EVALUATION OF THE EFFICACY OF HYDROCYN™ AQUA IN COMPARISON TO COMMERCIALLY AVAILABLE SUPER OXIDIZED SOLUTION AS DRESSING SOLUTION FOR DIABETIC FOOT ULCERS

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Abbreviations

ABSI:	Ankle Brachial Systolic Index
BMI:	Body mass index
CASOS:	Commercially available super oxidized solution
ESBL:	Extended Spectrum Beta Lactamase Producing Organisms
HOCL:	Hypochlorous Acid
HUSM:	Hospital Universiti Sains Malaysia
WHO:	World Health Organization
MDRO:	Multi Drug Resistant Organisms
MRSA:	Methicillin Resistant *Staphylococcus Aureus*
NaOH:	Sodium Hydroxide
NaClo:	Sodium Hypochlorite
ORP:	Oxygen reduction potential
ppm:	parts per million
SD:	Standard Deviation

PERBANDINGAN PENILAIAN KEBERKESANAN ANTARA HYDROCYN™ AQUA DAN LARUTAN "SUPER OXIDIZED" KOMERSIL PADA LUKA DI KAKI AKIBAT KENCING MANIS.

ABSTRAK

Latar Belakang:

Pesakit kencing manis secara am nya mempunyai 15% risiko untuk menghidapi luka di kaki mereka. Matlamat perawatan adalah untuk mendapatkan penyembuhan luka dengan menggabungkan rawatan pembedahan dan bukan pembedahan sekaligus mengurangkan risiko amputasi anggota badan. Larutan pembasmi infeksi secara tradisinya digunakan untuk pencucian luka namun mempunyai perbezaan pendapat di antara pengamal-pengamal kesihatan mengenai kesan toksik kepada sel-sel hos. Hydrocyn™ Aqua merupakan satu larutan antiseptik di dalam bentuk larutan "super oxidized" yang stabil dengan pH yang neutral dan tempoh tahan simpanan yang panjang.

Objektif:

Objektif kajian ini adalah untuk membandingkan keberkesanan larutan Hydrocyn™ Aqua dengan jenama komersil larutan "super oxidized" di dalam pengurangan saiz luka dan juga penyediaan dasar luka.

Cara Kajian:

Kajian klinikal prospektif terkawal secara rambang dengan kaedah "single blinded" ini melibatkan pesakit yang mempunyai penyakit kencing manis, luka kaki akibat kencing manis yang dijangkiti kuman dan yang mendapatkan rawatan pembedahan "debridement" di Jabatan Ortopedik, Hospital Universiti Sains Malaysia dari Januari 2015 hingga Disember

2015. Pesakit-pesakit yang memenuhi kriteria inklusif dan eksklusif di beri rawatan selama 2 minggu, ditentukan secara rambang sama ada rawatan dengan Hydrocyn™ Aqua (n=30) atau larutan "super oxidized" komersil (n=30) menggunakan perisian komputer untuk proses perambangan. Pelekat informasi pada kedua-dua botol larutan ditanggalkan supaya pesakit-pesakit tidak mengetahui rawatan yang diterima. Keputusan kajian ini meliputi data-data demografi, saiz luka, pemarkahan dasar luka dan juga komplikasi pada awal rawatan dan 2 minggu selepas rawatan.

Keputusan:

Pengumpulan dan analisis data dilakukan menggunakan perisian IBM SPSS Statistic Versi 22. Keputusan peratusan pengurangan saiz luka di antara kumpulan Hydrocyn™ Aqua dan larutan "super oxidized" komersil menunjukkan tiada perbezaan di antara mereka ($p = 0.9$). keputusan pemarkahan dasar luka juga menunjukkan tiada perbezaan yang ketara ($p = 0.09$). Tiada komplikasi direkodkan.

Kesimpulan:

Tiada perbezaan yang ketara di antara Hydrocyn™ Aqua berbanding larutan "super oxidized" komersil di dalam keberkesanan merawat luka di kaki akibat kencing manis terutama pada pengurangan saiz luka dan juga penyediaan dasar luka.

Evaluation of the Efficacy of Hydrocyn™ Aqua In Comparison To Commercially Available Super Oxidized Solution as Dressing Solution for Diabetic Foot Ulcers

Abstract

Introduction:

Diabetic patients will have a 15% of risk of developing diabetic foot ulcers during the disease course and usually this is complicated by infections. The principle aim of treatment is to promote wound healing combining surgical and non-surgical treatment. Disinfectants have been used traditionally but it has the argument of cyto-toxicity against host. Hydrocyn™ Aqua is an antiseptic solution in the form of stable super oxidized solution with neutral pH and longer shelf life which can be used in the treatment of diabetic foot ulcers.

