MICROBIOLOGICAL AND ANGIOGENIC PROPERTIES OF BURN INJURIES TREATED WITH TUALANG HONEY VERSUS SILVER-BASED DRESSING

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by

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In the name of Allah, The Most Gracious and The Most Merciful.

"*And thy Lord inspired the bee, saying: Choose thou habitations in the hills and in the trees and in that which they thatch; (68) Then eat of all fruits, and follow the ways of thy Lord, made smooth [for thee]. There cometh forth from their bellies a drink divers of hues, wherein is healing for mankind. Lo! herein is indeed a portent for people who reflect; (69) And Allah createth you, then causeth you to die, and among you is he who is brought back to the most abject stage of life, so that he knoweth nothing after [having had] knowledge (70).*" Al-Nahl.

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LIST OF SYMBOLS AND ABREVIATION

VEGF	Vascular endothelial growth factor
PDGF	Platelet derived growth factor
CONS	Coagulase negative staphylococci
S. aureus	*Staphylococcus aureus*
K. pneumonia	*Klebsiella pneumonia*
B. subtilis	*Bacillus subtilis*
E. clocae	*Enterobacter cloacae*
P. aeruginosa	*Pseudomonas aeruginosa*
spp.	Species
EC	Endothelial cell
BC	Before Christ
SSD	Silver sulfadizine
Ag	Silver
MRSA	Methicillin-resistant *Staphylococcus aureus*
RCT	Randomized control trial
MG	Methylglyoxal
p value	Is the probability of obtaining the same or more data assuming the null hypothesis of no effect; *p* values are generally (but) arbitrarily considered significant if $p < 0.05$
ECM	Extracellular matrix
FAMA	The Federal Agriculture Marketing Authority

D0	Day zero (day of admission)
D3	Day three
D6	Day six
D6+	After day six
g	Gravity
µL	Microliter
SEM	Standard error of the mean; is the standard deviation of the sample mean estimate of a population mean
cfu	Colony forming unit
g	Gram
TBSA	Total body surface area
TNF	Tumor necrosis factor
IGF	Insulin growth factor
KGF	Keratinocytes growth factor
TGF	Transforming growth factor
US	United States
VLU	Venous leg ulcer
pH	A measure of the acidity or basicity of a solution. It approximates p[H], the negative logarithm (base 10) of the molar concentration of dissolved hydronium ions
n	Number (sample number)
AIDS	Acquired immune deficiency syndrome
Inc.	Incorporated

UK	United Kingdom
UMF	Unique Manuka Factor; a standard used worldwide to identify and measure the antibacterial strength of a manuka honey
mL	Milliliter
cm	Centimeter
BA	Blood agar
MAC	MacConkey
^{o}C	Degree Celcius
USA	United State of America
MHA	Mueller Hinton Agar
TM	Trademark
PS	Phosphstildyserine
PI	Propidium Iodide
FITC	Fluorescein isothiocyanate
FSC-A	Forward scatter-A; measuring size of cells
SSC	Side scatter; measuring granularity of cells
DPBS	Dulbeco's Phosphate Buffer Saline
DMEM	Dulbeco's Modified Eagle Media
FBS	Foetal Bovine Serum
MTS	Tetrazolium compound [3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt
PMS	Phenazine methosulfate
DMSO	Dimethyl sulfoxide

EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
SPSS	Statistical Package for the Social Sciences
TNTC	Too numerous to count
kGy	Kilo gray
γ	Gamma
®	Registered
™	Trade mark

SIFAT-SIFAT MIKROBIOLOGI DAN ANGIOGENESIS LUKA TERBAKAR YANG DIRAWAT MENGGUNAKAN MADU TUALANG DAN BALUTAN BERASASKAN PERAK

ABSTRAK

Penggunaan madu sebagai balutan untuk luka terbakar telah digunakan secara meluas. Walau bagaimanapun, belum ada kajian yang dilakukan untuk mengetahui potensi dan mekanisma tindak balas madu Tualang. Projek ini dijalankan untuk mengkaji aktiviti-aktiviti mikrobiologi dan factor-faktor angiogenesis yang terdapat di dalam madu Tualang bagi merawat luka terbakar. Dua puluh pesakit yang mengalami luka terbakar separa-dalam telah bersetuju untuk menyertai kajian ini. Pesakit-pesakit ini diberi rawatan secara rawak sama ada menggunakan balutan hydrofiber®-madu Tualang (n=10) atau balutan hydrofiber®-perak (n=10). Sembilan mikroorganisma yang berlainan telah diisolat daripada specimen swab luka terbakar tersebut. Lima bakteria Gram positif diperolehi iaitu Staphylococci, coagulase negative staphylococci, *Bacillus subtilis*, *Proteus* spp. dan Streptococci. Manakala empat bakteria Gram negatif yang didapati adalah *Enterobacter clocae*, *Acinetobacter* spp., *Pseudomonas aeruginosa* dan *Klebsiella pneumoniae*. Jumlah bilangan bakteria didapati menurun pada hari ke-6 dan seterusnya. Kajian antibakteria secara *in-vitro* mendapati balutan hydrofiber®-perak dan hydrofiber®-madu Manuka mempunyai zon perencatan yang lebih baik terhadap bakteria Gram positif berbanding balutan hydrofiber®-madu Tualang. Walau bagaimanapun, keputusan yang hampir sama diperolehi di antara balutan hydrofiber®-madu manuka dan hydrofiber®-madu Tualang apabila diuji terhadap bakteria Gram negatif. Sampel tisu daripada luka

