CHAPTER 1: GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 REVIEW ON SKIN

1.1.1 FUNCTIONS OF SKIN

Skin serves a lot of functions; e.g. protection, sensory transduction, thermoregulation, metabolic function, excretion and flexibility (Martini, 2006; Wheater et al., 2006).

It serves as the first line of defence in our body that provides protections to the underlying tissues and organs against the external damaging stimuli such as ultraviolet light, chemical, thermal and mechanical insults. It also acts as a barrier against microbe invasion. Skin is involved in sensory transduction. It is the largest sensory organ in our body that contains various types of specialised nerve cells or receptors for touch, pressure, pain and temperature (Martini, 2006; Wheater et al., 2006).

Thermoregulation is another function of the skin. It is an important organ in maintenance of normal body temperature, either by heat insulation or evaporation. In mammals, skin is important in heat conservation and also heat lost by sweat evaporation (Martini, 2006; Wheater, et al., 2006). The glands found on the skin aid in excreting water, salt and other substances from the body through the skin (Wheater et al., 2006).

Skin also has metabolic functions: Skin functions as the storage of lipids. Besides synthesis of vitamin D3 (cholecalciferol) by the action of ultraviolet light on the precursor, the production of 7-dehydrocholestrol is also one of the important functions of the skin (Martini, 2006; Wheater et al., 2006). Skin also acts as a waterproof layer that allows flexibility of movement. Collagen and elastic fibres in it keep the skin smooth and supple (Wheater et al., 2006).

1.1.2 ANATOMY AND HISTOLOGY OF SKIN

As the largest organ in our body, skin accounts for around 16 % of body weight and varies in its thickness, texture and colour. It is a complicated structure that contains specialised cells and structures (Martini, 2006; Wheater et al., 2006).

Skin consists of three main layers, namely epidermis, dermis and hypodermis. Besides that, it also contains skin appendages such as hair follicles which mainly occupied the dermis and occasionally the upper hypodermis layer (Wheater et al., 2006).

1.1.2.1 EPIDERMIS

Epidermis is the outer layer of skin which is in contact with the exterior and consists of highly specialised self-regenerating stratified squamous epithelium (Martini, 2006; Wheater et al., 2006; Brannon, 2007).

It also contains other non epithelial cells such as melanocytes, Langerhans cells and Merkel cells (Wheater et al., 2006). It varies in thickness depending on the different parts of the body (Martini, 2006). This layer is dominated by keratinocytes, which form several layers and contain large amount of keratin. Keratin is tough, protective and partially water-resistant.

Epidermis is composed of 4 to 5 layers: stratum basale, stratum spinosum, stratum granulosum, stratum lucidum and stratum corneum (Martini, 2006; Wheater et al., 2006; Brannonn, 2007). Thin skin (roughly 0.08mm) which covers most of the body surface is composed of four layers while thick skin (roughly 0.5mm) as found at the palms and the soles is mainly composed of five layers (Martini, 2006).

Stratum basale or basal layer is the innermost epidermal layer (Wheater et al., 2006; Brannon, 2007). This epidermal layer is responsible for constant regeneration of new epidermal cells (Wheater et al., 2006; Brannon, 2007). Large basal cells which are arranged as a regimented single layer of cuboidal cells dominated this layers. The cells bound to the basement membrane by hemidesmosomes. Daughter cells from the stem cell mitotic division are pushed to the stratum spinosum, which is the upper layer of the epidermis (Martini, 2006).

Stratum spinosum is also known as prickle cell layer. Some of the cells which enter this layer from the basal layer continue to divide and thus, increasing the thickness of epithelium. This layer is composed of polyhedral keratinocytes that synthesized cytokeratin (Wheater et al., 2006; Brannon, 2007). Cytokeratines accumulate and form tonofibrils that converge into numerous desmosomes, forming the long contacts between adjacent keratinocytes (Martini, 2006; Wheater et al., 2006; Brannon, 2007).

Stratum granulosum or granular layer is also called as grainy layer in certain literature (Martini, 2006; Wheater et al., 2006; Brannon, 2007). Cells stop dividing and start to make large amount of keratin and keratohyalin in this layer (Martini, 2006). Cells lose their nuclei and cytoplasm and the cell shape becomes flattened; leaving the keratin that comprises the surface coating of the skin (Wheater et al., 2006).

Stratum lucidum or clear layer is only present in thick skin of palm and soles. It covers the stratum granulosum. Cells in this layer are flattened, densely packed and filled with keratin. It helps to reduce friction and shear forces between the stratum corneum and stratum granulosum (Martini, 2006; Brannon, 2007).

Stratum corneum or keratin layer is located at the exposed surface of the skin. It is normally composed of 15 to 30 layers of flat flakes sheets of keratinized cells, coated with anti-wetting agent (Martini, 2006; Wheater et al., 2006).

Generally, it takes 15 to 30 days for cells to move from the stratume basale to the upper most stratum corneum layer. The dead cells will remain in the exposed stratum corneum for an additional two weeks before they are shed off or washed away. This forms a protective barrier of dead, durable and expendable cells which is relatively dry and unsuitable for the microorganisms' growth (Martini, 2006).