Objective:

The objective of this study is to compare the efficacy of Hydrocyn™ Aqua with the commercially available super oxidized solution (CASOS) in term of wound size reduction and optimal wound bed preparation.

Methodology:

This was a prospective, single-blinded randomized controlled trial involving patients with diabetes mellitus, who attended Orthopaedic Department Hospital Universiti Sains Malaysia (HUSM), Kubang Kerian between January to December 2015 which have been surgically debrided. Patients who fulfilled the criteria were randomized to receive treatment with Hydrocyn™ Aqua (n = 30) or CASOS (n = 30) with daily dressing for 2 weeks. Outcome measures include demographic data, size of wounds, wound bed scoring and complications at baseline and at 2 weeks.

Results:

Wound reduction percentage after 2 weeks of treatment with Hydrocyn™ Aqua and CASOS showed no difference in both groups (p= 0.9). The wound bed scoring at 2 weeks of treatment showed no difference for both treatments. (p= 0.09) There was no adverse reactions recorded for both groups.

Conclusion:

There is no significant difference for the efficacy of Hydrocyn™ Aqua compared to the commercially available super oxidized solution in the treatment of diabetic foot ulcers.

CHAPTER 1: INTRODUCTION

1.1 Problem Statement

Diabetic foot ulcers is a known foot complications in diabetic patients and may consequently result in amputation rate as well as being life threatening. It has been estimated that around 15 % of patients with diabetes will develop lower extremity ulcer during the course of their disease (Palumbo PJ, 1995). The prevalence of foot ulceration in patients attending the diabetic outpatient clinics in Malaysia has been reported as 1.2% with almost double increase in the figure from year 2011 to 2012(Mustapha and Azmi, 2013). Diabetic ulcers and its complications are a major cause of morbidity, a leading case of hospital bed occupancy and account for substantial health care costs and resources(Girod *et al.*, 2003).

The primary treatment goal of diabetic foot ulcers is to ensure wound closure as soon as possible. It has been shown that, resolving foot ulcers and decreasing its recurrence rate can lower the probability of lower extremity amputation (Girod *et al.*, 2003). For optimum care, treatments are mainly combination of surgical and non-surgical.

Wound dressings after wound debridement of infected ulcers plays an important role for wound bed preparation and wound healing. Today, there are a variety dressing modalities have been developed. Traditionally this was done with just normal saline or povidone iodine alone.

Studies have shown that antiseptic solution is more effective than normal saline. Some experts argue against the use of antiseptic agents in wound treatment because of its cyto-toxicity against the host's dermal and epidermal cells(Luca *et al.*, 2006).

In the early 21st century, antiseptic solution in the form of stable super oxidized solution, which is produced by electrolysis of water and sodium hydrochloride to generate reactive species of chlorine and oxygen was developed. The solution has a neutral pH and a longer shelf life.

1.2 Study Justification.

To date, Dermacyn® has been the only commercially available neutral pH, super oxidized solution which is produced by Oculus Innovative Science Inc., United States and widely available worldwide. Recently, Vigilenz Medical Devices Sdn Bhd, a Malaysian company, began production of a neutral pH super oxidized solutions under the brand name Hydrocyn™ Aqua. It can be considered as a generic brand and based on both products information pamphlets, there is no significant difference in their properties (Table 1.1). However, the previous was produced from oversea and has been in the market for more than 10 years and many studies has been done showing its efficacy in the treatment of diabetic foot ulcers. Meanwhile, Hydrocyn™ Aqua has only been in the market for less than 5 years and so far there are no study, trials or clinical research testing this locally produced super oxidized solution. To date only one case report by Harikrishna et al (2012) available, where he reported 3 cases of infected diabetic foot ulcers and surgically debrided where Hydrocyn™ Aqua was used as a wound cleansing and irrigation solution. He found that all the 3 cases had reduced smell and there was improvement in terms of infection and the wounds healed well in concordance with wound bed preparation(Harikrishna, 2012).

In term of production, both products claim using their own technology of water oxidation and electrolysis producing the neutral pH super oxidized solution containing the reactive

chlorine and oxygen molecules. However, the details of the technologies used for both products could not be found in the literature and queries made to both companies were not answered due to their business strategies.

Although both brands have almost the same properties as a neutral pH super oxidized solution, the procedures and technologies of water oxidation and electrolysis they used could be different. Furthermore, no clinical trial or study has been done to show Hydrocyn™ Aqua efficacy as a neutral pH super oxidized solution Therefore, there is a need to investigate further Hydrocyn™ Aqua which may offer a better or equally good alternative to the commercially available neutral pH super oxidized solution which has proven efficacy in the treatment of diabetic foot ulcers.