terbakar (n=13) telah diambil and diuji untuk apoptosis menggunakan flositometri. Hanya dua daripada tiga belas sampel tisu yang diperolehi berjaya dikultur dan digunakan untuk melihat faktor pertumbuhan sel salur darah (VEGF) dan faktor pertumbuhan yang diperolehi daripada platelet (PDGF). Hasil kajian ini menunjukkan bahawa factor-faktor antibakteria dan apotosis adalah setara apabila luka terbakar separa-dalam dirawat menggunakan balutan madu Tualang atau balutan berasaskan perak. Kadar pertumbuhan kultur fibroblas daripada luka terbakar separa-dalam didapati berbeza-beza setelah dirawat menggunakan balutan madu Tualang atau balutan berasaskan perak. Luka terbakar separa-dalam yang dirawat menggunakan balutan berasaskan perak telah menunjukkan adanya faktor pertumbuhan sel salur darah (VEGF) dan faktor pertumbuhan yang diperolehi daripada platelet (PDGF).

MICROBIOLOGICAL AND ANGIOGENIC PROPERTIES OF BURN INJURIES TREATED WITH TUALANG HONEY VERSUS SILVER-BASED DRESSING

ABSTRACT

Honey as a dressing for burn wounds has been widely used. However the potential and the underlying mechanism of action of Tualang honey has not been studied. This project studied the microbiological activities and angiogenic properties of Tualang honey in the treatment of burn injuries. Twenty consented patients with partial thickness burn wound were included in this study. These patients were randomly treated either with hydrofiber®-Tualang honey dressing (n=10) or hydrofiber®-Ag (n=10) dressing. Nine different microorganisms were identified from the swab samples collected. Five were Gram positive namely, Staphylococci, coagulase negative staphylococci, *Bacillus subtilis*, swarming *Proteus* spp. and Streptococci. Whereas, Gram negative microorganisms were *Enterobacter clocae*, *Acinetobacter* spp., *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Total bacterial count decreased on day 6 and onwards. In the *in-vitro* antibacterial study, hydrofiber®-Ag and hydrofiber®-manuka honey dressings gave better zones of inhibition for Gram positive bacteria as compared to hydrofiber®-Tualang honey dressing. However, comparable results were obtained against Gram negative bacteria tested with hydrofiber®-manuka honey and hydrofiber®-Tualang honey dressing. Treated burn tissue samples (n=13) were harvested and tested for the apoptosis using flowcytometry. Only two from the thirteen tissue samples were managed to be cultured and tested for the VEGF and PDGF. The antibacterial properties and apoptosis

of partial thickness burn wound treated with hydrofiber®- Tualang honey and hydrofiber®- Ag dressings were comparable. Growth of fibroblast culture from partial thickness burn wound varied in proliferation rate when treated with hydrofiber®- Tualang honey or hydrofiber®- Ag dressings. Fibroblast culture from partial thickness burn treated with hydrofiber®-Ag dressing showed expression of VEGF and PDGF.

CHAPTER 1

INTRODUCTION

1.1 Research background

Various studies have been done to find the best treatment for burn wound. The most important in treating burn wound is to prevent and avoid burn wound infection as well as to accelerate wound healing. A very good dressing from natural product like honey is highly in demand to treat burn wound. Honey dressing was believed to have antibacterial properties to prevent infection and angiogenic properties to accelerate the wound healing (Subrahmanyam *et al.*, 2001; Molan, 2006; Iftikhar *et al.*, 2010; Raghukumar *et al.*, 2010).

The ideal and cheaper dressing was on demand since burn injuries normally produced large total body surface area (TBSA). The ideal dressings should be easy to apply and remove, odorless, lightweight and having good antibacterial as well as good angiogenic properties (Lionelli and Lawrence, 2003). The emergence of using bio-resources such as Tualang honey, as an alternative treatment, besides the currently available conventional treatment gives an option for cheaper dressing in treating partial thickness burn wound.

At present, Universiti Sains Malaysia in collaboration with Federal Agriculture Marketing Authority (FAMA) and Malaysia Nuclear Agency are engaged in a study on Tualang honey as an alternative treatment in burn wound. Tualang honey,

produced by giant honey bee *Apis dorsata* was obtained from Tualang tree (*Koompasia excelsa*), in the Malaysian rain forest. It contains carbohydrates such as fructose, glucose, maltose and turanose, gluconic acid (produced by enzymes), phenolic compound with low water activity and acidic pH (3.2 to 4.5), low water activity, vitamins, and essential nutrient for cells growth (Jeffrey and Echazarreta, 1996; Molan, 1998; Mohamed *et al.*, 2010; Kishore *et al.*, 2011). All these compounds are needed by human body as well as important for wound healing.

