BURN WOUND DRESSING USING HONEY HYDROGEL VERSUS HYDROGEL

by

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of the requirements for the degree
of Bachelor of Health Sciences (Biomedicine)

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CERTIFICATE

This is to certify that the dissertation entitled

"Burn wound dressing using honey hydrogel versus hydrogel"

is a bonafide record of research work done by

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ABSTRAK

Luka akibat kebakaran melibatkan kehilangan fungsi kulit. Kulit memainkan peranan penting untuk perlindungan, pertahanan badan, pengawalatur suhu badan,sentuhan saraf dan sebagainya. Kajian ini dijalankan untuk mencari kaedah alternatif yang paling berkesan untuk merawat luka akibat kebakaran. Madu digunakan sebagai perubatan tradisional yang boleh menyembuhkan luka kebakaran sama ada berpunca daripada elektrik atau haba. Kandungan madu yang unik dapat menghalang pertumbuhan bakteria, menyokong pertumbuhan sel baru dan juga untuk merawat luka terutama akibat kebakaran. Bagi membuktikan keberkesanannya, campuran madu hidrogel dan agar hidrogel digunakan sebagai produk kawalan. Sebanyak 12 ekor tikus Sprague Dawley betina digunakan dan dibahagikan kepada dua kumpulan iaitu 6 ekor dalam setiap kumpulan. Mereka disimpan di dalam bilik dan mendapat persekitaran dan makanan yang sama. Untuk mengelakkan pengaruh luar mempengaruhi, operasi dijalankan dalam bilik steril, peralatan steril seperti dalam bilik pembedahan biasa. Pada mulanya tikus akan dibiarkan dalam keadaan separa sedar dengan "ketamine" iaitu dengan mencucuk melalui otot. Alkohol dan iodin digunakan sebagai disinfektan. Luka terbakar dicipta dengan menggunakan besi panas dan dibiarkan selama 10 saat

untuk menghasilkan kebakaran peringkat ketiga. Mereka kemudiannya akan menerima rawatan dengan menggunakan hidrogel bermadu dan agar hidrogel. Madu hidrogel terdiri daripada hidrogel yang mana 6% kandungan airnya telah diganti dengan madu. Pada hari ke 7 dan 14 selepas prosedur, lesi luka akan diukur. Purata saiz luka bagi kumpulan hidrogel bermadu pada hari ke 14 ialah 0.24 cm^2 dan bagi agar hidrogel adalah 0.64 cm^2. Ini membuktikan penyembuhan luka adalah lebih cepat dalam rawatan hidrogel bermadu berbanding agar hydrogel. Hidrogel bermadu dilihat lebih berkesan berbanding agar hidrogel dalam penyembuhan luka akibat kebakaran kerana dapat menyediakan hamparan lembap untuk menggalakkan pertumbuhan sel-sel baru.

ABSTRACT

Burn injury results in either the loss or disruption of skin functions. Skin plays its main roles in protection, immunological, homeostatsis and thermoregulation, neurosensory, etc.. Honey is believed to be the best ancient medicine to cover wounds that causes by burn either thermally or electrically. The unique ingredients of honey acts by inhibiting gram positive and negative bacteria, promotes epithelialization and burn healing. This project is to assess the impact of honey on wound dressing for burn wound treatment. The study compared the effectiveness of honey hydrogel in burn wound healing with hydrogel agar as a control. Burn wounding rats were chosen as the study model. Twelve female Sprague-Dawley rats were divided into two equal of size n=6 and kept in same room with same food and environment. The operation was done under aseptic technique, in sterile condition. At first, rats were anaesthetized with ketamine injection and disinfectant with alcohol and povidone iodine. Burn lesion was created by applying hot metal plate for ten seconds on the back of the rats. The first group was treated by honey hydrogel while the other group was covered by hydrogel agar only. Honey hydrogel is actually a hydrogel whereby 6% of the water content replaced with honey. They were supervised daily and on the 7^{th} and 14^{th} day postoperatively the

burn lesion was measured. The average of burn wound size on day 14 for honey hydrogel and hydrogel was 0.24 cm^2 and 0.64 cm^2 respectively. This indicates that wound treated with honey hydrogel contracts faster and seem to be more effective than hydrogel as it provides moist and good bedding for epithelialisation that can heals the faster.

