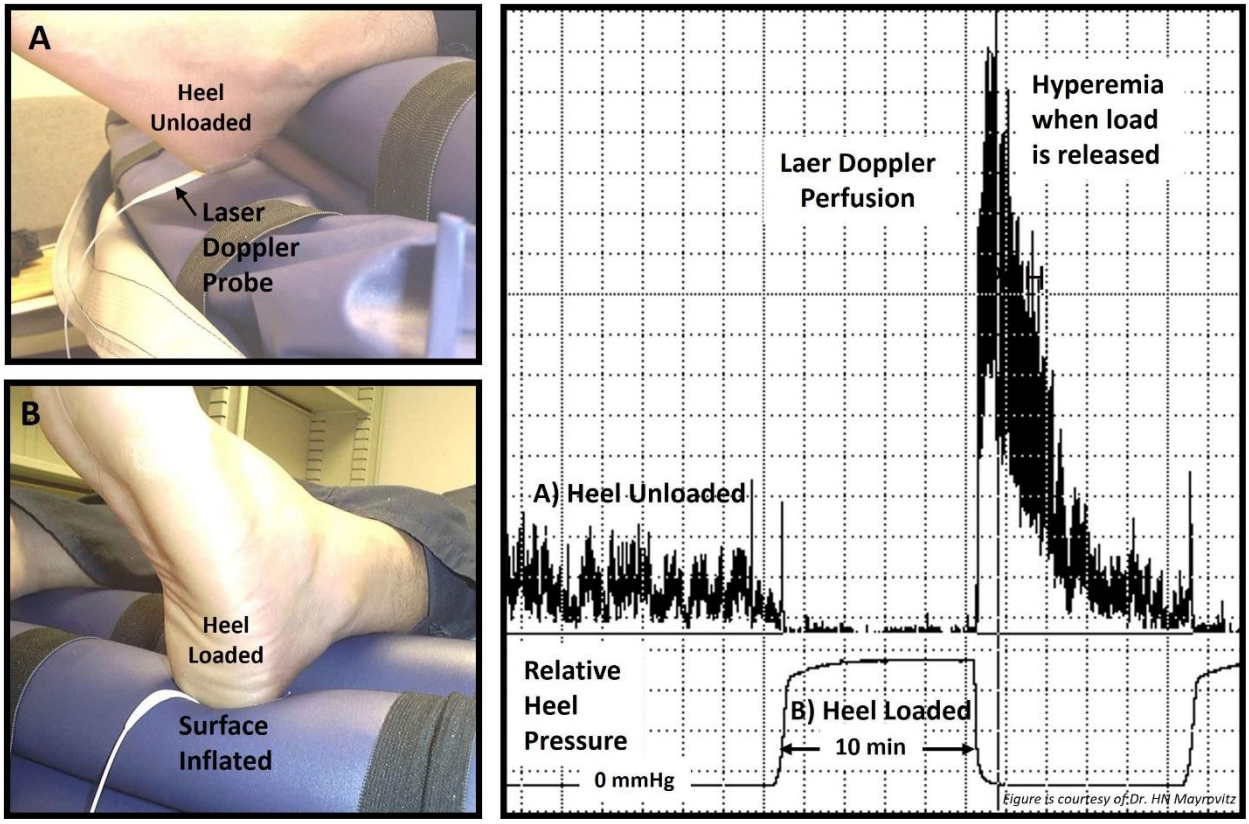

Figure 3. Normal hyperemic response to heel loading and off-loading.

A flat laser Doppler probe is placed on the heel in (A) with the heel unloaded since the support surface cell deflated. In (B) the surface is inflated to load the heel and the blood perfusion is seen to drop to unmeasurable levels as shown in the right panel. Upon deflation, after a loading time of just 10 minutes, a large hyperemic response is noted that is normal. In persons with compromised vascular and microvascular status the hyperemic response is much diminished or absent. Figure is provided as a courtesy of Dr. HN Mayrovitz

