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Healing of Diabetic Foot Wounds-The Role of Basic Science and Underlying Pathology in Management of Diabetic Foot Wounds

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Abstract

Introduction: While many articles exist on wound healing, little has been written on the healing of diabetic foot wounds. In a diabetic foot wound, in contrast to other wounds, special factors are at play including the elements of the 'diabetic foot triad' and underlying pathology involved. The latter must first be addressed and treated if the wound is to heal.

Impact of Basic Science of wound healing on healing of diabetic foot wounds: The basic science of wound healing included its four phases of Hemostasis, Inflammation, Proliferation and Remodeling. To promote healing chronic wounds resistant to healing, chronic wounds are converted by debridement into acute wounds to trigger acute healing process by releasing useful growth factors directing the phase of wound healing again. Wounds in the inflammatory phase with more exudate require dressings to be changed more frequently than wounds in the proliferation phase.

Impact of Community Factors on Healing of Diabetic Foot Wounds: Patients must be able to go to a nearby polyclinic or hospital as an outpatient for dressing of wounds. When no clinic is available nearby, arrangement must be made for a nurse to go to the patient's home to perform the dressings (from a Home Nursing Foundation). Alternatively, patients and caregivers are taught to do their own dressings.

Conclusions: Because of the complexity and multiplicity of factors at work in the healing of diabetic foot wounds, a holistic approach is required including control of diabetes, control of nutrition, selection of dressings, choice of antibiotics and the need for early and adequate surgical debridement. Adequate surgical debridement is often the key to good wound healing. Wound bed preparation is best performed using the TIME concept. The dressing to be selected can also be done following the TIME Guide. Off-loading is required for neuropathic ulcers. The treatment of the diabetic foot wound is therefore best performed by an inter-disciplinary diabetic foot team which discusses jointly all issues and efficiently promotes the healing of diabetic foot wounds.

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Introduction

Whilst much has been written on the healing of wounds in general, very little has been written specifically on the healing of diabetic foot wounds. Doctors and nurses are aware that diabetic wounds are one of the most difficult wounds to treat. Indeed, it is often wise to refer such wounds to a specialist trained for the management of such wounds.

There are several factors at work in diabetic wounds which are not seen with other wounds. These require attention if we are to succeed in healing the wound. Systemic factors which require special attention include the control of diabetes itself and also the nutritional status of the patient.

Control of Diabetes

With diabetes wounds, it is important to make sure that diabetes is well controlled. The wound would not heal with unless the blood sugar is under control. Endocrinologist and dietitian play a very important role. Hb1Ac must be controlled to less than 7%.

Nutritional Control

The albumin level must also be normal to ensure that enough proteins are available as building blocks to heal the wound. Hemoglobin must be above 10g/dl to ensure there is enough oxygen perfusing the wound and enough adenosine triphosphates to provide energy for healing the wound. Local factors at play in a diabetic foot wound include the "diabetic foot triad" and underlying pathology present.

Diabetic Foot Triad

In the diabetic foot, the 3 components of the Diabetic Foot Triad [1], namely Neuropathy, Immunopathy and Vasculopathy (Figure 1) play a big role in determining the outcome of healing of diabetic foot wounds.

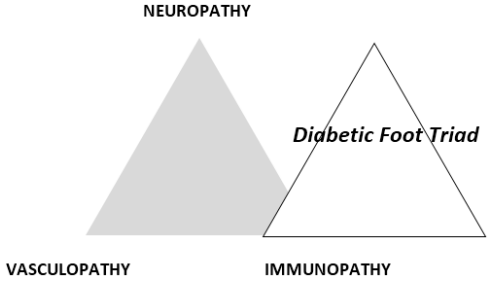

Figure 1: *The Diabetic Foot Triad.*

In each wound, the contribution by each component may be different. In some, vascular components predominate. In others, the problem is mainly infection due to immunopathy.

Underlying Pathology

In addition, one must pay attention to the underlying pathology prevailing in each wound. The pathology includes infection, ischemia, infection and ischemia, neuropathy or decubitus. An infective wound (Figure 2) is usually found on the dorsum of the foot. An ischemic ulcer (Figure 3) results when there is a lack of blood flow to the feet. Patients suffering from atherosclerosis have a higher chance of developing ischemic ulcers. A neuropathic ulcer (Figure 4) is usually found on the sole of the foot. A Decubitus ulcer (Figure 5) is usually found on the over the lateral malleolus, the lateral aspect of base of 5th metatarsal as well as over the heel.