1.0 INTRODUCTION

Burn injuries may result in either the loss or disruption of skin functions. The depth of the burn wound is used as a parameter to classify them into first degree, second degree or third degree burn. Different degrees of burn depth require different treatment. In order to cover the burn injury, wound dressing is required. Nowadays many researchers proposed variety of dressing. Burn patient suffered significantly especially when it involves major burns. Modern treatment like skin grafting is beneficial for the treatment but there are needs for dressing that is cheap, convenient and functional. The discovery of traditional materials like honey and tomato peels with healing properties, have made them possible to be applied in modern medicine.

1.1 Skin

Burn is closely associated with skin structure and functions. In order to study the burn wound healing mechanism, the knowledge of skin is mandatory. Skin is the largest organ of the body. It is not uniform in thickness. The average thickness of skin is about 1 to 2 mm. The thick region of skin is in the sole of the foot, palm of the hand and in the interscapular measuring about 5mm. All other region of the body has thin skin. It is thinnest over the eyelids and penis measuring about 0.5mm. There are two layers that made up skin. The first layer structured by outer epidermis and beneath is a dermis.

The epidermis is formed by stratified epithelium which is made up of five layers. They are stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum and last layer is stratum germinativum. The epidermis does not have blood vessels and therefore the nutrition is provided by the capillaries of the dermis. Dermis is a connective tissue layer made up of dense stout collagen fibers, fibroblasts, and histiocytes. The collagen fiber exhibit elastic property and capable of storing or holding water. The collagen fibers contain the enzyme collagenase, which is responsible for wound healing. Dermis is made up of two layers: superficial papillary layers and deeper reticular layer.

Skin covers all parts of the body. Its main role is protection. This include protect against bacteria and toxic substances, mechanical blow and ultraviolet rays. As skin covers organ of the body, it protects organ by preventing bacterial invasion from having direct contacts with external environment. Bacterial invades skin when it is injured and allows exposure to external environment. Stratum corneum of epidermis is the layer responsible for protective functions of skin. Besides, this layer also makes skin resistant against toxic chemicals like acids and alkalis.

Other functions of the skin are as sense organ, storage, synthesis of Vitamin D, regulation of body temperature, water and electrolyte balance, excretory function, absorptive and secretory functions.(Sembulingams, 2000)