1.1.2.2 DERMIS

Dermis lies between epidermis and hypodermis. The average thickness is approximately 1 to 2mm. It has two major layers which are papillary dermis and reticular dermis (Martini, 2006; Wheater et al., 2006).

The outer surface of the dermis which is in contact with the epidermis is usually uneven and elevated into papillae that project into the concavities between the ridges on the deep surface of the epidermis. This layer is called **papillary layer**. Papillary dermis is narrow, loose and contains very fine interlacing collagen and elastic fibres. It contains arterioles, capillary loops and venules, lymphatic and fine nerves twigs from the sensory nerve endings (Martini, 2006; Wheater et al., 2006).

Beneath the papillary layer is the **reticular dermis layer** (Martini, 2006; Wheater et al., 2006; Brannon, 2007). The thickness of this layer varies greatly at different parts of the body. This layer contains interwoven meshwork of irregular collagen bundles and elastic fibres that are larger compared to the one found in papillary dermis. It also contains blood vessels and nerves, and other skin appendages

such as hair follicles and sweat glands (Martini, 2006; Wheater et al., 2006). Macrophages and lymphocytes will be present in this layer (Wheater et al., 2006).

Hair follicles with erector pili muscle being attached to each follicle are found in this layer. The follicles produce hair shafts that composed largely of keratin arranged in an organised manner (Martini, 2006; Wheater et al., 2006; Brannon, 2007).

There are two types of **exocrine glands** (sebaceous gland and sweat glands) found in skin (Martini, 2006; Wheater et al., 2006). Sebaceous glands or oil glands are holocrine glands that discharge sebum (an oily lipid secretion) into hair follicles that helps to inhibit the growth of bacteria, lubricates and protects the keratin of hair shaft and skin. **Exocrine sweat glands** that produce sweat are widespread throughout the skin and located around the junction between dermis and hypodermis. The sweat evaporation helps in reduction of body temperature. (Martini, 2006; Wheater et al., 2006; Brannon, 2007)

Blood vessels which transport oxygen and nutrients to the skin and remove waste product can also be found in this layer. Other than that, there are also nerve processes found in this layer which transmits various sensations such as pain, itch, and temperature. Lymph vessels in this layer supply lymph for the skin tissue to fight microbes (Brannon, 2007).

1.1.2.3 HYPODERMIS

Hypodermis or subcutaneous layer is the innermost layer of the skin (Martini, 2006; Wheater et al., 2006; Brannon, 2007; Slomianka, 2009). This layer is not a part of the integument but it is important in stabilizing the position of the skin in relation to the underlying tissue, such as skeletal muscles or other organs, while permitting independent movement. This layer consists of loose connective tissue and is a deeper

continuation of the dermis. Its superficial region contains large arteries and veins (Martini, 2006; Wheater et al., 2006).

1.2 REVIEW ON WOUND AND WOUND HEALING

Wound is defined as a breach in epidermis or dermis, which initiates the process of healing caused by the trauma and pathological changes within the skin or the body (Davis et al., 1992). Wound healing is defined as a process which leads to the restoration of tissue integrity and functions of injured skin (Thomas, 1990). It is the process of the body's replacement of destroyed tissue by living tissue (Watson, 2006). A typical wound may take over a year to fully mature in the primary wound healing. The scar may dramatically change during this time, thus, scar revision is advised to be done after 1 year of post-injury (Gabrie et al., 2009).

1.2.1 CLASSIFICATION OF WOUNDS

Wounds can be classified into different types according to the cause of injury: include mechanical injuries, burns and chemical injuries and chronic ulceration (Thomas, 1990).

Mechanical injuries include abrasion, laceration, penetrating wound, bites and surgical or traumatic wound (Aljady, 2003). Burn wounds could be cause by thermal, chemical, electrical and radiation. Chronic ulceration wound is divided into different types depending on the underlying causes decubitus ulcers, ulcers associated with certain systemic infection and ulcers resulting from radiotherapy or malignant disease (Thomas, 1990).

1.2.2 CATEGORIES OF WOUND HEALING

There are various categories of wound healing but the interaction of cellular and extracellular constituents are similar. The three main categories of wound healing are primary wound healing, delayed primary wound healing, and secondary wound healing (Merchandetti and Cohen, 2008).

Primary wound healing (healing by first intention) occurs within hours to repair a full-thickness wound. It only involves the mortality of a minimal number of cellular constituents (Merchandetti and Cohen, 2008).

If the wound edges are not closed immediately, **delayed primary wound healing** (tertiary wound healing) occurs. It takes place when primary or secondary wound healing is unsuccessful due to infection or wound contamination (Thomas, 1990; Merchandetti and Cohen, 2008).

Secondary wound healing (healing by second intention) is the third category of wound healing. Full thickness wound is allowed to close and heal. This type of healing process results in a more intense inflammatory response compared to primary wound healing. This healing category also resulted in pronounced contraction of wounds (Merchandetti and Cohen, 2008).

1.2.3 PRINCIPLES OF WOUND HEALING

Wound healing is defined as a complex and dynamic process of cell structures and tissue layers restoration. This process consists of tissue regeneration and repair (Watson, 2006; Merchandetti and Cohen, 2008).