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Figure 4

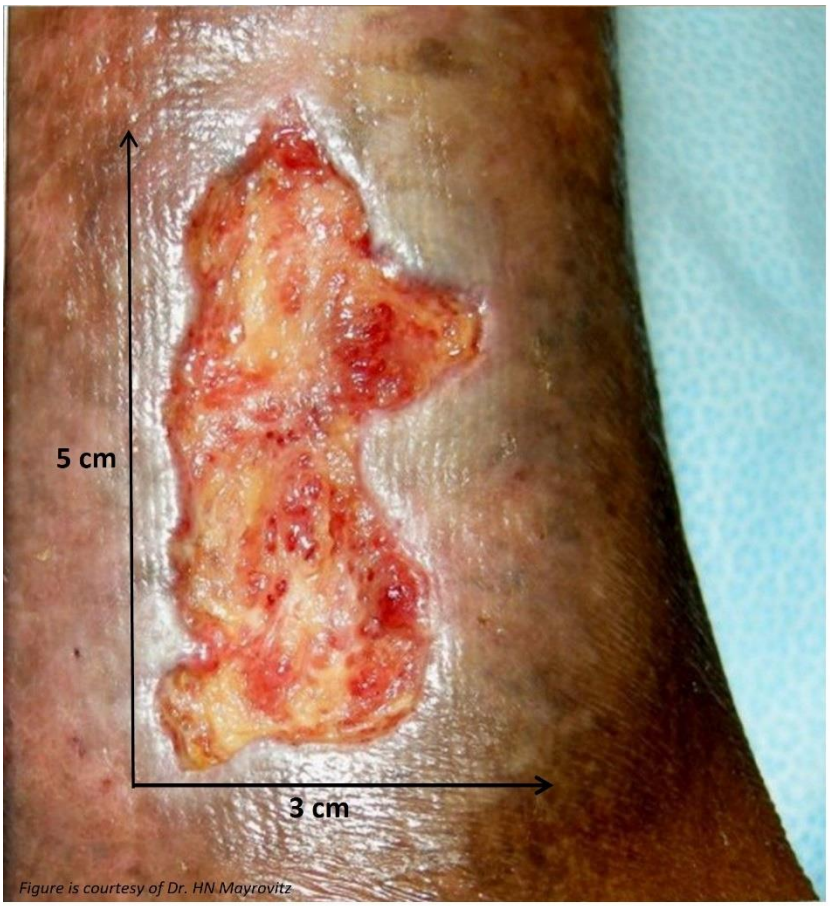

Figure 4. a venous ulcer located on the lateral gaiter area.

These ulcers typically have an irregular shape and characteristic wound bed granulation tissue and surrounding tissue hyperpigmentation. Figure is provided as a courtesy of Dr. HN Mayrovitz

Return to text.