Malaysian Tualang honey was believed and has been proved by previous studies to have good antibacterial (Tan *et al.*, 2009; Khoo *et al.*, 2010) and angiogenic properties for wound healing (Rodzaian *et al.*, 2011; Nawfar *et al.*, 2011). Thus, this gives us the opportunity to conduct this study and to have a better understanding about the antibacterial and angiogenic properties of the Malaysian Tualang honey.

On the other hand, commercial products particularly silver-based dressings are widely used in clinical practice as a burn wound treatment. This silver-based dressing has been well characterized to have antibacterial properties especially nanocrystalline silver dressing which can modulate the inflammatory process at or above the level of TNF-[alpha] expression, thus generating an anti-inflammatory effect and induces apoptosis and prevent cells undergoing necrosis (Atiyeh *et al.*, 2007; Poon and Burd, 2004).

The Tualang honey used in this study was supplied by FAMA and irradiated by Malaysia Nuclear Agency. The irradiated Tualang honey has potential to be used as burn wound dressing by incorporating it with hydrofiber® dressing (Aquacel® plain).

The established hydrofiber®-dressing was chosen in this study and was combined with the irradiated Tualang honey.

The purposes of this study were to evaluate the potential of hydrofiber®-Tualang honey as an antibacterial and angiogenic dressing and its effectiveness compared to hydrofiber®-Ag to treat partial thickness burn wounds. Different types of bacteria were characterized, bioburden of the treated samples was determined, and the effectiveness of each dressing used against different bacteria isolated from burn wounds were determined by *in-vitro* study. Besides that, angiogenesis tests were performed to determine the apoptosis stages and viability of fibroblast cells isolated from burn wound tissues treated with hydrofiber®-Tualang honey and hydrofiber®-Ag. In addition, fibroblast's cell proliferation and its growth factors were determined from the tissue samples collected from the burn wounds treated with hydrofiber®-Tualang honey and hydrofiber®-Ag to determine the wound healing process.

1.2 Hypothesis

Hydrofiber®-Tualang honey dressing is better or equivalent to hydrofiber®-Ag dressing as an antibacterial and angiogenic dressing in treating partial thickness burn wound.

1.3 General objective

The main aim of this study is to determine the potential antibacterial activities and angiogenic properties of the Tualang honey compared to silver-based dressing in treating partial thickness of burn wounds.

1.4 Specific objectives

1) To compare the antibacterial properties of Tualang honey dressing and silver-based dressing in treating partial thickness burn wounds.
2) To determine the cell apoptosis of fibroblast of burn wound tissue treated with Tualang honey and silver-based dressing.
3) To determine the growth of fibroblasts in cultures obtained from burn wound tissue treated with Tualang honey or silver-based dressings.
4) To determine the angiogenic properties of fibroblasts: Vascular endothelia growth factor and platelet derived growth factor harvested from the burn wound after treatment with Tualang honey and silver-based dressing.

CHAPTER 2

LITERATURE REVIEW

2.1 Skin and its normal flora

Skin is the largest organ in human covering the entire external surface of the human body and is the principle site of interaction with the surrounding world. It serves as a protective barrier preventing internal tissues from exposure to trauma, ultraviolet radiation, temperature extremes, toxins and microbial invades (Revis, 2006; Molan, 2006). Skin also continuously produces substances that can be employed as nutrients by bacteria. Although the habitats are widely diverse, ranging across such territories as the ear canal, perineum, scalp, face, intertriginous area, hands and feet, the types of organisms that have managed to colonize these specialized sites are remarkably limited. The native floral is simple and the same organisms are found in all areas, although in vast number (Klingman *et al.*, 1976). These organisms include anaerobic and aerobic diptheroids (Propionibacterium and Corynebacterium), aerobic cocci (Micrococcus and Staphylococcus) and yeast like fungus (Pityrosporum). Gram negatives occur in the wetlands such as the axilla. These are not the same organisms habitating the bowel as stringent requirements needed for an organism to become part of the microflora. The same specificity is noted for mouth and vaginal organisms (Klingman *et al.*, 1976).

McBride *et al.* (1977) reported that bacterial populations were significantly larger from the back, axillae and feet in individuals from high temperature and high

humidity environment compared to the moderate temperature, low humidity environment. No significant effect on total populations from high humidity and low temperature. It was concluded that climatic changes may cause the fluctuation in microbial population from certain sites but not influence in the ecology of the microbial flora of normal skin environment.

Skin was formed by 2 anatomic layers of epidermis or outermost nonvascular layer consists of several layers of epidermal cells that vary in thickness over various body surfaces. The inner layer dermis or corium is largely made of collagen and contains the microcirculation, a complex vascular plexus or arterioles, venules and capillaries. These two layers are bound together by complex mechanism that is essential for normal function (Martin, 1997; Revis, 2006; Molan, 2006). Nevertheless the epidermal also contains skin normal floral which is not harmful to human in a normal skin condition because skin itself provides an immunologic barrier and serves as an antigen presenting the source for immune cells. Skin glands that produced sebum has also been reported to have antibacterial properties (Church *et al.*, 2006).