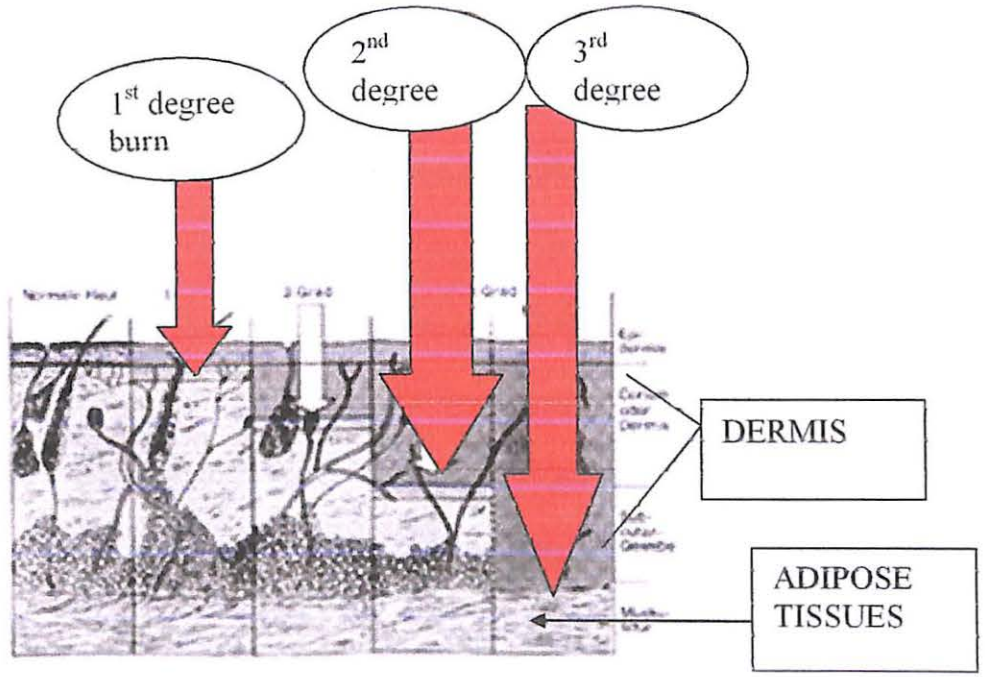
1.2 BURN

Burns are one of most complex and harmful physical injuries that often require initial trauma care, followed by careful evaluation and appropriate wound management. Hundreds of thousands patients seek medical attention for burn injuries. In United States alone, more than 2.4 million people suffered burn injuries. A burn is an injury that damages and destroys skin layers. The sources of burns are by heat, electrical, chemicals or radiation. (Rook *et. al*). In facilitating proper treatment, burns are classified into first, second and third degree depending on how deep is the damage to skin layers.

A first degree burn is limited to the epidermis. It is characterized by heat, pain, moistening and reddening of the burn surface, but rarely shows blistering or charring of tissue. First degree burns often heal in three to seven days and seldom has scar. Typical first degree burns include sunburn and minor scalds.

The second degree burns are sometimes referred to as partial thickness burns. It is further subclassified as either "superficial" or "deep." Both types penetrate deeper than a first degree burn and destroy the epidermal layers, extending into the dermis layer. They can cause damage to sweat glands and hair follicles and are extremely painful, often with intense swelling. A superficial second degree burn is moist, red and weepy. Most superficial second degree burns heal in 10 to 21 days, but leave changes in skin color and pigmentation. A deep second degree burn can be ivory or pearly white in color and may require a process known as debridement and additional skin grafting treatments.

A third degree or full thickness burn completely damage all of the epidermis and dermis and may even destroy the underlying structures. (http://www.fda.gov) The tissue damage extends below hair follicles and sweat glands to subcutaneous (fat) tissue. The skin becomes charred and leathery and often appears depressed relative to surrounding tissue. The skin can be bright red, waxy white, tan or brown. There are no blisters; and third degree burns may cause massive swelling and usually painless because the injury has destroyed nerve endings. This third degree burn is used as the study model for the evaluation of the burn wound dressing.



Figures 1: Depth of skin (http://www.biomed.metu.edu.)

1.3 Wound Healing

Wounds are physical injuries that result in an opening or breaching of the skin continuity. Proper healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin. This is a product of the integrated response of several cell types to injury. Wound healing is a complex process that results in the contraction and closure of the wound and restoration of a functional barrier. (Raghavan *et. al*, 2004) Factors that will enable healing to occur include wound care, good nutrition, and maintenance of function, positive attitude and co-operation from the patient. (Kavanagh *et. al*). Edema reduction, prevention of burn wound infection and adequate analgesia will also contribute to optimal patient outcome.

1.4 Wound Dressing

After any injury or wound, it requires coverage for protection. This lead to the creation of wound dressing. A wound dressing is applied to stop bleeding, absorbs exudates, ease pain, and facilitate epidermal resurfacing. (http://www.biomed.metu.edu.tr/biomeds.htm). Earlier, the main role of dressing is to provide an environtment which is dry as possible because microorganism tend to be proliferating in moist environment but later variety of occlusive and semiocclusive dressings were shown to increase the rate of epithelialization by as much as 40 percent. Since the tissue under the dressing remains moist, treatment with these types of dressing has been termed *moist wound healing*.

Wound dressing is responsible to replace the functions of lost skin, to protect wounds from protein and fluid losses, prevent bacterial invasion, dissipiate mechanical stress (external) and to improve and stimulate wound healing. Dressing is classified according to its properties, either from the usage or from the nature of materials. However, the classification of dressings more frequently used is one based on the nature of its material rather than the mode of application. Based on the type of material used for the preparation of dressing they may be classified as conventional, biological and synthetic dressings. Within each category, the dressings may be further classified into primary, secondary and island dressing. Another category is an alternative treatment, using the ancient and traditional materials like honey and potato peels.