Figure 5

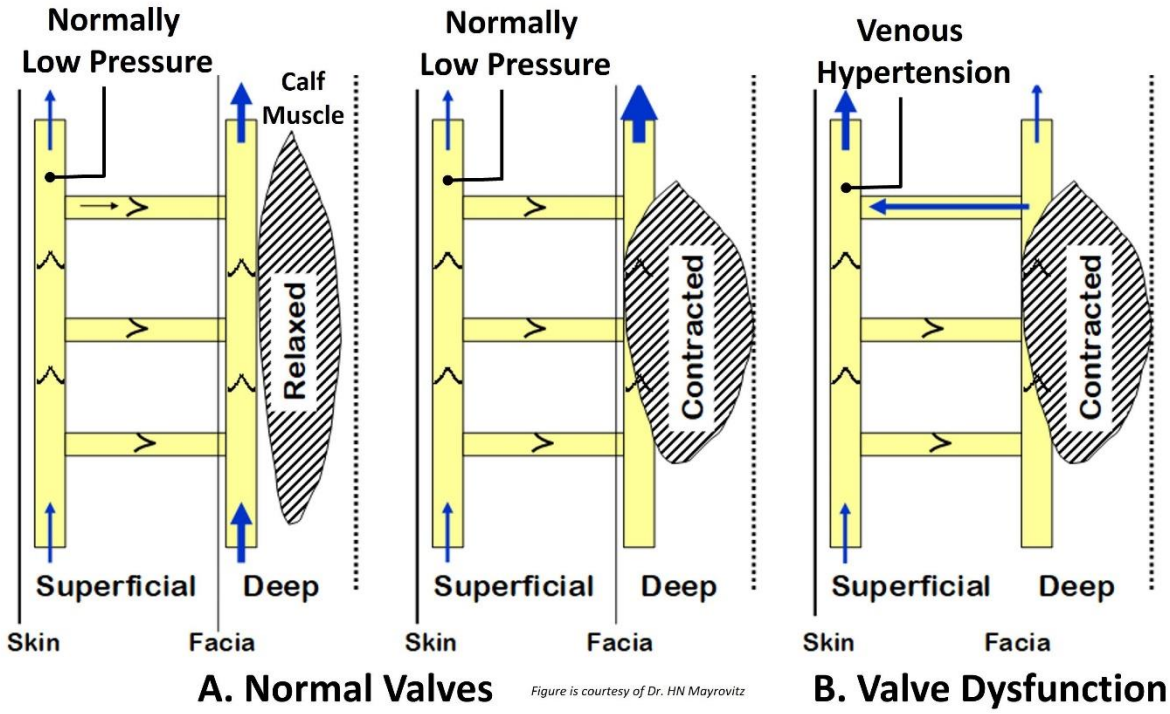

Figure 5. Schema of impact and hemodynamics of incompetent venous valves.

The normally low pressure experienced by the superficial veins is subject to high pressures in the presence of the valve incompetency as shown in part B. This elevated pressure is not well tolerated and causes venous injury that triggers a sequence of events that may lead to the development of a venous ulcer. Figure is provided as a courtesy of Dr. HN Mayrovitz

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Figure 6

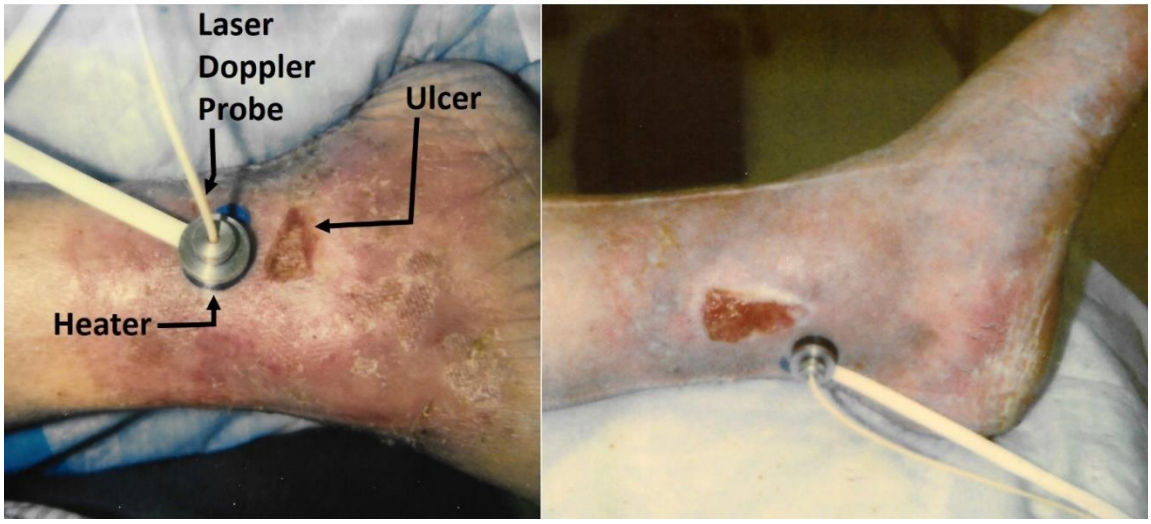

Figure 6. Peri-wound skin blood perfusion measurement in a venous ulcer. A laser Doppler probe is fitted through a concentric hole in the heater that is in contact with skin. Localized heating produces an increase in microvascular perfusion in healthy skin but with a differ pattern in peri-wound skin as shown in figure 7. Figure is provided as a courtesy of Dr. HN Mayrovitz