2.1.1 Burn injury and wound

Burns are one of the most common and devastating forms of trauma (Rafla and Tredget, 2011). When thermal injury occurs, a variety of skin functions are lost. Most important is the protective barrier against infection and other environmental stimuli. As the thermal burn injuries happen, increase in molecular collisions occurs, resulting in altered molecular conformation and the disruption of intermolecular bonds (Church *et al.*, 2006; Steed, 1997). The burn wounds are classified by depth of

tissue penetration as superficial, partial thickness and full thickness burn wounds (Johnson and Richard, 2003).

Superficial burns normally affect only the epidermis of the skin. The usual cause is ultraviolet radiation by sun. No blister is present and the surface is dry. It is painful to touch after a few hours of exposure to the sun. The burn area will heal by itself without evidence of scarring usually in 3 to 5 days (Michael and Richard, 2003).

Partial thickness burns are divided into superficial partial thickness burns and deep partial thickness burns (Johnson and Richard, 2003). Superficial partial thickness happens when burns extend through the epidermis downward into the papillary or superficial layer of the dermis. A hallmark of superficial partial thickness is the burn area will blanch but demonstrated a brisk or rapid capillary refill upon releasing the pressure (Devgan *et al.*, 2006; Watts *et al.*, 2001). This wound is extremely painful as the blisters break and exposed the nerve endings. The wound will be moist because of the characteristic waterproofing of the epidermis has been lost, allowing body fluid to leak on the wound surface and moderate edema usually present.

However, when the burns extend downward into reticular or deeper layer of the dermis with presentation of mixed waxy red and white, it is called deep partial thickness burns. When pressure is applied, areas of redness will continue to blanch but capillary refill may be absent or may be sluggish when pressure is released. Blisters are usually absent and the exposed surface of the wound is wet or moist similar to superficial partial thickness burns. Edema is marked and sensation is altered in areas of a deep partial thickness burn (Johnson and Richard, 2003).

Full thickness burns affect every body system and organ. This injury has destroyed both epidermis and dermis. Wound appears white without blanch. Within hours, fluid and protein shift from capillary to interstitial space causing edema. An immediate immunologic response to injury makes wound sepsis a potential threat. An increase in metabolic rate after a burn injury mandates aggressive nutritional support and this wound will not heal without surgical intervention (Dries, 1997; Johnson and Richard, 2003) .

2.1.2 Burn wound infection

Appelgren *et al.,* (2002) reported that burn wound infection is very important and potential serious complications may occur in the acute period following injury. Burns become infected because the environment at the site of the wound is ideal for the multiplication of the infecting organism. The immune suppressive status of the patient and lack of antibodies allow the microorganism to multiply freely. There is plentiful supply of moisture and nutrient and physical environment such as temperature and gaseous requirements (Edwards-Jones and Greenwood, 2003). The burn wound infection can be recognized by; i) Pus and/or foul smelling discharge from burn wound area, ii) Change in burn wound appearance or character such as dark discolouration of the eschar, increased bleeding tendency, sign of inflammation in or around the wound, and iii) Positive swab culture from burn wound (Appelgren *et al.,* 2002). Appelgren *et al.,* (2002) reported that older patient and patient with larger burn wound were infected more than other burn wound patient.

The most frequent organism causing burn wound infection was Methicillin resistant *S. aureus* (MRSA), *P. aeruginosa*, *Streptococcus* spp., coagulase negative staphylococci, *Enterococcus* and *Enterobacter* (Appelgren *et al.,* 2002). Basualdo *et al.*, (2007) reported that most of the undiluted honey samples inhibited the growth *S. aureus* and *S. epidermidis*. Whereas, some honey samples inhibited the growth of *S. uberis*, *P. aeruginosa*, *E. coli* and *K. pneumonia* although to a lesser extent.

2.2 Wound healing

The healing of an adult skin wound is a complex process requiring the collaborative efforts of many different tissues and lineages. This process can be roughly divided into 3 overlapping phases. Inflammatory phase which involves vascular responses characterized by blood coagulation and hemostasis as well as cellular event, including infiltration of leukocytes with varied functions in antimicrobial and cytokine release to initiate the proliferate response for wound repair and remodeling phase (Falanga *et al.*, 1988; Steed, 1997; Li *et al.*, 2007; Schreml *et al.*, 2010) . Wound healing processes seem to be strictly regulated by multiple growth factors and cytokines release at the wound site.

As soon as the wound on skin happen, most will cause blood leakage from damaged blood vessels and wound will be dominated by inflammatory reactions mediated by cytokines, chemokines, growth factors and their actions on cellular receptors. Intracellular signaling cascades are activated, contributing to cell proliferation, migration and differentiation. In addition, chemo-attractant factors recruit different

cell types such as granulocytes and macrophages to the wound site thus, initiating wound repair (Li *et al.*, 2007; Schreml *et al.*, 2010).

During this hemostasis stage too, platelet activating mediator and vasoactive and chemotactic mediator was released. The formation of clot that consists of platelets embedded in a mesh of cross-linked fibrin fibers derived by thrombin cleavage of fibrinogen, together with smaller amount of plasma fibronectin, vitronectin and thrombospondin.