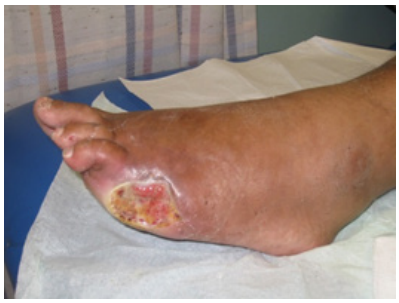

Figure 2: *An infective wound over 5th metatarsal area.*

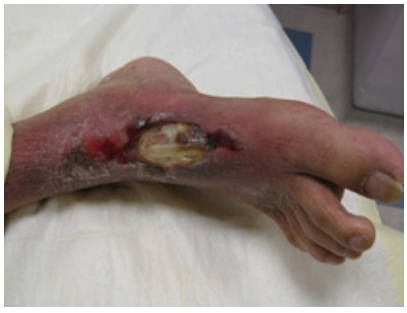

Figure 3: *An ischemic wound on dorsum of foot.*

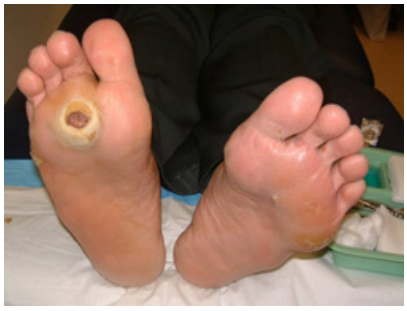

Figure 4: *A neuropathic ulcer over 2nd metatarsal head.*

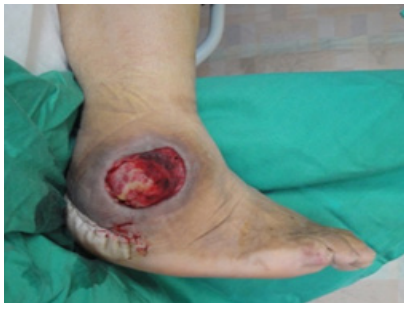

Figure 5: *A decubitus ulcer over lateral malleolus region.*

Impact of Underlying Pathology on Healing of Diabetic Foot Wound

Before a diabetic wound will heal the underlying pathology must be addressed and treated. In an infected wound, adequate surgical debridement must be performed to remove all devitalised and infected tissue and the appropriate antibiotics administered. In an ischaemic ulcer, the foot needs to be revascularised. Duplex ultrasound is performed to identify blockages in the anterior tibial, posterior tibial or peroneal artery and angioplasty performed to improve the blood flow supplying the foot and the wound. Only then can the wound heal. In neuropathic ulcers mechanical factors must be addressed. Off-loading the ulcer is mandatory in addition to dressing of wounds. In decubitus ulcers, in addition to the debridement and antibiotics, supportive measures must be instituted including 2 hourly turning of patient and the use of ankle wraps to help off-load the ulcer.

Importance of Team Approach in Healing Diabetic Wounds

Because of the complexity and multitude of factors at play in the healing of diabetic foot wounds, the latter is best treated by an inter-disciplinary diabetic foot team including endocrinologist, orthopaedic surgeon, vascular surgeon, infectious disease consultant, podiatrist, wound care nurse, education nurse, dietitian and medico-social workers.

Anatomy of the Skin

It is also important to understand the anatomy and functions of the skin in order to better comprehend the basic science of wound healing. The skin is composed of 2 major layers: the epidermis and the dermis. Both skin layers contain cells and extracellular structures. The epidermis is a multi-layered sheet of cells with little extracellular matrix. It is the outermost, continuously renewing part of the skin which is composed of several layers and which includes several cell types. (Figure 6)

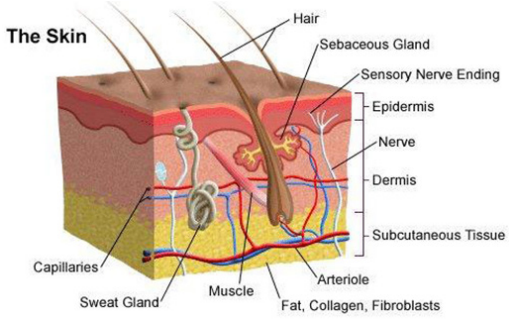

Figure 6: *(from http://hospitals.unm.edu/burn/skin_anatomy.shtml): Cross section of the skin Different depths of wound heal differently. A Wagner 1 wound is a partial thickness wound which heals by epithelialisation. A Wagner 2 wound is a full thickness wound which heals by scar formation.*