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Figure 7

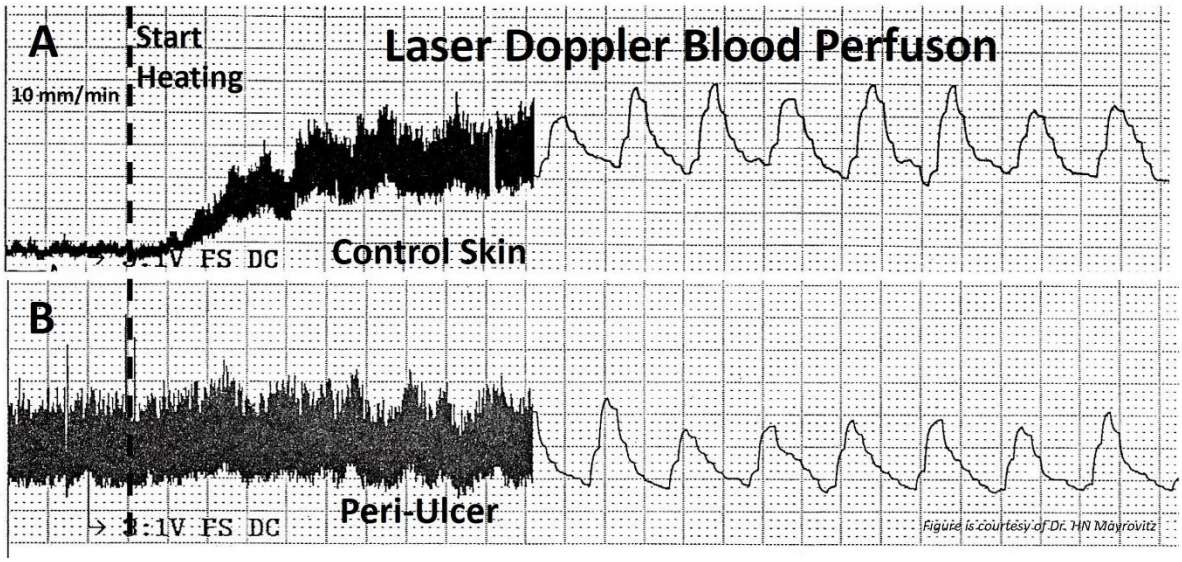

Figure 7. Skin blood perfusion response to heat in healthy vs. peri-ulcer skin.

The responses show a normal response to localized heating (A) and a common finding associated with venous ulcers (B). In B, an elevated peri-ulcer basal resting perfusion is noted with little if any microvascular reserve when stimulated with heat. Contrastingly, in control skin as shown in part (A) a normal active hyperemia is noted in response to the heating.