2.2.1 Apoptosis

Apoptosis is derived from the Greek word for "falling off" of leaves from a tree. It is also known as programmed cell death which describes the characteristic mode of cell death common to various cell types (Huang *et al.*, 2003). It is an active and physiological mode of cell death, in which the cell itself designs and executes the program of its own demise and subsequent body disposal (Darzynkiewicz *et al.*, 1997). Apoptosis is responsible for the removal of inflammatory cells and the evolution of granulation tissue into scar. Dysregulation in apoptosis can lead to abnormal wound healing such as hypertrophic scar, keloid formation (Huang *et al.*, 2003) and abnormal neovascularization (Zagzag *et al.*, 2000). The four basic steps of apoptosis are induction, detection, effector and removal. Various external stimuli can trigger apoptosis including nutrient deprivation, cytokine depletion, ionizing radiation and oxidative stress (al-Rubeai and Singh, 1998; Teraki and Shiohara, 1999).

As wound healing involves a series of rapid increases in specific cells population that prepare the wound for repair, deposit new matrices and finally matured the wound. Upon completing their task, these specific cell types must be eliminated from the wound prior to the progression to the next phase of healing. Apoptosis allows for eliminations of the entire population without tissue damage or an inflammatory response (Greenhalgh, 1998). At the morphological level, it is characterized by cell shrinkage rather than swelling seen in necrotic cell death (Plasier *et al.*, 1999). The most characteristic feature of apoptosis is condensation of nuclear chromatin. This change is a very unique characteristic to apoptosis (Darzynkiewicz *et al.*, 1997).

2.2.2 Angiogenesis in wound healing

Angiogenesis refers to new vessel growth by the sprouting of preexisting vessels adjacent to the wound. Angiogenesis is very important in wound healing process. In response to the injury, microvascular endothelial cells initiate an angiogenic process consisting of activation of endothelial cells, local degradation of their basement membrane, sprouting into the wound clot, cell proliferation, tubule structure formation, reconstruction of the basement membrane and eventually regression and involution of the newly formed vascular as tissue remodeling (Marx *et al.*, 1994).

Angiogenesis involves many growth factors such as vascular endothelial growth factor (VEGF) and platelet growth factor (PDGF).

2.2.2(a) Vascular endothelia growth factor

Vascular endothelial factor (VEGF) or vascular permeability factor is a key mediator to angiogenesis. VEGF performs multiple function and is a very potent mitogen for endothelial cells and induce endothelial cells migration and sprouting by regulation of several integrin receptors (Senger *et al.*, 1997). Many cell types such as keratinocytes, fibroblasts and endothelial cells can produce VEGF. VEGF is expressed at low level in normal human skin but highly up-regulated during wound healing. Major inducer for this factor is low-oxygen tension that occurs in tissue hypoxia during tissue injury (Detmar *et al.*, 1997).

Vascularization may be enhanced by VEGF secretion by the fibroblast as well as increased VEGF secretion by the thicker epidermis with more keratinocytes (Erdag and Sheridan, 2004; Marx *et al.*, 1994). It was reported that PDGF-BB inside the surrounding matrix induced the microvascular endothelia cells to proliferate. The migration and proliferation of endothelial cell and the functional maturation of endothelial cell into mature blood vessels is very important in angiogenesis (Senger *et al.*, 1997).

VEGF also known as vascular permeability factor is a potent angiogenic cytokine that stimulates endothelial cells (EC) through two receptor tyrosine kinase. VEGF is particularly important to induce angiogenesis in a variety of experiment models as well as conversely antagonism in their function to inhibit angiogenesis. Inactivation of a single allele can disrupt normal blood vessel development resulting in embryonic death in utero. Whereas elevated expression of VEGF and its receptor

have been shown to correlate with neovascularization associated with embryogenesis, wound healing cancer, rheumatoid arthritis, psoriasis, delayed hypersensitivity reactions and proliferative retinopathy (Senger *et al.*, 1997).

2.2.2(b) Platelet derived growth factor

Platelet derived growth factor (PDGF) is a sulfide linked dimeric protein, variably composed of two related polypeptide chains A and B which are expressed from different genes and assembled as a homo or heterodimers (Oefner *et al.*, 1992). The size of unreduced, active and reduce, inactive of PDGF forms ranging from 23 – 35 kDa and 12 – 18 kDa respectively (Antoniades *et al.*, 1979). Three possible isoforms of PDGF, AA, BB and AB have been identified and shown to bind with different affinities to homo and heterodimers of two different but homologues receptor gene products, denoted α and β (Matsui *et al.*, 1989). The crystal structure of the homodimeric BB isoform of human recombinant PDGF-BB is made of polypeptide chain, which is folded into two highly twisted antiparallel pairs of β-strands. It contains an unusual knotted arrangement of three intramolecular disulfide bonds. Dimerization leads to the clustering of three surface loops at each end of the elongated dimer, which most probably form the receptor recognition sites (Oefner *et al.*, 1992). PDGF-BB was more potent mitogen compared to equivalent concentration of PDGF-AB (Marx *et al.*, 1994).