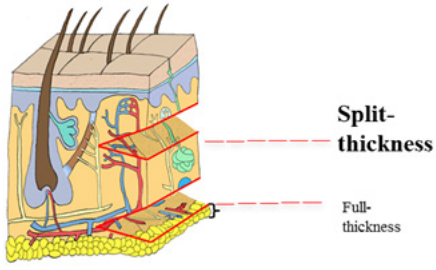

Figure 7: *(Adapted from Pearson Education Inc 2004): Difference in amount of dermis harvested for split and full thickness skin grafts.*

The Wagner-Meggitt Wound Classification [2] (Figure 8) is a useful six-grade system to classify ulcers according to the depth and extent of wound.

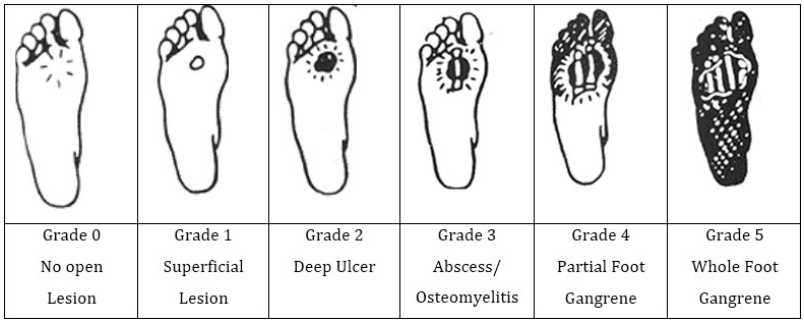

Figure 8: *(Adapted from http://www.oandp.org/): The Wagner-Meggitt Wound Classification Advantages of the Wagner-Meggitt Wound classification include its simplicity in usage. It also provides a useful guide for practitioners to plan treatment.*

Basic Science of Wound Healing

Wounds have the intrinsic capacity to heal. It is a complex process. The process occurs in 4 phases, namely hemostasis, inflammation, proliferation and remodeling (Table 1, Figure 9).

Phase	Time After Injury	Cells Involved	Activity
Hemostasis	Immediately	Platelets	Clotting
Inflammation	Day 1 to 4	Neutrophils Macrophages	Phagocytosis
Proliferation	Day 4 to 21	Fibroblasts Myofibroblasts Angiocytes Lymphocytes Keratinocytes Macrophages	Collagen secretion Wound contraction Angiogenesis Epithelialisation Forms new granulation tissue
Remodeling	Day 21 to 2 years	Fibrocytes	Develop tensile strength

Table 1: *(Adapted from Orsted., et al[3].): 4 phases of wound healing.*

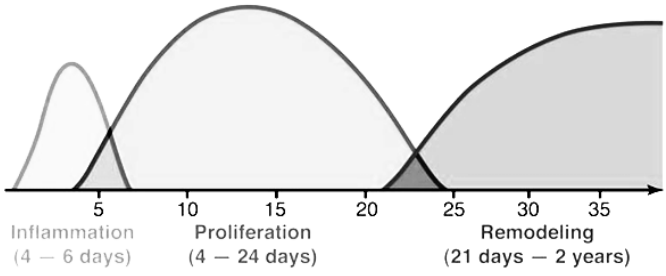

Figure 9: *(Adapted from http://woundeducators.com/): Phases of wound healing.*

Hemostasis

Hemostasis takes place immediately after injury [3,4]. During this phase, adenosine diphosphate (ADP) from damaged tissues cause platelets to adhere to the exposed Type I Collagen. This activates the platelets and stimulates the clotting cascade, leading to the formation of a clot to seal damaged blood vessels [3,5]. The activated platelets also secrete platelet-derived growth factor (PDGF) which recruits neutrophils and monocytes [3].