There are several different ways to divide the entire process of wound healing. The allocations of the phases commonly used in the process of wound healing are the inflammation phase, the proliferation phase and the remodelling phase (Watson, 2006; Merchandetti and Cohen 2008; Torre and Chambers, 2008).

The initial phase of the wound healing process is haemostasis (the stopping of the bleeding phase). This is a short phase that starts immediately after the injury, trauma or other similar insult (Watson, 2006; Merchandetti and Cohen, 2008).The phase is followed by the vasoconstriction and is continue with platelets adherence to the wound site and discharge of adenosine diphosphate (ADP) that promote thrombocyte clumping. The release of cytokines by platelets initiates the inflammatory phase of the wound healing process (Merchandetti and Cohen, 2008).

1.2.3.1 INFLAMMATORY PHASE

Inflammation is an essential component of the tissue repair process (Watson, 2006). The clinical recognizable cardinal signs of inflammation are rubor (redness), calor (warmth), tumor (swelling), dolor (pain) and *functio leasa* (loss of function) (Torre and Chambers, 2008).

There are two essential elements involved in the inflammatory events: vascular and cellular cascades (Watson, 2006; Thomas Romo III et al., 2008). The inflammatory event occurs within the first six to eight hours post injury with the polyporphonuclear leukocytes (PMNs) engorging the wound. PMNs migrate from the surrounding blood vessels and attain their maximal numbers in 24 to 48 hour, then commence their departure by 72 hour (Merchandetti and Cohen, 2008).

In the vascular event, there are changes in vessels wall and blood flow. Vasoconstriction that leads to the temporary blanching of wound is mediated by the vasoconstrictors. This process functions to limit the haemorrhage and keep the healing factor within the wound (Watson, 2006; Thomas Romo III et al, 2008; Gabrie et al, 2009).

Platelets attach to the sub endothelial collagen surfaces through the adhesive glycoprotein (fibrinogen, fibronectin, thrombospondin, and von Willebrand factor). It discharge a number of pro-inflammatory substances such as ADP, tissue growth factor beta (TGF-ß) and also platelets derived growth factor (PDGF) (Torre and Chambers, 2008; Merchandetti and Cohen, 2008). This aggregation results in the formation of the primary plug. The aggregation and attachment of the platelets cause activates the activity that lead to platelet degranulation. The release of the chemo tactic and growth factors such as PDGF, proteases and vasoactive agents (e.g. serotonin and histamine) will also occur (Thomas Romo III et al, 2008). These growth factors stimulate chemo taxis of neutrophils, monocytes and fibroblasts to the area of injury (Torre and Chambers, 2008).

As the healing process progresses, monocytes or macrophages exude from the vessels and continue the cleansing process and producing various growth factors during Day 3 or Day 4 (Merchandetti and Cohen, 2008). Collagen exposed during wound formation activates the clotting cascade (Gabrie et al, 2009). There are two coagulation pathways occurring in this phase: intrinsic pathway and extrinsic pathway. The intrinsic pathway is initiated by the activation of factor XII (Hademan Factor) when the blood is exposed to the extra vascular surfaces (Thomas Romo III et al, 2008). Injury of the vascular tissue initiates the extrinsic coagulation pathway. This pathway occurs through the activation of tissue factor found in extra vascular cells in the presence of factor VII and VIIa (Torre and Chambers, 2008).

Both of these pathways activate the thrombin that function to converts the fibrinogen to fibrin. Thrombin also facilitates the migration of the inflammatory cells to the injured site by increasing the vascular permeability. The increased vascular permeability helps the factors and cells that are necessary to the healing process flow from intravascular space to extracellular space (Thomas Romo III et al, 2008). Fibrin is essential in the wound healing process. The inflammatory cells, platelets and plasma protein migrate to this primary component. The absence of fibrin matrix causes the delay of wound healing (Thomas Romo III et al., 2008).

Clot formation occurs in the site of injury as the result of platelets aggregation and coagulation. Clot formation only occurrs at the site of injury because the nearby uninjured endothelial cells produce prostacyclin. In the uninjured adjacent areas, antithrombin III binds with thrombin; protein C to form a complex which binds the coagulation factors (factors V and VII), thus stopping the coagulation process (Thomas Romo III et al, 2008).

After vasoconstriction, a persistent vasodilation that leads to erythema, edema, and heat occurs. Increased blood flow supplies the wound with the inflammation that helps to fight infection. Alteration in pH (due to bacterial degradation), swelling, and tissue hypoxemia at the wound site cause the sensation of pain (Thomas Romo III et al, 2008).

The cellular aspect of the inflammatory phase occurs within hours of injury. Neutrophils predominate over the wound site for the first 48 hours after injury but do not appear as essential in the wound healing process. It helps in bactericidal activity and necrotic matter debrimentation in wound tissues. Besides that, it also releases the inflammatory mediators and bactericidal oxygen free radicals metabolites (Mercandetti

and Cohen, 2008; Thomas Romo III et al, 2008; Torre and Chambers, 2008; Gabrie et al, 2009).