Platelet derived growth factor (PDGF) is a known modulator of fibroblast cell mitosis and chemotaxis (Ross *et al.*, 1986; Vij *et al.*, 2008). It was secreted by platelets, macrophages, keratinocytes and endothelia cells (Steed, 1997). It is a potent

and important growth factor especially in the early inflammatory phase of wound healing (Li *et al.*, 2007). PDGF is chemotactic for fibroblasts and smooth muscle cells at low concentration. The PDGF receptor is found on cells that show a mitogenic response to PGDF. Thus, the receptor is present on fibroblasts, glial cells and vascular smooth muscle cells. The PDGF receptor has an extracellular ligand binding domain and an intracellular effector domain with tyrosine kinase activity and the peptide receptor complex is internalized by endocytosis (Ross and Vogel, 1978; Grotendorst, 1984; Steed, 1997; Takehara, 2000).

2.2.2(c) Fibroblasts

Human dermal fibroblasts play a central role in skin tissue regeneration. Fibroblasts appear at the injury site at a very early stage and proliferate rapidly as wound healing progresses. Fibroblast migration to the wound site was caused by the chemo attractant from PDGF (Antoniades *et al.*, 1979; Li *et al.*, 2007). They accelerate the healing process by regulation of matrix deposition (type I, type IV collagen, elastin, laminin) epidermal differentiation and dermal regeneration (Marks *et al.*, 1991; Demarchez *et al.*, 1992; Bennett and Schultz, 1993).

Fibroblasts can be derived from different tissues and appeared very similar. They are amoeboid, migratory and taking spindle shaped morphology. Fibroblasts can generate a ligament or lay down tendon, under *in vivo* condition, an extracellular matrix that differs greatly from that produced by fibroblasts from the skin, the liver or the vitreous humor of the eyes (Mansbridge, 2002).

In addition, fibroblasts synthesize various growth factors; insulin, growth factor (IGF), keratinocytes growth factor (KGF), platelet derived growth factor A (PDG-A), transforming growth factor (TGF), vascular endothelial growth factor (VEGF) and cytokines that stimulate wound healing. Fibroblasts enhance the vascularization and endothelial cells proliferation as well as vascular basement membrane formation (Erdag and Sheridan, 2004). Erdag and Sheridan, (2004) also reported that fibroblasts promote early keratinocytes proliferation and stratification that promote dermo-epidermal regeneration. Besides epidermal regeneration, fibroblasts also play a major role in dermal regeneration.

Fibroblasts come from the second layer of the skin (often called dermis) that involved in partial thickness burn wound. As partial thickness burn always needed surgery, it is easier to harvest fibroblasts and used in burn wound angiogenesis test. The dermis layer, the inner layer of the skin, provides tensile strength, mechanical support and protection to the underlying muscle, bones and organs. It contains mostly connective tissue and few skin cells. Collagen (a tough, fibrous protein), blood vessels, fibroblasts and nerves are found in the dermal layer (Waugh A, 2001).

Desmouliere and Gabbiani, (1995) reported the main function of fibroblasts in normal adults is to secrete the extracellular matrix components while smooth muscle cells exert contractile activities. However, during pathological situations (e.g. during wound healing) fibroblasts may develop contractility whereas smooth muscle cells may secrete important amounts of collagen.

During tissue aggression, fibroblasts and smooth muscle cells, which are both mesenchymal cells, adopt similar feature. They migrate and proliferate to replace the defect of injured tissue. In pathological situation, these migration and proliferation can induce the development of organ fibrosis or the constitution of an atheromatous plaque respectively. In some situation, the proliferation can be counterbalanced by cell loss. The apoptosis could be the process through which the cells are eliminated (Desmoulier *et al.*, 1997).

2.3 Burn wound dressing

In history, washing the wound, making plasters with mixtures of herbs, ointment and oil to be applied to wound and bandaging known as "three healing gestures" as described in (circa) 2200 BC is to aid wound healing (Yardley, 1998). In 1867, Lister introduced the first antiseptic dressings. Tulle gras, which consisted of gauze, impregnated with paraffin used in World War I until now, it is one of the earliest non-adherent dressings (Lionelli and Lawrence, 2003).

The principles of burn wound dressings in this twentieth century were to protect bacteria and foreign material from invading the wound. The dressing must have the properties such as able to absorb exudates from wound, haemostatic, non-adherent to limit wound disruption, maintain the wound moist to accelerate wound healing, durable, comfort, cost effective and esthetically attractive (Qiunn *et al.*, 1985; Ryan, 1990; Lionelli and Lawrence, 2003; Ovington, 2007).

Based on the burn dressing principles and understanding of burn wound needs, many studies have been conducted to search for the best dressing for burn wound. However no single dressing is suitable for all types of wound (Mat Saad, 2011). The dressing must have antibacterial properties as well as angiogenic properties to hasten wound healing. To date, many modern dressings emerged to aid in wound healing such as silver, hydrofiber as well as an alternative treatment using natural product like honey.