Inflammation

The inflammation phase occurs from day 1 to day 4 following injury [3,5]. Platelets continue to release PDGF and transforming growth factor-beta (TGF-b) from their alpha granules to attract neutrophils and macrophages [5]. The extracellular matrix interacts with the integrin receptors of cells, resulting in platelet activation, epithelial cell migration and fibroblast movement. Neutrophils and macrophages phagocytose bacteria to prevent infection [3]. The macrophages, fibroblasts and other cells involved in wound healing secrete various growth factors. Key growth factors include the fibroblast growth factor, the epidermal growth factor, transforming growth factor-beta, and interleukins. These play an important role in directing the next phase of wound healing [3,5]. Table 1 shows some of the functions of important growth factors.

Proliferation

Proliferation can take place from day 4 to day 21 [3]. Several processes occur during this phase. Fibroblasts migrate to the wound site under the influence of growth factors such as TGF-beta and PDGF [4], and secrete mainly Type III Collagen [6]. Pericytes are involved in angiogenesis, regenerating the outer layers of capillaries and the endothelial cells. Keratinocytes are responsible for epithelialization and differentiate to form the protective outer layer of the skin [3].

MMPs and TIMPs

During proliferation, there is a balance between two types of enzymes, namely Matrix Metalloproteases (MMPs) and Tissue Inhibitors of Metalloproteases (TIMPs). This balance is vital in ensuring that there is a net production of new tissue to allow for proper wound healing. MMPs are zinc-dependent endopeptidases made up of 3 domains, namely the pro-peptide domain, the catalytic domain and the haemopexin-like domain. They are secreted by macrophages, neutrophils, fibroblasts and other cell types during wound healing. TIMPs are proteases which inactivate MMPs by reversibly binding to them [3].

23 human MMPs have been identified, with MMP-1, MMP-2, MMP-8 and MMP-9 being the particular focus of research in wound healing. MMPs play several roles throughout the process of normal wound healing. These include the removal of damaged tissue and bacteria during inflammation, facilitating epidermal cell migration during proliferation and the contraction of scar extracellular matrix during remodeling.

Although controlled levels of MMPs are important for normal wound healing, elevated levels of MMPs can result in the degradation of essential growth factors and their receptors, as well as extracellular matrix proteins [6]. During the normal wound healing process, the levels of proteases (including MMPs) peak at about day 3 and begin to fall by about day 5 [7]. An excess of MMPs and a decrease in the level of TIMPs has been identified as a cause of impaired healing [8,9,10]. When wound healing is impaired, it is due to an imbalance between the levels of MMPs and TIMPs. The reason for the imbalance is not fully understood [8].

Collagen

Collagen plays a key role in wound repair and is a major protein component of the extracellular matrix. The collagen molecule is composed of three alpha chains that collectively form a triple helix structure. Collagen fibrils consist of collagen molecules linked by intermolecular cross-links [11]. These give collagen high tensile strength and thermal stability [12].

Collagen in the skin are predominantly Type I and Type III. Both types of collagen are found in all layers of the dermis. However, type III collagen is concentrated in the outer layers of the skin [13,14]. Collagen in the skin is about 80% Type I and 20% Type III [4,5]. During wound healing, the fibroblasts primarily produce Type III Collagen which is subsequently replaced by Type I Collagen in the next stage of wound healing [5].

Remodeling

The remodeling phase begins at day 21, and can continue for up to 2 years after wounding [3]. During this phase, the initial Type III Collagen is replaced by Type I Collagen until a Type I: Type III ratio of 4:1 is reached. This is similar to the ratio found in normal skin [5,16].

Collagen is reorganised along lines of tension and crosslinks to give added strength [6]. The strength of collagen eventually reaches 80% of that of unwounded tissue [4,15]. Cell density and vascularity decrease as a result of apoptosis [17]. Fibroblasts differentiate into myofibroblasts which express smooth muscle alpha-actin and actin-myosin stress fibers. These myofibroblasts are responsible for wound contraction that leads to wound closure [18].

Impact of Basic Science of Wound Healing on Healing of Diabetic Foot Wounds

Several diabetic wounds are chronic and resistant to heal. Debridement of such wounds can play a role in promoting the healing of such chronic "anergic" wounds by converting such chronic wounds into acute wounds. This will trigger the acute healing process by releasing the useful growth factors which direct the phases of wound healing again.

The frequency of dressing changes required can also be determined by the phase of the wound healing the wound is in. In wounds undergoing inflammatory phase of healing, with much inflammation and exudate occurring, dressings need to be changed more frequently than in wounds undergoing the proliferation phase.