Approximately 72 hours of post injury, T lymphocytes migrate into the wound (Thomas Romo III et al, 2008; Torre and Chambers, 2008). T lymphocytes attracted to wound by the release of interleukin-1 (IL-1) that contributes to the regulation of collagenase. Lymphocytes secrete lymphokines that help in the cellular immunity and antibody production (Thomas Romo III et al, 2008).

Circulating monocytes enter the wound and mature into tissue macrophages which are the most important cells in this early phase of wound healing. It phagocytoses debris and bacteria and also secretes collagenases and elastase (Thomas Romo III et al, 2008; Torre and Chambers, 2008). Collagenase and elastase break down the injured tissue and release cytokines. Besides that, macrophages also release PDGF (Thomas Romo III et al, 2008). Finally, it secretes substances that attract the endothelial cells to the wound and stimulate their proliferation to promote angiogenesis. Unlike neutrophils, macrophages depletion will impair wound healing due to the absent of the fibroblast proliferation, debriment, and angiogenesis (Torre and Chambers, 2008).

1.2.3.2 PROLIFERATIVE PHASE

Proliferative phase is involved in the generation of the repair materials (Watson, 2006). This phase consists of different sub phases (e.g. fibroplasias, matrix deposition, angiogenesis, and re-epithelisation) that do not happen in discrete time frames. It is an overall and ongoing process in wound healing. (Merchandetti and Cohen, 2008).

This phase has a rapid onset within 2 to 3 days of post injury but takes considerably longer to reach its peak activity, which is usually 2 to 3 weeks of post

injury (Watson, 2006). It can last up to 4 weeks in the clean and uncontaminated wounds that undergo the normal wound healing process (Merchandetti and Cohen, 2008).

The formation of granulation tissue is a central event during this phase. Granulation tissue is the combination of inflammatory cells, fibroblasts, neovasculature on a matrix of fibronectin, collagen, glycosaminoglycans, and proteoglycans. Its formation occurs within day 3 to 5 of post injury and overlaps with the preceding inflammatory phase (Thomas Romo III et al., 2008).

Fibroblasts are the most important cells in proliferative phase (Gabrie et al., 2009). They are responsible for the production of collagen, elastin, fibronectin, glycosaminoglycans, and proteases. Fibroblast increases in number in the wound as the number of inflammatory cell decreases (Thomas Romo III et al, 2008). Fibroplasia begins on day 3 to day 5 post injury, and may last as long as 14 days (Thomas Romo III et al., 2008). During the first week of wound healing, fibroblasts begin to produce glycosaminoglycans and proteoglycans, the ground substance for granulation tissue and also collagen. Fibroblasts soon predominate the wound area at the first or second weeks (Torre and Chambers, 2008). The cells are responsible for initiating angiogenesis, epithelialization and collagen formation (Gabrie et al., 2009).

The synthesis and deposition of collagen are critical events in this phase. Fibroblasts assemble collagen molecules into fibers. Collagen which is the major component in the acute wound connective tissue is secreted into extracellular space in the form of procollagen, called tropocollagen. Aggregation of tropocollagen forms collagen filaments. Collagen filaments arranged in a staggered fashion are joined by the intermolecular cross-links. The aggregations of filaments form collagen fibrils, while the aggregations of the collagen fibrils lead to the formation of collagen fibers. Tensile

strength of the wound increases as the content of the wound collagen increases (Mercandetti and Cohen, 2008; Thomas Romo III et al., 2008; Torre and Chambers, 2008).

Approximately 80% of the collagen in normal skin is type I collagen, while the remaining is mostly type III. In the injured skin, fibroblasts migrate into the wound laying down the new collagen of type I and type III. Type III collagen (primary component of early granulation tissue) predominates in the early stage of the normal wound healing and replaces type I collagen at the later phase (Mercandetti and Cohen, 2008; Thomas Romo III et al., 2008).

The proliferation of keratinocytes and endothelial cells also occur during this stage. The expansion of the endothelial cells contributes to angiogenesis. Neovascularization facilitates the growth of fibroblast into wound thus providing it with nutrients and cytokines (Torre and Chambers, 2008).

Angiogenesis is the formation of new blood vessels and it is important as the blood supply is vital to sustain the newly formed tissue. It is a complex process that relies on several angiogenic factors, such as vascular endothelial growth factor, angiogenin and angiotropin. This is the product of the parent vessel off shoots (Mercandetti and Cohen, 2008; Thomas Romo III et al, 2008; Torre and Chambers, 2008).

Epithelialization is the formation of the epithelium over a denuded surface that involves the migration of cells at the wound edges from the periphery of wound and adnexal structures over a distance of less than 1mm. The division of peripheral cells results in a thin epithelial cell layer that bridges the wound. This process occurs within 48 to 72 hours of post-injury (Mercandetti and Cohen, 2008; Thomas Romo III et al., 2008). Epidermal cells undergo structural changes and become detached from their

connection to the other epidermal cells and their basement membrane (Thomas Romo III et al., 2008).

Macrophage is essential to the stimulation of angiogenesis and produces the macrophages-derived angiogenic factor that fuctions as chemoattractant for endothelial cells (Thomas Romo III et al., 2008; Torre and Chambers, 2008). The basic fibroblast growth factor (FGF) secreted by macrophages and vascular endothelial growth factor secreted by the epithermal cells are believed to modulate angiogenesis (Mercandetti and Cohen, 2008; Thomas Romo III et al., 2008). This sub phase results in greater blood flow to the wound and consequently increases the perfusion of healing factors. When the demand of the new blood vessels ceases, the process of angiogenesis will stop. The unnecessary new blood vessels disappear by apoptosis (Thomas Romo III et al, 2008).