Table 1.1: Components of Hydrocyn™ Aqua and Commercially available super oxidized solution

	Hydrocyn™ Aqua	Commercially available super oxidized solution
Electrolyzed water	99.8-99.9%	99.98%
Hypochlorous acid	0.02-0.03%	0.025%
Sodium Hypochlorite	0.03-0.04%	0.036%
Sodium Chloride	0.05%	0.11%
pH	7.5	6.2-7.8

1.3 Benefit of the study

This study aims to demonstrate that this locally produced super oxidized solution is comparable in its efficacy to the commercially available super oxidized solution in terms of treatment of diabetic foot ulcers. This may help to reduce the cost of diabetic foot ulcer management as Hydrocyn™ Aqua is produced locally and is cheaper.

CHAPTER 2: LITERATURE REVIEW

2.1 Diabetic foot

2.1.1 Definition

The World Health Organization (WHO) and the International Working Group on the Diabetic Foot defined diabetic foot as the foot of a diabetic patient that has the potential risk of pathological consequences including infection, ulceration and or destruction of deep tissues associated with neurological abnormalities, various degrees of peripheral vascular disease and or metabolic complications of diabetic foot ulcers and or metabolic complications of diabetes in the lower limb.

2.1.2 Epidemiology

It has been projected that by 2030 there will be estimated 366 million diabetics worldwide (Wild *et al.*, 2004). With increasing number of diabetes patients, there will be an increase in the number of diabetic foot. It has been documented that about 15% of diabetic patients will develop lower extremity ulcers as the disease progress. The cost of treating diabetic foot ulcers to the patient and healthcare system is enormously high. This has been estimated to be USD 20 billion in the United States (Ramsey *et al.*, 1999). Generally, the prevalence of diabetic foot ulcers has been reported to occur in 2 % of the population. National Diabetes Registry in 2013 reported that the prevalence of foot ulceration in patients attending diabetic outpatient clinics in Malaysia has been reported as 1.2% in 2012 but with increasing number of patients from 2011. Diabetic foot ulceration has been considered the most likely predictor of eventual lower extremity amputation in patients with diabetes mellitus with 85% of lower extremity amputations were preceded by foot ulcers (Palumbo PJ, 1995).

2.1.3 Pathophysiology

Ischaemia and neuropathy are the principle pathophysiology in the development of diabetic foot ulcers. The aetiology of foot ulcer classically has been categorized into mainly pure neuropathic (60%), neuroischaemia (40%) and lastly pure ischemic in origin (10%)(Clayton Jr and Elasy, 2009). However , there is a changing trend in the etiology of diabetic foot ulcers where neuroischaemic type is slowly gaining predominance (52%) followed by neuropathic (36%) and (11%) ischemic (Oyibo *et al.*, 2001). This might suggest the greater awareness of patients and health care providers towards certain categories of foot ulcer aetiology.

Ischemia and neuropathy are the principle pathophysiology in the development of diabetic foot ulcers. Both ischemia and neuropathy are frequently the initiating factors of foot ulcers with infection as the consequence of these.

In a diabetic foot, all layers of the foot can get involved from the skin, subcutaneous tissue, muscles, bones and joints to the blood vessels and nerves. Development of foot ulcers in diabetic patients are multifactorial involving neuropathy, minor foot trauma and foot deformity. Other existing risk factors such as uncontrolled hyperglycemia, unable to offload pressure over the specific area such as the first metatarsal head, and poor nutrition will worsen the wound and impair the healing process.

1. Neuropathy

A majority (60%) of diabetic foot ulcers are caused by underlying neuropathy (Dyck *et al.*, 1999). The neuropathy can be either focal or diffuse. The latter is more common and includes autonomic neuropathy and chronic sensorimotor polyneuropathies. Sensorimotor

polyneuropathy which involves lower extremity symmetrically leads to loss of protective sensation for the foot.

The pathomechanics of diabetic neuropathy has been described with regards to the metabolic and microvascular components. Hyperglycemia-induced metabolic abnormalities has been shown in animal and in vitro models as the main mechanism in the development of neuropathy in affected patients (Zochodne, 2008). These hyperglycaemic induced metabolic abnormalities has been described as the polyol pathway. Hyperglycemic state leads to an increase in enzyme aldose reductase and sorbitol dehydrogenase resulting production of sorbitol and fructose from intracellular glucose (Clayton Jr and Elasy, 2009). These metabolic products accumulate and in turn result in a decrease in the synthesis of nerve cell myoinositol which is required for normal neuron conduction. The conversion of glucose also results in depletion of the nicotinamide adenine dinucleotide phosphate storage which is important in detoxification of reactive oxygen species and for synthesis of the vasodilator nitric oxide. This leads to nerve cell injury and death cause by increase oxidative stress and vasoconstriction. Hyperglycemia and oxidative stress also cause abnormal glycation of nerve cell proteins and the inappropriate activation of Protein Kinase C resulting in further nerve dysfunction and ischemia (Clayton Jr and Elasy, 2009).