Factors Influencing Healing of Diabetic Foot Wound

Several factors affect wound healing, namely the patient, his home and his community (Figure 10).

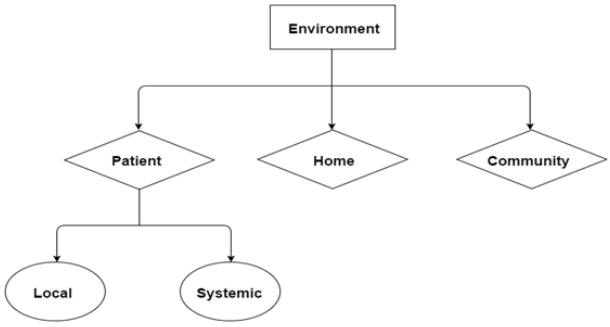

Figure 10: *Factors affecting wound healing.*

Patient factors include local and systemic factors. Local factors include the 3 components of the diabetic foot triad (Figure 1), namely neuropathy, vasculopathy and immunopathy. Systemic factors include endocrine control, haemoglobin level and level of nutrition. For wounds to heal, it is important to control the blood sugar level. The level of haemoglobin must also be adequate – more than 10 g/dL. This is necessary to carry sufficient amount of oxygen via haemoglobin to the tissue. HbA1C should preferably by < 7%. Oxygen is needed for aerobic respiration to generate adenosine triphosphate (ATP). ATP is a source of energy for cell proliferation and collagen synthesis [18]. Nutrition also plays a key role in influencing wound healing. Albumin and other proteins from foods are needed to support wound healing.

Home factors include sufficient space in the home to provide for patient mobility, and cleanliness of the home to provide a clean environment for wound healing. Thirdly, family support must be available to promote foot hygiene and to dress the wounds of the patient. Where no family member is present, a caregiver is useful.

Community factors play an important role in influencing wound healing. These include the accessibility of the community to the patient. Walking aids and wheelchairs should be made available to them to facilitate their movement in the community. It is also useful to have a polyclinic and a hospital in close proximity to the home. This facilitates healing of the patient's wound.

Impact of Community Factors on Healing of Diabetic Foot Wounds

It is therefore important to ensure that the patient with diabetic foot wound should be able to visit a polyclinic or a hospital nearby for the dressing of wound as an outpatient. If there is no clinic available nearby, arrangement must be made for a nurse to go to the patient's home to do the dressing. Such a nurse need to be contracted from a Home Nursing Foundation providing such nursing.

Nowadays, we advocate teaching our patients and caregivers to do their own dressings in their home. Our experience shows that better results are obtained when patients perform their own dressings. However, for this to succeed patients and caregivers must be carefully trained by our nurses how to perform their own dressings.

Concepts in Management of Diabetic Foot Wound

Holistic Approach

Wound healing is both an art and a science [19]. It requires a holistic approach. One has to treat the whole patient and not just the wound alone.

General Assessment

General assessment of the patient must be performed. This includes assessment of endocrine control, nutritional assessment of the patient and choice of antibiotics to be given.

Local Assessment

The foot and wound must then be examined methodically to assess for all three components of the diabetic foot triad (Figure 1) – vasculopathy, neuropathy and immunopathy[1]. The underlying pathology must also be recognized and treated (Figure 2,3,4,5). The wound is classified according to the Wagner-Meggitt classification [2] for better management.

Medical Treatment

Medical treatment of the wound must be instituted first. Blood sugar level must be controlled. Antibiotics needed is started after tissue is sent for culture and sensitivity.

Surgical Treatment

The need for surgical debridement is made by the clinician after assessing the wound.

Wound Bed Preparation

This is performed following the TIME Concept (Table 2) [20]: T for Tissue, I for Infection and Inflammation, M for Moisture and E for Epidermal Margin.