2.3.1 The silver-based dressings

One of the most important strategies for burn management and therapy is to heal and re-epithelize the wound as soon as possible. This is to prevent infection, to reduce functional disability of burn sites and prevent poor aesthetic after-effect by using noble metal antimicrobials agent. The most prevalent metal used in burn wound therapy is silver (Wright *et al.*, 1998). For centuries silver has been known to have antibacterial properties. As early as 1000 B.C., the antimicrobial properties of silver in rendering antibacterial were appreciated (Richard *et al.*, 2002; Russell and Hugo, 1994).

Topical silver cream and silver nitrate solution has a broad antibacterial spectrum (Moyer *et al.*, 1965). However, it can slow epithelialization (Bellinger and Conway, 1970). Silver sulfadiazine (SSD) has a broad spectrum of antibacterial, antifungal and antiviral activity (Ballin, 1974) but it showed toxicity to fibroblast *in-vitro* (McCauley *et al.*, 1992). However, SSD was noted to accelerate epithelialization of partial thickness wounds (Geronemus *et al.*, 1979).

Atiyeh and colleagues (2007) found that silver has reemerged as viable treatment option for infections encountered in burns, open wounds and chronic ulcers. The gold standard in topical burn treatment is silver sulfadiazine (Ag-SD), a useful antibacterial agent for burn wound treatment. Recent findings indicated that the compound in the silver-based dressing delays the wound-healing process (Subrahmanyam, 1998; Subrahmanyam *et al.*, 2001). This silver may have serious cytotoxic activity on various host cells. The beneficial effects of silver on wound biology due to its potent antimicrobial activity have been overlooked in general until recently. Irrespective of the source of silver, whether released from solutions, creams and ointments or nanocrystalline silver released from commercially available new dressing, silver is highly toxic to both keratinocytes and fibroblasts. Fibroblasts appear to be more sensitive to silver than keratinocytes. Poon and Burd (2004), showed that silver is highly toxic to both keratinocytes and fibroblasts in monolayer culture but when in optimized individual culture, the fibroblasts appear to be more sensitive to silver than keratinocytes. However, when both cell types were grown in the same medium their viability was the same (Poon and Burd, 2004). The present review aims to determine the practical therapeutic balance between antimicrobial activity and toxicity (Poon and Burd, 2004; Atiyeh *et al.*, 2007). Recent advancement in wound healing dressings is hydrofiber® dressing and hydrofiber®-Ag dressing.

2.3.2 Honey as a wound dressing

Honey was known to be varied in colour, taste, texture and other chemicals contained inside make it very unique. This uniqueness and differential in chemical contents make it a good antibacterial agent (Cooper, 1999; Molan, 1999; Molan, 2002). It is

also good in skin care and wound healing as honey can boost angiogenesis. The precise composition of honey varies according to the plant species on which the bee forages but the main constituents are the same in all honey (Jeffrey and Echazarreta, 1996). US Federal Drug Administration approved the use of honey as an option in wound care dressing in 2007. Honey exerts anti-inflammatory and antibacterial effects without antibiotic resistant, promotes wound healing and facilitates debridement (Pieper, 2009). Honey also can be regarded as a safe ointment and used without dilution (Molan, 2002). Majtan and Majtan (2009), in their letter to the editor highlighted the potential beneficial properties of honey during the healing process at cellular level because honey not only contains the antibacterial properties but also contains effective molecule(s) with non-antimicrobial characteristics that stimulates cells involved in wound healing. Aljady *et al*., (2000) in their animal study also reported that Malaysian Gelam honey stimulates the fibroblast function, enhance synthesis of glycosaminoglycans and deposition of collagen. Gelam honey can also increase the rate of wound contraction and epithelialization and improves the nutritional state of the animal under study when given orally. Randomized control trial (RCT) on manuka honey exhibited an increased incidence of wound healing on venous leg ulcer (VLU), more efficacious desloughing and lower the rate of infection as compared with the controls (Gethin and Cowman, 2008). Majtan, (2010) claimed that manuka honey had more therapeutic advantages over other honeys. Antibacterial activity of manuka honey is due, at least in part, to reactive methylglyoxal (MG). The concentration of MG in manuka honey is up to 100-fold higher than in conventional honeys but MG also may delay wound healing in diabetic patients. Iftikhar *et al*., (2009) in their study on efficacy of Acacia honey on wound healing confirmed that honey can aid wound healing when applied topically in several models. They also

suggested that honey may enhance wound healing by accelerating glycolytic enzyme activity, and supplying sufficient energy for cellular repair.

There are many studies on honey either to assess the antimicrobial properties or angiogenic properties or as a food or supplements. Jeffrey and Echazarreta, (1996) reported that honey is composed of more than 95% carbohydrate such as fructose, glucose, sucrose, maltose, gluconic acid and gluconolactone. Other compounds listed are ash, nitrogen, mineral and vitamins with moisture and good pH range. Honey also contains a number of amino acids such as proline, phenyalanine and aspartic acid. Last but not least the enzymes that work out to make a great honey are invertase, catalase, acid phosphatase and amylase. Invertase was derived from the hypopharyngeal glands of worker honeybees, which inverts sucrose to glucose and fructose, glucose oxidase to gluconic acid and hydrogen peroxide in the presence of water and amylase which breakdowns the starch. Catalase originated from plant is a regulator of glucose activity. Hydrogen peroxide is a good antibacterial as well as cytotoxicity agent (Bang *et al.,* 2003).