The motor nerve fiber damage, specifically to the intrinsic foot muscles leads to an imbalance flexion and extension of the intrinsic foot muscles. The small muscles will be atrophied while the bigger muscles will only be affected later. This results in the metatarsal phalangeal joint pulled in to flexion resulting in clawing of the toes and anatomic foot deformity. Skin breakdown will occur over areas of abnormal bony prominences and pressure points will

gradually will lead ulceration. These subtle changes, in addition to loss of sensation from the neuropathy, may not be noticed by the patients. Thus further repeated assaults and injuries to the areas may lead to skin breakdown and ulcer formation.

In autonomic neuropathy there will also be loss of sympathetic tone. With loss of the sympathetic tone, there will be an increase in arteriovenous shunting in the foot and peripheral arterioles dilatation. Autonomic dysfunction also leads to drying of skin due to lack of normal glandular function. This predispose the skin to skin breakdown as a result of anhydrosis and cracking of dry skin.

2. Ischemia (Vascular Disease)

Up to 50% of diabetic foot ulcers are contributed by peripheral vascular disease (Simmons and Feldman, 2002). Diabetic patient are four times more common to have peripheral vascular disease compared to non-diabetics (Kannel and McGee, 1979). Peripheral vascular disease is correlated with poor glucose control and for every 1% increase in hemoglobin A1c, there is corresponding increase of 25 - 28% in the relative risk of vascular disease.(Selvin *et al.*, 2004)

In diabetes, there is alteration in vascular function at both microvascular and macrovascular level. The microvascular involves capillaries and arterioles of all organs such as the kidneys, retina and peripheral nerves. This has been described as non-occlusive type. At macrovascular level, it is the occlusive type which is caused by atherosclerotic changes of the coronary and peripheral arteries. Usually this affects the tibial and peroneal arteries of the calf (Clayton Jr and Elasy, 2009). In microvascular or non-occlusive type, there is

thickening of the capillary basement membrane which subsequently impaired migration of leukocytes and increased the risk of infection (Williamson *et al.*, 1988)

A persistent hyperglycaemic state resulting endothelial cell dysfunction and smooth cell abnormalities in the peripheral arteries (Zochodne, 2008). Both condition will lead to lack in endothelium derived vasodilators which cause the constriction of the vessels. The hyperglycaemic state also is associated with increase in thromboxane A2. Thromboxane A2 increase the risk for plasma hypercoagulability since it is a vasoconstrictor and platelet aggregation agonist(Paraskevas *et al.*, 2008). Development of peripheral arterial disease in diabetic patient is also contributed by factors such as smoking, hypertension and hyperlipidaemia (Armstrong and Lavery, 1998)

3. Infection

The above mechanisms, either physiologically or mechanically, increase the risk of ulcer formation in diabetic foot. The ulcers which is exposed to the surrounding area and given the biological medium of the tissues, are prone to be infected. In diabetic foot ulcers management, it is important to understand the anatomy of the affected area as infection may spread from one compartment to other compartments in the foot. The infection may cause increase in inter compartmental pressure which can impair the capillary blood flow and leads to progressive tissue ischemia and necrosis.

The common organism associated with diabetic foot ulcers are the aerobic Gram-positive cocci including *Staphylococcus Aureus*, coagulase negative *staphylococci* and group B *streptococci*. Gram negative organism frequently cultured are *Proteus sp, Escherichia coli*,

pseudomonas and other Enterobacteriaceae. Emergence of multiple drug resistant organism (MDRO) are on the rise such as Methicillin-Resistant Staphylococcus aureus (MRSA), bacteria producing extended spectrum beta-lactamase (ESBL), *Pseudomonas aeruginosa* resistant to ceftazidime and imipenem, and *Acinetobacter baumannii* resistant to imipenem (Hartemann-Heurtier *et al.*, 2004) and brings more challenges to the treatment of infected diabetic foot ulcers.

2.14 Classification of Diabetic Foot Ulcers

In diabetic foot ulcers, the wound healing tendency can be evaluated by accurate classification of diabetic foot ulcers (Santema *et al.*, 2015). There are two widely used classification system which are the Megitt-Wagner and the University of Texas classification.

1. The Megitt-Wagner system(Wagner, 1981).

This first ever diabetic specific classification system was produced in 1981 by Wagner. It is based on depth of penetration and extent of tissue necrosis. The simple system consists of 6 grades (Table 2.1) with the first three grades are relating to the depth; making it a popular classification among clinicians (Game, 2016). However, it has limitations where neuropathy, ischaemia or infection are not addressed.