Wound contraction that results in the decrease of wound size begins almost concurrently with collagen synthesis but the wound contration does not depend on it. The maximal rate of the contraction is 0.75 mm/day and depends on the degree of tissue laxity and shape of wound, e.g. square wound contracts more than circular wound (Thomas Romo III et al., 2008). Wound contraction occurs to a greater extent in secondary healing compare to primary healing (Mercandetti and Cohen, 2008). Besides that, wound contraction also depends on myofibroblast proliferation and myofibroblasts located at the periphery of the wound since these cells are the connection to the components of the extracellular matrix (Thomas Romo III et al., 2008).

1.2.3.3 REMODELING PHASE

Remodeling phase or the maturation phase is the last phase of the wound healing process. It begins when the net collagen content of the wound is stable and may last for years after injury (Mercandetti and Cohen, 2008; Thomas Romo III et al., 2008; Torre and Chambers, 2008).

For the first 6 weeks of post injury, the wound healing process is dominated by collagen production. The collagen is deposited randomly in the wound granulation tissue. As wound matured, collagen is remodelled into a more organised structure with increased tensile strength (Torre and Chambers, 2008).

Collagen remodelling in this stage depends on continued collagen synthesis in the presence of the collagen destruction. Collagen is degraded and deposited in an equilibrium-producing manner. This results in a balance between the formation of new collagen and removal of old collagen, thus, there are no changes in the overall amount of the collagen in the wound (Mercandetti and Cohen, 2008; Thomas Romo III et al., 2008).

In the remodelling stage, collagen becomes increasingly organised. Fibronectin disappears and proteoglycans replace hyaluronic acid and glycosaminoglycans. Gradually, type III collagen is replaced by type I collagen until the normal skin ratio of 4:1 is achieved (Thomas Romo III et al., 2008; Torre and Chambers, 2008; Gabrie et al., 2009). Water reabsorption from the scar occurs and causes the collagen fibres to lay close, thus facilitating collagen cross-linking and ultimately decreasing the thickness of the scar (Thomas Romo III et al., 2008).

In normal wound healing process, collagen deposition reaches the peak by third week after wound injury. The tensile strength of wound is measured by its load capacity per unit area. By week 12 of post injury, the wound will achieve the peak of

tensile strength. Approximately 1 year post injury ultimate resultant scar or a healed wound has only approximately 80% of the tensile strength of the original skin (Mercandetti and Cohen, 2008; Thomas Romo III et al, 2008; Torre and Chambers, 2008).

Migration of epithelial cells continues until the defect is covered. The replacement of injured tissue volume with new tissue is carried out by the tissue contraction (Torre and Chambers, 2008). Vascularity decreases, producing a less hyperic and more cosmetically appealing wound as this phase progresses (Gabrie et al., 2009).

1.2.4 PROBLEMS AND FACTORS AFFECTING WOUND HEALING

Wound healing process is affected by both intrinsic and extrinsic factors (Table 1.1) (Aljady, 2003). Control of these factors may help in improving the wound healing process (MacLellan, 2000).

Intrinsic factors are related to the underlying pathology of the patient such as health status, age and nutritional status (Table 1.1) (Aljady, 2003; MacLellan, 2000). These factors may affect the skin integrity for wound healing. The general health status of the patient has important implication of the process of wound healing. Medical condition such as diabetes may affect the rate of wound healing and some may cause specific healing problems (MacLellan, 2000). Aging will affect the wound healing process by slowing down the rate of inflamation, cell migration, proliferation and maturation (Eaglstein, 1989). This may delay the repair process due to the delay of the cellular response to the stimulus of injury, delay of collagen deposition and also decrease in tensile strength during the tissue remodelling process (Sussman and Bates-Jansen, 2001). Nutritional status of the patient may also be one of the important factors

that affect the wound healing process. Normal reparative process needs an adequate demand of calories. Vitamins (e.g. Vitamin C, Vitamin A, Vitamin K and Vitamin B) and trace elements (e.g zinc and copper) are essential in the wound healing process (MacLellan, 2000).

Table 1.1: Intrinsic and extrinsic factors that affect wound healing process (Adapted from MacLellan, 2000)

Intrinsic factors	Extrinsic factors
▪ Health status: • Diabetes • Blood circulation • Anaemia • Immune status ▪ Age ▪ Nutritional Status	▪ Mechanical stress ▪ Debris ▪ Temperature ▪ Desiccation ▪ Infection ▪ Chemical stress ▪ Medication

As doe extrinsic factors, they are factors that come from sources in the environment that affect the body or the wound (Aljady, 2003; MacLellan, 2000). Examples of the extrinsic factors are mechanical stress, debris, temperature, desiccation, infection, chemical stress and medication (Table 1.1). Unrelieved pressure on the wound may contribute to the ongoing tissue destruction (MacLellan, 2000). Some patients are having multiple drugs that may affect the wound healing process. Other than medication, the most common complication of wound healing is bacterial infection (Aljady, 2003).