Clinical Observations	Proposed Pathophysiology	WBP clinical actions	Effect of WBP actions	Clinical outcome
Tissue non-viable or deficient	Defective matrix and cell debris impair healing	Debridement (episodic or continuous): • Autolytic, sharp surgical, enzymatic, mechanical or biological • Biological agents	Restoration of wound base and functional extracellular matrix proteins	Viable wound base
Infection or Inflammation	High bacterial counts or prolonged inflammation Inflammatory cytokines Protease activity Growth factor activity	Remove infected foci Topical/systemic: • Antimicrobials • Anti-inflammatories • Protease inhibition	Low bacterial counts or controlled inflammation: Inflammatory cytokines Protease activity Growth factor activity	Bacterial balance and reduced inflammation
Moisture imbalance	Desiccation slows epithelial cell migration Excessive fluid causes maceration of wound margin	Apply moisture- balancing dressings Compression, negative pressure or other methods of removing fluid	Restored epithelial cell migration, desiccation avoided Oedema, excessive fluid controlled, maceration avoided	Moisture balance
Edge of wound — non-advancing or undermined	Non-migrating keratinocytes Non-responsive wound cells and abnormalities in extracellular matrix or abnormal protease activity	Re-assess cause or consider corrective therapies: • Debridement • Skin grafts • Biological agents • Adjunctive therapies	Migrating keratinocytes and responsive wound cells. Restoration of appropriate protease profile	Advancing epidermal margin

Table 2: *TIME Concept [20].*

The wound dressing used is selected according to the TIME Guide (Table 3).

	Aim of care	Exudate	Dressing	
			Contact layer	Outer dressing
Tissue Necrotic	If vascular supply is good, debride eschar and promote moisture balance	Dry or Low	Hydrogel	Hydrocolloid or foam
		Moderate	hydrocolloid	Gauze or film
		Heavy Alginate, foam or hydrofibre		Gauze or foam with pad
	If vascular supply is compromised, keep eschar dry	Dry	Tulle gauze, film	Film or gauze
Sloughy	Deslough, provide moisture balance	Low	Hydrocolloid, hydrogel	Gauze or film
		Moderate	Alginate	Hydrocolloid with pad or gauze
		Heavy	Foam, hydrofibre	Foam with pad or gauze
			Negative Pressure Wound Therapy(NPWT)	
Granulating	Provide moisture balance	Low	Non adherence	Film or gauze
		Moderate	Hydrocolloid, foam	Gauze or contact layer with pad
			NPWT	
Epithelializing	Provide moisture balance	Low	Non adherence material	Film or gauze
		Moderale	Hydrocolloid or foam	Gauze
Infection	Get rid of infection (biofilm)	Low Nano-crystalline or ion silver containing material, Iodine cream		Hydrocolloid with pad or gauze
		Moderate Silver containing, iodine containing material		Foam with pad or gauze
Moisture balance	Maintain moist environment	Low	Film, hydrogel	Gauze
		Moderate Alginate	Hydrocolloid,	Contact layer with pad or foam
		Heavy	Foam, hydrofibre, NPWT	Contact layer with pad or gauze
Edge	Promote advance of wound edge	Low	Film, hydrogel	Gauze
		Moderate Alginate, NPWT	Hydrocolloid,	Contact layer with pad or foam
		Heavy Foam, hydrofibre, NPWT		Contact layer with pad or gauze

Table 3: *TIME Guide [21].*

New Trends in Wound Healing

Over the last few years, new trends have emerged in the healing of wounds. These include the use of silver dressings, Platelet-Rich Plasma, Mesenchymal Stem Cells, Matrix Metalloproteinases and the Protease Test. It is important to evaluate whether these new developments are effective in promoting the healing of wounds.

So far, there is no evidence that silver dressings can promote wound healing compared to conventional non-silver dressings [22]. With regards to the role of Platelet-Rich Plasma (PRP), there is also no evidence that PRP promotes wound healing [22]. Conflicting evidence exists in literature. With regards to Mesenchymal Stem Cells, present evidence does not support its role for wound healing [22].

Literature suggests that Matrix Metalloproteinases (MMP) level can be an accurate marker for wound healing if it can be measured. At present, it is very hard to measure MMP activity in wound fluids or biopsies [23]. A new diagnostic tool is needed to measure MMP activity in wounds. Such a device is still not available [7].

Conclusions

In a diabetic foot wound, special factors are at play include the elements of the 'diabetic foot triad' and underlying pathology present. The latter must first be addressed and treated before the wound can heal. Because of the complexity and multiplicity of factors at work in the healing of diabetic foot wounds, a holistic approach is required including control of diabetes, control of nutrition, selection of dressings, choice of antibiotics and the need for early and adequate surgical debridement when indicated. Adequate surgical debridement is often the key to good wound healing. Wound bed preparation is best performed using the TIME concept. The dressing to be selected can also be done following the TIME Guide. Off-loading is required for neuropathic ulcers. The treatment of diabetic foot wounds is therefore best performed by an inter-disciplinary diabetic foot team which discusses jointly all issues involved and efficiently promotes the healing of such wounds.