Table 2.1: Megitt-Wagner classification (1981)

Grading	Features
0	Pre-ulcer. No open lesion. May have deformities, erythematous, areas of pressure or hyperkeratosis
1	Superficial ulcer. Disruption of skin without penetration of subcutaneous fat layer.
2	Full thickness ulcer. Penetrates through fat to tendon or joint capsule without deep abscess or osteomyelitis.
3	Deep ulcer with abscess, osteomyelitis or joint sepsis. It includes deep plantar surface infections, abscesses, necrotizing fasciitis and tendon sheath infections
4	Gangrene of a geographical portion of the foot such as toes, forefoot or heel
5	Gangrene or necrosis of large portion of the foot requiring major limb amputation

2. The University of Texas Classification(Lavery *et al.*, 1996).

This classification was developed in 1996 by Lavery *et al* at the University of Texas San Antonio in response to their observation that poor outcomes are often associated with wounds of increasing depth, increasing severity of infection and presence of peripheral vascular disease. The University of Texas San Antonio Diabetic Classification uses a system of wound grade and stage to categorize wounds by severity (Table 2.2). Higher grades and higher stages are associated with worse outcomes (Game, 2016). Although it includes the infection and ischaemia in the classification, neuropathy is not part of the classification. However it is still widely used in view of its simple designs and clear definition.

Table 2.2: The University of Texas Classification

Grading	Features
0	Pre or post ulcerative or healed wound
I	Superficial wound not involving tendon, capsule or bone
II	Wound penetrating to tendon or capsule
III	Wound penetrating to bone or joint
Stages	
A	No Infection or ischemia
B	Infection present
C	Ischaemia present
D	Infection and ischaemia present

2.15 Principles of Management

The management of diabetic foot ulcers crucially requires multidisciplinary approach including the podiatrist, dietitian, nurses, general practices, physicians and surgeons. Each disciplines are important in diabetic control, recognizing risk and prevention, recognizing infection and starting appropriate antibiotics, limb at risk and surgical requirements and wound care.

Offloading together with debridement are vital in the healing process of diabetic foot wounds (Armstrong *et al.*, 2004). Offloading can be done by a number of methods such as total contact cast, half shoes, removable cast walkers, wheelchairs and crutches. The aim of offloading is to redistribute force from ulcers sites and pressure points at risk to a wider area of contact (Clayton Jr and Elasy, 2009). Patients' compliance, wound condition and infection must be considered in choosing the offloading methods.

Presence of necrotic or unhealthy tissues might preventing the ulcer from healing thus requiring surgical debridement. Surgical debridement range from callus removal to removal of dead or unhealthy tissues and bones to major or minor amputation which aid in removal of colonizing bacteria and allowing assessment of the deep tissues. Removal of surrounding callus help in reducing pressure point surrounding the ulcer (Clayton Jr and Elasy, 2009). Deep tissue or bone can then be send for culture of colonizing bacteria and sensitivity of the colonizing bacteria towards antibiotics.

After surgical debridement, wound debridement is important in aiding the healing process. Many modalities have been developed recently such as silver based dressings, hydrocolloid,

foam, growth factors and skin substitutes. Wounds size, depth, location, surface and discharge are the factors that determine the type of dressings. Wound dressings were done traditionally with normal saline or antiseptic solution such as povidone or chlorhexidine. The type of wound dressing must be tailored to the individual's wound factors as described before. An ideal wound dressing should contribute to a moist wound environment, absorb excessive exudates and does not increase the risk of infection (Foster AVM, 1994)

In the presence of infection, oral or parenteral antibiotic is required. The choice of antibiotic will take into consideration of the common organism for empirical treatment and the sensitivity of the bacteria to antibiotics from the tissue culture. The common classes of antibiotics used includes cephalosporin, fluoroquinolones and penicillin with B-lactamase inhibitor (Clayton Jr and Elasy, 2009). In a non-limb threatening infection, oral antibiotic can be prescribed to patients with regular follow up. Parenteral antibiotic is required when there are presence of limb threatening infection or life threatening and require hospital admission.

Adequate blood supply to the limb is necessary to facilitate wound healing and clearing the infection(Clayton Jr and Elasy, 2009). Patients with peripheral vascular disease with abnormal ankle brachial systolic index (ABSI) should be referred to the vascular surgeons or interventional radiologists. Further identification of the diseased vessels can be assessed using angiogram. Base on severity; treatment will range from angioplasty to bypass of the diseased artery. Surgical bypass of the lower extremity has demonstrated up to 90% 10 years limb salvage rate (Shah *et al.*, 1995).

Adjunctive therapies are also available for the treatment of diabetic ulcers such as hyperbaric oxygen chambers, granulocytes colony stimulating factor and vacuum assisted dressings. Usage of human skin equivalents and a recombinant platelet derived growth factors for promoting wound healing are also currently being used. However, there is insufficient evidence for implementation of these adjunctive modalities in the treatment of diabetic wounds (Lipsky *et al.*, 2004).