1.3 REVIEWS ON WOUND CARE

1.3.1 WOUND DRESSINGS

Wound dressing is used to promote wound healing and prevent further harm to the wound. It acts to provide necessary environment for different cells that are involved in the wound healing process (Mortimer, 2007).

Several researchers had proposed the ideal characteristic of wound dressing and wound environment. The optimal healing environment varies depending upon the characteristics of the treated wound and also according to the tissue repair progress (Mortimer, 2007). The properties of the dressing include being able to remove the excessive exudates but not causing the wound to dry out. Besides that, the dressing also functions to avoid wound infection and excessive slough. The wound dressing should also be free of toxic contaminants. Its usage should be non traumatic and it does not adhere to the wound, so that no damage occurs to the granulating tissue on removal. A good dressing should also allow gaseous exchange. In addition to provide optimum environment for healing, some other properties should be included like cost ease of application and removal, so that its application of wound treatment would be simplified (Sussman and Bates Jensen, 2001). A cost effective wound dressing is also welcomed.

Wound healing process occurs more quickly in moist environment rather than dry conditions. Winter (1962) demonstrated the benefits of moist wound healing environment and a huge number of the modern dressing materials with similar basis have been introduced. Moist wound dressings include the major categories of antibiotics oiments, antiseptics, alginates, collagen, films, foams, hydrocolloids, and hydrogels (Aljady, 2003). However, the uses of these modern dressings are expensive and for some their uses are associated with some complications (Ballard and Bazter,

2000). Currently, interest of researchers on wound management by using natural products as wound dressing is on the rise all over the world.

1.3.2 NATURAL PRODUCTS AND WOUND HEALING

In recent years, wound management in conventional medicine was returned to the roots of medicine and embraced some of the remedies used ages ago. The natural products being used include honey, aloe, cocoa and oak bark extracts. These had recently been used for their efficacy in wound healing (Davis and Perez, 2009). Many natural products have been described to improve wound healing process. And some were validated clinically for human used, while some were not (Aljady, 2003). Although some have claimed to be effective in wound healing, the lack of standardization of these natural products made it difficult to determine their true efficacy (Davis and Perez, 2009).

Aloe vera (*Aloe barbadensis*) has been used as folk remedies for centuries in treating various ailments especially on wound healing (Marshall, 1990). Animal study proves that *Aloe vera* treatments decrease the inflammation and promote cellular repair (Chithra et al., 1998). Topical treatment with *Aloe vera* has shown improvement in dealing with frostbite and electrical injury in animal study. The use of *Aloe vera* in wound management appears to be safe and improve wound repair (Mackay and Miller, 2003).

Cocoa has been reported to have a variety of benefits on skin, such as soothing burns, acting as moisturizer for the skin and best known for removing scar. However, studies are still needed to determine the efficacy of cocoa in wound healing (Davis and Perez, 2009).

Honey and sugar paste have been used for decades in treating dermal wound. They are both considered to be antimicrobial and have been associated with scarless healing. Besides the natural products mention above, tea tree oil has also report supporting its use in wound treatment (Davis and Perez, 2009).

Due to the lack of clinical trials using these natural products, it may be hard to warranty the pharmaceutical intervention in wound care and also delay the application of these products in a clinical setting (Davis and Perez, 2009).

1.4 REVIEW ON HONEY IN WOUND DRESSING

Honey is a popular sweetener and common household product throughout the world (Bansal et al., 2005). Honey has been used in traditional medicine since ancient times (Zumla and Lulat, 1989; Molan, 2001; Khalil et al., 2012). The use of honey is traced to some 8000 years ago as depicted by Stone Age painting (Bansal et al., 2005). Honey is the most popular Egyptian drug; it has been mentioned 500 times in 900 remedies. It was used for wounds and gut diseases by the ancient Egyptians, Assyrians, Chinese, Greeks and Romans. During the Biblical era, it received the religious endorsement by both Christianity and Islam. In twentieth century, honey was reported in wound healing due to it good antimicrobial properties. The Russians used it in World War I to prevent infection and to accelerate the healing process (Basal et al., 2005).

In the past, scientific opinions on the medicinal use of honey are different and clash with that of the community to large (Zumla and Lulat, 1989). Nowadays, interest in the use of honey in treating ailments is on the increase although the mechanisms of the action of its properties remain obscure and need further investigation (Molan, 2001; 2006).

Honey has proven to have its value in treating infected surgical wounds, burns and decubitus ulcers. Wound healing was accelerated and less bacterial colonization noted at local application of honey in patients (Zulma and Lulat, 1989). Even though there is published review of clinical evidence for the use of honey, not all has been persuaded to believe in its usage (Molan, 2006).

1.4.1 NOMENCLATURE OF HONEY

Different types of Honeys are named according to floral and non-floral nectars sources and geographical origin. It can be named according to the specific floral source; the primary flowers from which that the bees gather nectar. Bees can also gathere nectar from sources other than flowers, such as honeydew. Honey can also be named according to the geographic origin such as the area of production, state and region; e.g. Malaysian honey (National Honey Board, 1996).