This study was done using Malaysian local Tualang honey that are combined with hydrofiber® as a recent advancement in burn wound treatment to evaluate the effectiveness of Tualang honey dressing compared to silver-based dressings.

2.3.2(a) Tualang honey as a wound dressing

Tualang honey is made by colonies of bee, *Apis dorsata* and is collected from comb built very high on the Tualang tree (*Koompasia excelsa*). Tualang honey is well

known by Malaysians and has been used as either food or medicine (Ainul Hafiza *et al.*, 2005; Ghazali, 2009). A study done by Nawfar *et al.*, (2011) suggested that Tualang honey could be used as an alternative therapeutic agent for diabetic foot wounds with similar beneficial effects as those expected for manuka honey. Honey is also considered to be a suitable option to replace the other modern dressings in treating partial thickness burn wounds because of its antibacterial properties, its high osmolarity, pH, low water activity, vitamins and rich in essential nutrient for cell growth (Molan, 1998; Tan *et al.*, 2009; Mohamed *et al.*, 2010; Khoo *et al.*, 2010; Nasir *et al.*, 2010; Kishore *et al.*, 2011). A study by Kishore *et al.*, (2011) showed that Tualang honey has higher phenolic content and greater radical scavenging activity compared with other honey source. The phenolic and other compounds within honey are responsible for free radical scavenging.

2.3.3 The hydrofiber dressings

Hydrofiber® dressing (Aquacel®) is a white, soft, sterile, non-woven pad composed of hydrocolloid fibers (sodium carboxymethylcellulose). This conformable and highly absorbent dressing absorbs wound fluid and creates a soft gel. This dressing maintains a moist environment that supports the body healing process and aids in the removal of unnecessary material from the wound (autolytic debridement) without damaging newly formed tissue.

2.3.3(a) The hydrofiber®-Ag dressings

This dressing composition is the same as hydrofiber® plain (Aquacel®) dressing but impregnated with 1.2 % ionic silver which allows for a maximum of 12 mg of silver

for a 4 inch x 4 inch dressing. The silver ions will be released when it is hydrated, and the carboxymethylcellulose will sequester the bacteria (Mooney *et al.*, 2006). This mechanism is thought to be the key to the product's effectiveness.

2.3.3(b) The hydrofiber®-Tualang honey dressings

Hydrofiber® dressing was soaked into Tualang honey to create a hydrofiber®-Tualang honey dressing. Hydrofiber® dressing will absorb Tualang honey to create a soft gel to maintain wound moist and keep the important antibacterial properties of Tualang honey on wound (Khoo *et al.*, 2010; Nasir *et al.*, 2010; Halim A.S. *et al.*, 2011; Mat Saad *et al.*, 2011). The colour of the dressing will be changed according to the colour of the honey (Figure 3.4). Tualang honey is easily absorbed by the hydrofiber® dressing and is easy to place on the wounds.

CHAPTER 3

MATERIALS AND METHODS

3.1 Sample collection

This study was carried out at the School of Medical Sciences, Health Campus, Universiti Sains Malaysia and Burn Unit, Hospital Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia with approval from Ethics Committee (Human) of Universiti Sains Malaysia [Reg. No: 198.3(4)].

Eighty eight burn patients were screened and twenty consented patients with partial thickness burn wound were included in this randomized control trial study. These patients were randomly treated either with hydrofiber®-Tualang honey dressing (n=10) or hydrofiber®-Ag dressing (n=10).

3.1.1 Inclusion criteria

Partial thickness burn patients aged between 2 to 60 years old with total body surface area (TBSA) less than 30% and requiring tangential excision. These patients are expected to survive. Upon signing the consent form, patients were enrolled for this study.

3.1.2 Exclusion criteria

Patients with electrical burn or chemical burn, superficial or full thickness burn wound, delayed referral patients or deep infection, patients that had been treated more than 3 days with silver-based dressing or silver antibiotic, patients on long term antibiotic usage (more than 3 doses), diabetic patients, patients on steroid, patient with skin disorder, hypersensitivity to silver, clinical AIDS or immunocompromised patients, chronic inflammation diseases, patients from whom culture cannot be obtained, patients who are recent abusers of alcohol and/or any other drug that would result in his/her inability to participate in this study.

3.2 Dressing materials used

3.2.1 Tualang honey

Agromas® pure Tualang honey (Figure 3.1) was supplied by Federal Agriculture Marketing Authority, FAMA Negeri Kedah Darul Aman, Malaysia and was irradiated with 25 kGy of Gamma (γ) radiation at Malaysia Nuclear Agency. No significant loss of antibacterial activity of honey was reported with Gamma-irradiated (Molan and Allen, 1996).

3.2.2 Hydrofiber® dressings

Hydrofiber®-plain or hydrofiber®-Ag dressing (ConvaTec Inc, UK) were used for patients' treatment and laboratory work. These dressings were used as control.