Prevention of diabetic foot ulcers also requires a multidisciplinary team approach with involvement of the patient. This is done by early detection of the risk factors that can contribute to development of diabetic foot ulcers. Regular visits, examination and foot care advices by podiatrician can help identifying the risk factors and to start appropriate treatment. For patient at risk, use of specialized protective shoes with extra depth and custom-molded shoes deemed necessary in order to avoid continuous pressure to deformed areas or ulcers. One the most important components in prevention is through patient and healthcare personal education. For patients they need to be educated regarding the need for daily foot inspection and necessity for early intervention. Compliance to their diabetic medication and adequate blood sugar control are also important in preventing complication to foot such as neuropathy and angiopathy. For healthcare provider, they need to know the significance of foot lesions, the importance of regular foot examination and current concept of diabetic wound management. The Primary Care team also plays an important role in ensuring good control of blood sugars by regular and proper adjustment of patients' diabetic medication requirement.

2.2 Wound Healing

Wound can be defined as a breakdown in protective function of the skin where there is a loss in continuity of the epithelium with or without loss of underlying tissue following injury to the skin or underlying tissue or organs caused by surgery, a blow, a cut, chemicals, heat or cold, friction or shear force, pressure or as a result of a disease such as leg ulcers or carcinomas (Leaper DJ, 1998).

Wound healing can be defined as a complex and well-orchestrated process of restoring the integrity of injured tissues by replacement of dead tissue with viable tissue(Clayton Jr and Elasy, 2009). The process can be divided to 4 phases which are hemostasis, inflammation, proliferation and remodeling and scar formation.

A wound is considered healed when the connective tissues has been repaired and the wound completely re-epithialized by regeneration followed by returns to its normal anatomical structure and function without requiring continues drainage or dressing (Clayton Jr and Elasy, 2009).

2.2.1 Types of wound healing

There are three types of wound healing which are primary, delayed primary and secondary healing. In primary healing, the healing is by first intention where the wound is closed within 12 to 24 hours. The wound edges are approximated primarily usually by using sutures, tissue glue, tapes or staplers. The wound will usually heal well and gives complete closure since the incision only causing focal disruption and damages of the epithelial basement membrane.

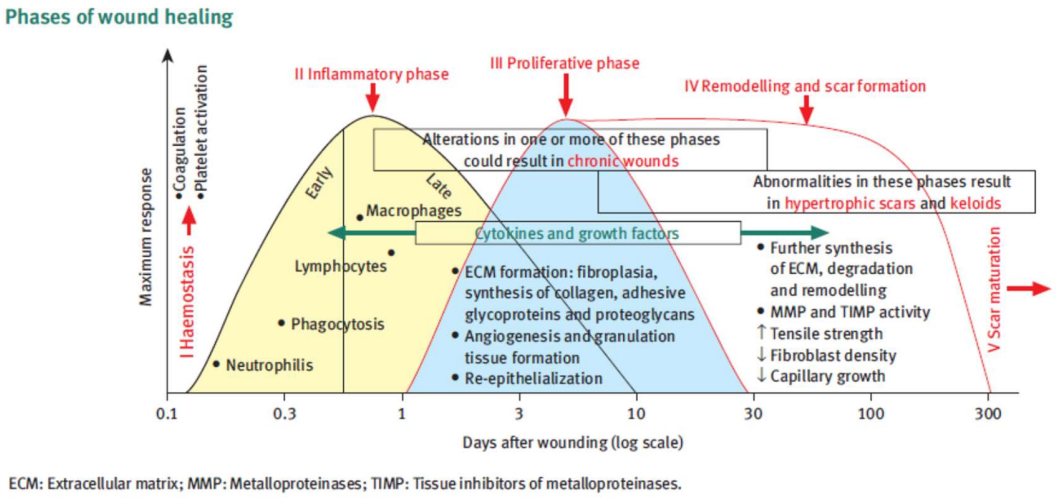
When the wound is closed after a few days in an open contaminated wound that has been left open, this is considered as delayed primary healing. By doing so, the infection can be cleared by the host defenses before closure of the wound. The collagen metabolism usually preserved and the tensile strength is intact.

In secondary healing, the wound will be left to heal by secondary intention where there is usually extensive loss of soft tissue such as in infection or extensive trauma due to degloving injuries. The normal skin architecture can't be regenerated and restored by the epithelial cells alone. When left open, ingrowth of granulation tissue from the wound margin followed by extracellular matrix with collagen within will occur. The closure of the wound is caused by wound contraction and epithelialization. The healing process is slower and might cause functional restriction due to wound contraction.