1.4.2 GENERAL CHARACTERISTICS OF HONEY

Honey is carbohydrate rich syrup produced by bees from plants nectars. The source of honey determines many of its attributes such as aroma, flavour, colour and composition. The chemical composition of honey may also vary depending on plant sources, seasons, and production methods (Table 1.2) (Office of Complementary Medicines, 1998), but the main constituents in all honey are the same (Jeffrey and Echazarreta, 1996).

Table 1.2: General chemical composition of honey (Bodganov, 2010c).

	Average	Min-Max
Water content	17.2	15-20
Fructose	38.2	30-45
Glucose	31.3	24-40
Sucrose	0.7	0.1-4.8
Other disaccharides	5.0	28
Melezitose	<0.1	
Erlose	0.8	0.56
Other oligosaccharides	3.6	0.5-1
Total sugars	79.7	
Minerals	0.2	0.1-0.5
Amino acids, proteins	0.3	0.2-0.4
Acids	0.5	0.2-0.8
pH	3.9	3.5-4.5

In honey, approximately 95% of its dry weight is made up of carbohydrates (Jeffrey and Echazarreta, 1996; Bogdanov, 2010c). 25 different sugars have been detected in honey. Fuctose (approximately 38%) and glucose (approximately 31%) are the two major sugars present in honey. These sugars are the products of the hydrolysis of dissacharides sucrose Sucrose amount is less in honey, which is only approximately 1% (Office of Complementary Medicines, 1998; Bogdanov, 2010c). These constituents are maltose, isomaltase, nigerose, turanose, maltulose and kojibiose. Many of them are formed during the ripening and storage effects of bee enzymes and the acids of honey (Jeffrey and Echazarreta, 1996).

Most honey has low moisture content. Approximately 17 % of moisture content can be found in honey from temperate climate. It is mildly acidic with the pH ranging from 3.2 to 4.5. Most acids are naturally added by bees and it is important for the taste of the honey. Gluconic acid is the predominant acid found in honey. It is the product from the activity of glucose oxidation by glucose oxidase added by bees during ripening (Jeffrey and Echazarrete, 1996; Office of Complementary Medicines, 1998; Bogdanov, 2010 c).

Honey contains relatively small amount of other acids, proteins, enzymes, amino acids, minerals and vitamins (Office of Complementary Medicines, 1998; Jeffrey and Echazarreta, 1996). It contains almost all physiologically important amino acids. The proteins in honey are mainly enzymes (Bogdanov, 2010c). The main enzymes that are present in honey (e.g. invertase, glucose oxidases, and amylase) are derived from hypopharyngeal glands of worker bees (Jeffrey and Echazarreta, 1996b; Bansal et al., 2005). Invertase (saccharase, α-glucosidase) inverts the sucrose to glucose and fructose (Jeffrey and Echazarreta, 1996; Bogdanov, 2010c). Glucose oxidase changes glucose to gluconic acid and hydrogen peroxide in the presence of water (Jeffrey and Echazarreta, 1996; Office of Complementary Medicines, 1998). Besides that, there are also enzymes which originate from plants that are found in honey. These enzymes are catalase, acid phosphotase, and amylase. Catalase in honey acts as a regulator of the glucose oxidase activity (Jeffrey and Echazarreta, 1996).

Mineral contents in honey are mostly between 0.1 to 0.3 %. It is possible to differentiate between different unifloral honey by the determination of different trace elements. Some examples of the trace elements are shown in Table 1.3 (Bogdanov, 2010c).

Hydroxymethylfurfuraldehyde (HMF) is the decomposition product of fructose. Only small amount of HMF can be traced in fresh honey. HMF concentration increases with storage and prolonged heating of honey. This is used as a standard for assessing freshness and honey overheating (Bogdanov, 2010b).

The volatile compounds in honey are responsible for the honey aroma. Most volatile compounds originate from plant and bees. This could be the marker for the determination of botanical origin of honey. Dark colored honey cotained more phenolic

acid derivatives but less flavanoids than light colored honey. Determination of the flavonoid patterns is useful for the classification of some uniflower honeys.

Table 1.3: Trace elements in honey (Adapted from Bogdanov, 2010c).

Element	mg/100 g	Element	mg/100 g
Aluminium (Al)	0.01-2.4	Lead (Pb)*	0.001-0.03
Arsen (As)	0.014-0.026	Lithium (Li)	0.225-1.56
Barium (Ba)	0.01-0.08	Molybdenum (Mo)	0-0.004
Boron (B)	0.05-0.3	Nickel (Ni)	0-0.051
Bromine (Br)	0.4-1.3	Rubidium (Rb)	0.040-3.5
Cadmium (Cd)*	0-0.001	Silicium (Si)	0.05-24
Chlorine (Cl)	0.4-56	Strontium (Sr)	0.04-0.35
Cobalt (Co)	0.1-0.35	Sulfur (S)	0.7-26
Floride (F)	0.4-1.34	Vanadium (V)	0-0.013
Iodine (I)	10-100	Zirkonium	0.05-0.08

*- elements regarded as toxic, can be partially of anthropological origin

1.4.3 HONEY AS WOUND DRESSING

There are many reasons that contribute to the effects of honey on wounds. The physical and biochemical properties of honey play a part in its effectiveness as wound dressing. The physical properties of honey, (e.g. high osmolarity, low pH, hydrogen peroxide content and the uncharacterised compounds) play the important role in the wound healing process (Office of Complementary Medicines, 1998; Molan, 2001).