References

1. Nather A and Tan TF. "Diabetic Foot Wounds- Types of Wounds and Classification Systems. In:Surgery for Diabetic Foot". *World Scientific* (2016): 1-9.
2. Wagner FW. "The Dysvascular foot: A system for diagnosis and treatment". *Foot & Ankle International* 2.2 (1981): 64-122.
3. Orsted HL., *et al*. "Basic Principles of Wound Healing". *Wound Care Canada* 9.2 (2004): 4-12.
4. Velnar T., *et al*. "The wound healing process: An overview of the cellular and molecular mechanisms". *Journal of International Medical Research* 37.5 (2009):1528-1542.
5. Gabriel A, Molnar J. Wound Healing and Growth Factors. Medscape.
6. Gibson D., *et al.* "MMPs Made Easy". Wound International. Published November 18, 2014. Accessed April 18, 2016.
7. International consensus. The role of proteases in wound diagnostics. An expert working group review. London: Wounds International, 2011.
8. Muller M., *et al*. "Matrix metalloproteinases and diabetic foot ulcers: The ratio of MMP-1 to TIMP-1 is a predictor of wound healing". *Diabetic Medicine* 25.4 (2008): 419-426.
9. Schultz GS and Wysocki A. "Interactions between extracellular matrix and growth factors in wound healing". *Wound Repair and Regeneration* 17.2 (2009): 153-162.
10. Yager DR., *et al.* "Wound fluids from human pressure ulcers contain elevated matrix Metalloproteinase levels and activity compared to surgical wound fluids". *Journal of Investigative Dermatology* 107.5 (1996): 743-748.
11. Moore K. "*The Scientific Basis of Wound Healing. In: The Scientific Basis of Tissue Transplantation*". *Ed. Nather A Singapore: World Scientific*. (2001): 379-398.
12. Shoulders MD and Raines RT. "Collagen structure and stability". *Annual Review of Biochemistry* 78.1 (2009): 929-958.
13. Meigel WN., *et al.* "Dermal architecture and collagen type distribution". *Archives for Dermatological Research* 259.1 (1977): 1-10.

14. Ackerman AB., *et al.* "Embryologic, Histologic and Anatomic Aspects. In: *Histologic diagnosis of inflammatory skin diseases: An Algorithmic method based on pattern analysis"*. Ed. Ackerman AB, Boer A, Bennin B. 3rd ed. Ardor Scribendi, United States, 2005.
15. Wang C., *et al*. "The content and ratio of type I and III collagen in skin differ with age and injury". *African Journal of Biotechnology* 10.13 (2011): 2524-2529.
16. Broughton G and Rohrich R. "Wounds and Scars". *Selected Readings in Plastic Surgery* 10.7 (2005): 1-54.
17. Van De Water L., *et al*. "Mechanoregulation of the Myofibroblast in wound contraction, Scarring, and fibrosis: Opportunities for new therapeutic intervention". *Advances in Wound Care* 2.4 (2013): 122-141.
18. Schreml S., *et al*. "Oxygen in acute and chronic wound healing". *British Journal of Dermatology* 163.2 (2010): 257-268.
19. Teo ZL., *et al*. "Basic Science of Wound Healing. In: The Diabetic Foot". Ed. Nather A:World Scientific; 2012: 89-97.
20. Schultz GS., *et al*. "Wound bed preparation: A systematic approach to wound management". *Wound Repair and Regeneration* 11.s1 (2003): S1–S28.
21. Nather A., *et al.* "Best practice guidelines for ASEAN Plus: Management of diabetic foot wounds". *Sri Lanka Journal of Diabetes Endocrinology and Metabolism* 5.1 (2015): 1.
22. The International Working Group on the Diabetic Foot. Prevention and Management of Foot Problems in Diabetes Guidance Documents and Recommendations 2015. IWGDF.
23. Beidler SK., *et al*. "Multiplexed analysis of matrix metalloproteinases in leg ulcer tissue of patients with chronic venous insufficiency before and after compression therapy". *Wound Repair and Regeneration* 16.5 (2008): 642-648.