2.2.2 Phases of wound healing

When there is tissue injury, wound healing will take place in four overlapping phases. These phases are haemostasis, inflammation, proliferation and remodeling (Figure 2.1). Haemostasis occurs immediately after an injury. Blood component, namely the platelets play important roles in this phase where they release alpha granules which contain growth factors like platelet-derived growth factor (PDGF), insulin-like growth factor-1 (IGF-1), epidermal growth factor (EGF), transforming growth factor-β (TGF-β) and platelet factor-IV. All these growth factors will activate the fibroblast, endothelial cells and macrophages. The complement cascade, clotting mechanism, kinin cascade and plasmin generation are also activated. The clot formed provides provisional matrix for cellular migration and comprise of fibrin, fibronectin, vitronectin, von Willibrand factor and thrombospondin.

Figure 2.1: Phases of wound healing.



Taken from Basic Science of Wound Healing (Enoch and Leaper, 2005)

The inflammation phase can be divided into early and late phases. The early phase occurs 24 to 48 hours after the injury with the activation of the complement cascade. At this phase, neutrophils granulocytes (polymorphonuclear leukocytes) infiltrates the wound from surrounding vessels and engulf bacteria and foreign particles. During the same time, basal cells will increase their mitotic activity and epithelial cells start to migrate and proliferate along the dermis and deposit components of basement membrane. In the late stage, within 48 to 72 hours, the macrophages take over the important role as the key regulatory cells to initiate repair. Besides phagocytosing the bacteria and foreign particles, proteolytic enzymes are also released. Growth factors responsible for proliferation of smooth cells and endothelial cell resulting in angiogenesis and extracellular matrix by fibroblast are release by macrophages.

The proliferation phase starts at day three and continue for two weeks after the injury. It starts with fibroblasts migration to the wound which is attracted by various growth factors

including platelet-derived growth factor and transforming growth factor-β. Fibroblasts proliferates and produces extracellular matrix. The extracellular matrix contain fibrous structural protein (such as collagen and elastin) and adhesive glycoprotein. It provides soft tissue turgor, rigidity to bone, adhesion area for cells, regulate growth, movement and differentiation of the cells within. At around the third to fifth day, granulation tissue starts to form. This appears as pink, soft and granular in appearance. The formation of granulation tissue is hallmarked by angiogenesis at site of injury. Epithelization occurs within a few hours of injury where the epithelial cells from wound edges migrate over the wound to form a complete sheet of cells cover and attach to the matrix below it. In primary healing, it completes within 24 hours but in secondary healing, a longer time is required since there is a larger epithelial gap is larger.

The remodeling phase begins at the first week and may take up to two years to complete. Continuous collagen synthesis and breakdown occurs while the extracellular matrix are constantly remodeled and achieve steady state about 21 days after injury (Enoch and Leaper, 2005). Type 3 collagen replaced type 1 collagen which are usually found abundant on skin before injury. All this results in normal epithelization and eventually scar maturation. The strength of the scar is 50% of its original tensile strength at three months and 80% maximum strength at long term (Enoch and Leaper, 2005).

2.2.3 Wound healing in diabetic foot infection

In diabetic patients, the wound healing cascade more often is interrupted with complications and remain in different phases leading to loss of sequence of ideal wound healing processes (Loot *et al.*, 2002). The complications include infection, thrombosis and ischemia. Diabetic foot ulcers commonly become chronic wound and difficult to heal.

With infected wound, the healing process is unable to proceed unless the source, in this case the infection, is removed. Wound debridement, is a surgical process whereby dead tissues are removed and antibiotics are essential in order for wound healing to take place. However, the heterogenicity of diabetic foot infection made the management approach rather difficult and complicated.

As mentioned before, vascular disease is common in diabetic patients. For a wound to heal orderly, there has to be a good blood supply surrounding the wound is mandatory. With microvascular deficiency, there will be reduction of capillary size, thickening of basement membrane and hyaline arteriosclerosis. With inadequate blood supply it is not possible for all the components which initiates and support wound healing as mentioned above to cross to the wound and initiate wound healing. Important inflammatory reaction against infection and delivery of antibiotics will also impaired due to the lack of blood supply. The abnormal thickening of the basement membrane also interferes with physiological exchange and leads to altered leucocytes migration, decrease maximal hyperaemia and abnormal autoregulatory capacity (Dinh and Veves, 2005)

With neuropathic foot, there will also be autonomic neuropathy which will then lead to blood flow maldistribution as discussed above. This results in dry and sensitive skin which together with repetitive injuries, and in addition to infection, further impair wound healing.