Honey acts as a protective barrier that prevents cross-infection due to its viscosity (Molan, 2001).The low water activity (WA) is one of the factors that inhibits microbial growth (e.g. bacterial, yeasts and moulds) (Office of Complementary Medicines, 1998; Bansal et al., 2005). WA of the honey is ranged from 0.56 to 0.62 (Bansal et al., 2005). Honey diluted by water from wounds still retains a low WA which sufficiently inhibits the bacteria. Some studies reported that, sugar syrups with same WA as honey were found to be less effective than honey at inhibiting microbial growth *in vitro*. *Staphylococcus aureus* which survived in wound treated with the

concentrated solutions of pure sugars having same WA as honey was inhibited by honey treatment (Office of Complementary Medicines, 1998)

Osmolarity of honey draws fluid out from tissues and creates a moist healing environment for wound in topical application. This process helps in drying the infected tissue and reduces bacterial growth in the wound. Osmotically induced outflow by honey creates “drainage” that flushes away the harmful substances from bacterial contaminants (Office of Complementary Medicines, 1998; Molan, 2001). The moist healing environment contributes to optimum healing as tissue growth is not reduced by drying of wounds, fibroblasts are able to pull wound closed, and epithelial cells grow to cover the skin surface to stop the scar formation. Honey also prevents wound dressing from sticking on the wound surface.

Sugar content in honey helps to remove malodour from wound. Bacteria use glucose in preference to amino acids. Hence, lactic acid instead of bad-smelling amines and mercaptants are produced (Molan, 2001).

The growth of organisms such as *Staphylococcus aureus, Enteropathogens* and *Candida albicans* are inhibited in undiluted honey (Zumla and Lulat, 1989; Office of Complementary Medicines, 1998). The growth of various gram-negative and gram-positive bacterial can be cleared by honey at a concentration of 40%. Studies shown that the use of honey concentrations of between 30% to 50% honey was found to be superior to antibiotics (e.g. ampicilin, gentamycin, nitrofurantoin and nalidixic acid) in inhibiting growth of nine types of pathogenic organisms isolated from urine samples of patients with urinary tract infection (Zumla and Lulat, 1989).

The average pH value in honey (3.9) does not support growth of bacteria. The low pH level of honey used in by topical applications is one of the main factors causing inhibition of the growth of various pathogenic bacteria. Honey contains glucose oxidase

which becomes active when honey is diluted. This process produces the hydrogen peroxide by the action of glucose oxidase on glucose (Office of Complementary Medicines, 1998; Molan, 2001). Hydrogen peroxide is identified as the main factors of the antibacterial activity in honey. In the undiluted honey, gluconic acid lower the pH of the honey until it reaches the point that inhibits further enzyme activity to produce peroxide. Dilution of honey result in 2,500 to 50,000 times increase in enzyme activity and a slow release of antiseptic that is non-damaging to skin. Small contribution in antibacterial activity also comes from the presence of small amount of non-peroxides, such as flavonoids (pinocembrin), benzylalcohol, and terpenes (Basal et al, 2005).

1.5 RESEARCH OBJECTIVES

According to the Annual report of Ministry of Health in Malaysia from year 1996 to 2000, injuries are one of the causes of the mortality and morbidity. Huge cost from the health budget is being spent each year in resolving the problems faced in the field of wound management.

Wound management continues to be a significant problem in modern medicine, although there is development of wound care product. The rates of wound infection and non healing wound are still high. These problems consume a considerable portion of health care finances all over the world. The cost of the modern therapies on wound healing is expensive and may lead to some unwanted side effects such as erythema. With the increasing number of bacterial strains resistant to the current antibiotics, it is a good reason for us to search for alternative and effective ways in dealing with wound healing. Some local applications which are cheaper, easily available and effective for wound healing have become the interest of the practitioners and researchers all over the world with application of honey as one of the options.

In Malaysia, a few local honey are available, e.g. Gelam honey, Nenas honey and Tualang honey. The application of local honey in wound healing is not widely accepted due to the lack of scientific evidence and no honey based wound care products are well established in the market. More research on wound healing using honey, especially locally produced honey should be done to provide an important platform for its clinical use based on the acceptable scientific evidences.

The general objective of this study was to collect data that could be useful in fingerprinting the selected Malaysian honey for wound healing purposes. The specific objectives of this study were:

1. To check the purity of the selected Malaysian honey to be used for wound treatment by using selected techniques of Honey Purity Test in this research.
2. To establish a suitable *in vivo* wound model (quantitative, repeatable and reliable method) that could contribute to the development of novel therapies (local Malaysian honey in this study) in wound healing research.
3. To evaluate the acute wound healing process and honey treatment from macroscopic and histological approaches.
4. To identify and correlate the contribution of topical honey treatment to the wound healing process.
5. To compare the efficacy of the two selected Malaysian honey (Gelam and Nenas honey) in wound healing.