Figure is provided as a courtesy of Dr. HN Mayrovitz

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Figure 8

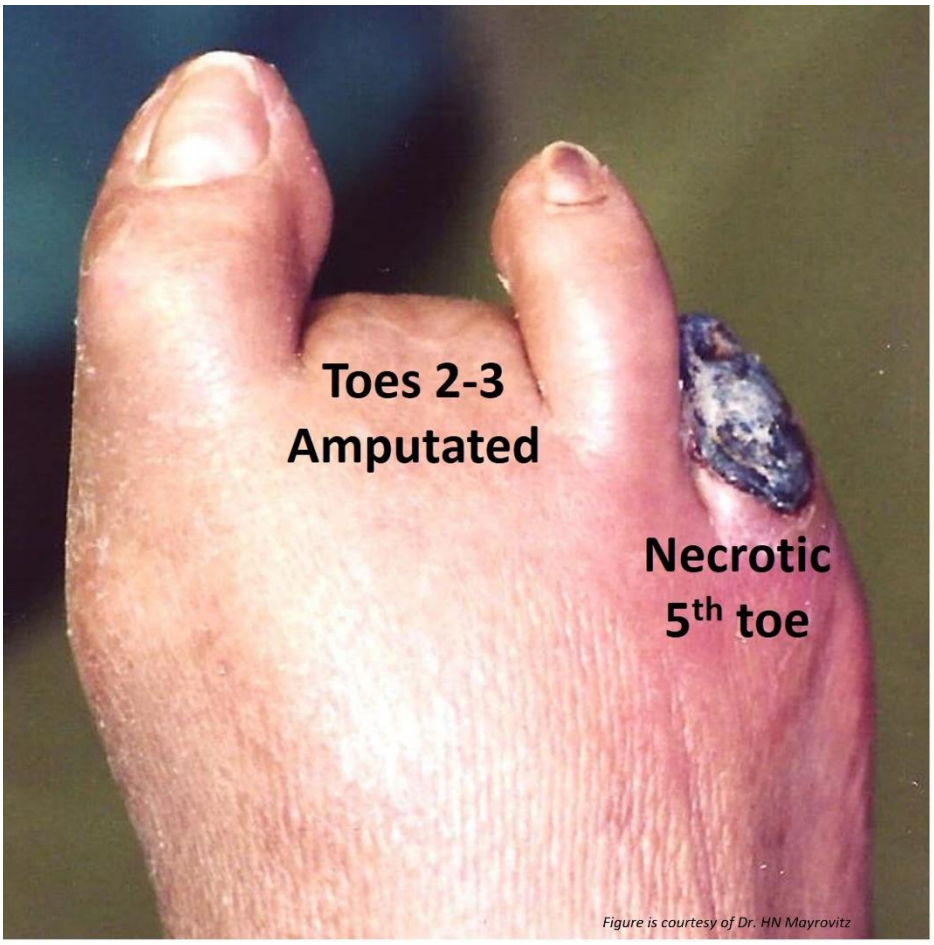

Figure 8. Illustrating aspects of an arterial ulcer.

A patient with critical ischemia due to significant PAD in whom toes 2-3 were previously amputated and toe 5 is necrotic.

Figure is provided as a courtesy of Dr. HN Mayrovitz

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Figure 9

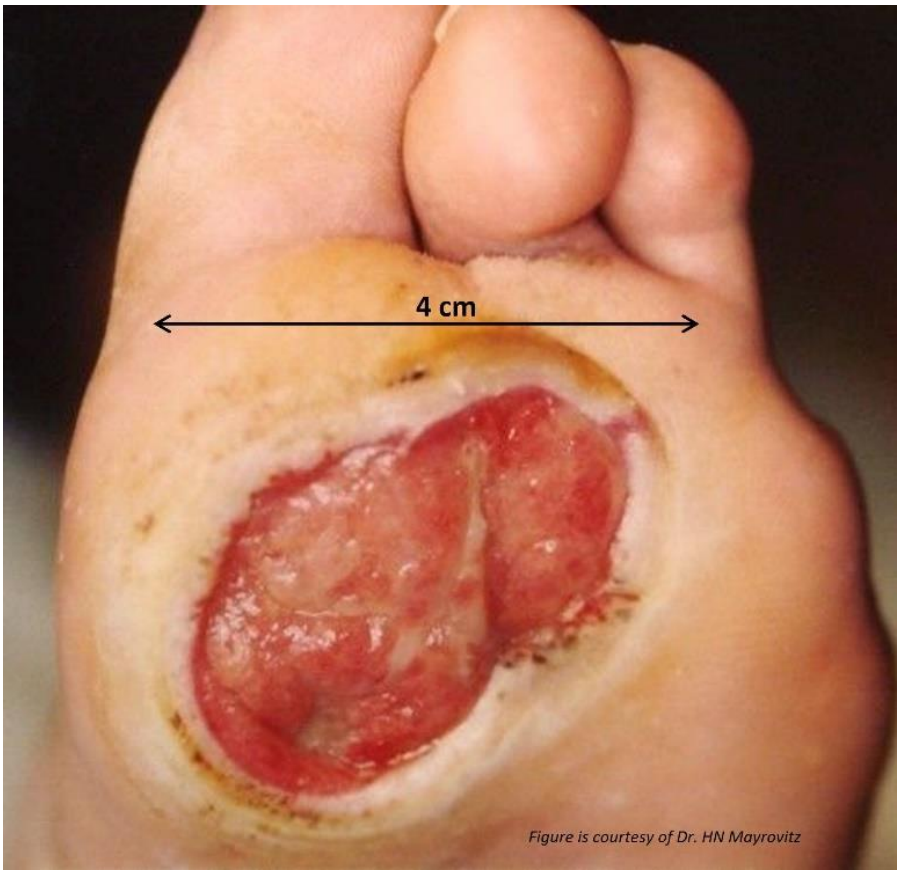

Figure 9. An example of a plantar diabetic neuropathic ulcer

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