In diabetic patients, some important cells for wound healing might undergone phenotypic alteration. It has been shown that fibroblast of diabetic ulcers shows a decrease proliferative response to growth factors. In term of infection, some diabetic patients will also have defect in host defense against infection especially bacterial infection (Naghibi *et al.*, 1987) for example, the macrophages has also been shown to have a reduced cytokines release which include tumor necrosis factor, interleukin and vascular endothelial growth factor (Zykova *et al.*, 2000)

2.3 Super oxidized solution.

The super oxidized solution is produced from pure water mixed with sodium chloride using a multi chambers electrolysis cells with the ability to stabilize the solution via the oxygen reduction potential (ORP) stabilization process. Electrolysis is an electrochemical process in which electrical energy is the driving force of chemical reactions where substances are decomposed by passing an electric current through them. The history of water electrolysis dated back in 1800 and there were already 400 industrial water electrolysis units in use (Zoulias *et al.*, 2004). However they have properties of low or high pH, unstable and short shelf life which made them not suitable for human usage (Rutala and Weber, 2001). It was mainly used to clean hard inanimate object and also for sterilization of medical devices.

The super oxidized solution is produced using multiple electrode chambers where purified water containing trace amounts of chloride are oxidized creating oxidized water. The water is then broken down into oxygen, ozone and other oxidized acid and chlorine. Chlorine reacts with water forming hypochlorous acid (HOCl) and neutralization with Sodium Hydroxide (NaOH) converts a portion of HOCl to Sodium Hypochlorite (NaClo). In simpler words, during the electrolysis process, the water molecules are dissociated and reactive species of chlorine and oxygen are formed (Gonzalez-Espinosa *et al.*, 2007).

Recent advances in electrolysis process makes the super oxidized solution safer and more effective for human usage with a non-toxic neutral pH and a longer shelf life (Jesús *et al.*, 2007; Luca *et al.*, 2006; Piaggesi *et al.*, 2010a). Components of this solution includes superoxide-neutral pH water, chlorine <85 parts per million (ppm), reactive oxygen species, free radicals, oxidised water (99%), sodium hypochlorite (<50ppm), hypochlorous acid

(<60ppm), hydrogen peroxide (<4ppm), ozone (<0.2ppm), chlorine dioxide (<1.5ppm), sodium carbonate (<21ppm) and sodium chloride (<110ppm).

In the treatment of infected diabetic foot ulcers, this neutral pH super oxidized solution has the antimicrobial property, improves vascularity of the wound and also promotes healing process. Super oxidized solution derives its antimicrobial property from its content of reactive oxygen species and free radicals which are similarly produced and released inside mitochondria during respiratory burst. During microbial elimination, leucocytes generates reactive oxygen species and various microbicidal enzymes and peptides(Jesús *et al.*, 2007). With its oxidising capacity, the super oxidized solution are highly reactive with bacterial cell wall and membrane component and signals protease activation leading to direct killing of the bacteria (Thomson, 2000). It also creates a high concentration of potassium ion environment which deactivate the bacterial proteases.

The hypochlorous acid content in super oxidized solution is a highly microbicidal chemical. In a normal oxidative stress and body primary and secondary defences, the super oxidized solution anion dismutes to form hydrogen peroxide and several other reactive oxygen species and reactive intermediates. Catalysed by the granule enzyme myeloperoxidase, hydrogen peroxide combine with chloride to form the hypochlorous acid which is highly microbicidal.

Super oxidized solution has electrical energy that able to destroy microorganism including fungi, viruses, mycobacteria, spirochetes and bacteria (Thomson, 2000). It kills them in short periods of time from seconds to few minutes (Gonzalez-Espinosa *et al.*, 2007).The former acidic or alkalotic super oxidized solution loses its electrical potential and its germicidal

action when come into contact with multicellular organic matter and turn back into ordinary water. A neutral pH super oxidized solution is more effective in retaining its germicidal action in this situation because of the trigger of reactive oxygen species in a potassium and pH dependant mechanism (Jesús *et al.*, 2007)

The healing properties of super oxidized solution has hypothetically arise from the reactive oxygen species which might trigger early wound healing through fibroblast migration and proliferation (Yahagi *et al.*, 2000). It also aids in improving wound bed vascularity by increasing capillary perfusion and acceleration of angiogenesis.

Most chemical disinfectants used for current wound for example hypochlorite, chlorohexidine and povidone iodine, may cause damage to the skin or granulation tissue. This can lead to interference with wound healing process and also could be cytotoxic or deleterious for the underlying tissues or proximal skin (Fowler *et al.*, 1999; Nakae and Inaba, 2000). The traditional dressing with normal saline is considered non cytotoxic but inadequate to remove surface contaminate and does not have antiseptic properties to control infection (Jesús *et al.*, 2007). Study on super oxidized solution toxicity showed that it does not alter DNA or RNA stability and produce only limited damage to plasma membranes of fibroblast with no effects to cell nuclei (Gonzalez-Espinosa *et al.*, 2007). The same study also showed the neutral pH super oxidized solution does not accelerate ageing in fibroblast after 1 month of exposure.

The problem with the previous super oxidized solution is its stability once exposed to the environment ranging from a few hours to days only. Aging studies for neutral pH super