The conventional dressing are made up of fabric material such as gauze. They provide little or no occlusion and allow evaporation of moisture resulting in a dry dessicated wound bed. Since the conventional dressings had limitations for application on full thickness wounds, there has been many research and development of more advanced wound dressings in the form of synthetic and biological dressings. Biological dressings are derived from natural tissues usually consisting of various formulations and combinations of collagen, elastin and lipid. They have properties to restore a water vapor barrier and prevent dehydration of the wound; decrease evaporational heat loss; decrease protein and electrolyte losses in wound exudates, prevent bacterial contamination of the wound and hence protect the wound and patient from sepsis, permit less painful dressing changes, permit painless movement over joints, facilitate debridement of wounds, create good granulation tissue bed for autografting of deep wounds, can be used to test for successful subsequent autograft, decrease healing time of partial thickness burns and donor sites and improve quality of healing, inhibit excessive fibroblasts and decrease contraction. Examples of biological dressing are allograft, heterografts from pigs, dogs and other species, to embryonic membranes, embryofetus and neonatal skins, films of reconstituted collagen from bovine and other sources, fibrin, cultured epidermal grafts, dermal matrix grafts and cultured dermal matrix composite grafts.

The synthetic polymer dressing derived from petroleum products can be easily manufactured using conventional technology into films, fibers, sheets, and

sponges. Therefore these materials have received attention as potential wound dressings for deep wounds.

Synthetic polymers have several advantages such as ability to adhere to the wound edges, ability to drape to the wound contour, and ease of use. The major disadvantage is the lack of biological properties such as enhancment of wound healing via attraction of cells involved in healing process. These dressings are used as coverings for deep (full-thickness) burns and skin ulcers. In these applications synthetic polymeric dressings create an inert environment that controls water and heat passage from the wound while preventing bacterial infiltration

In some countries the use of expensive commercially produced dressing products is not viable. Economic reality is a fact of life. Many centers have been very creative in the development of alternative dressings. The use of amniotic membrane, banana leaf dressing, honey, papaya and boiled potato peel bandage, have all been reported in the literature (Kaghavan et. al)

1.5 HONEY

Honey is a viscous fluid produced by bees and other insects from the nectar of flower (*Honey Scientific Report*, 1998) The National Honey Board had defined honey as pure product that does not allow for the addition of any substance.

The application of honey in wound and burn treatment has become very famous and had discussion points among scientist and researchers. Indeed honey is not a new medicine because it actually has been applied for medicinal purposes since ancient times. Despite the Al-Quran and Bible as well as Ayurvedic books have stated clearly about the use of honey in healing but modern practitioners still hesitate to apply honey for local treatment of wounds. (Kalam *et. al*, 2003).Honey was subjected to laboratory and clinical investigation during the past few decades and until now the honey contents is still being researched. The most remarkable discovery in honey was the anti-bacterial activity of honey that has been confirmed in numerous studies. This antibacterial activity is related to four properties of honey. The healing of any wounds is dependant in the presence of microorganism at the wound area and these microorganisms are water lovers. This makes the first criteria for honey in wound healing. Honey is a supersaturated sugar solution and this result in a strong interaction between the sugar molecules and water molecules. This 'osmotic effects' leave very few water molecules for growth support of microorganisms. The rate of inhibition of growth depends on the species of bacteria and the concentration of honey.

Secondly, the pH of honey is between 3.2 and 4.5 and this acidity is low enough to inhibit the growth of most microorganisms that cannot stand acidic environment. Honey also have specific enzyme called glucose oxidase. Hydrogen peroxide produced by glucose oxidase is the third and probably most important antibacterial component. Lastly, several phytochemical factors like antibacterial activity had been found in honey. When applied to wounds, the osmotic effect and acidity of honey decrease on dilution in wound fluids. This is more so because these fluids are well buffered. Contrary to this decrease, hydrogen peroxide activity increases 2.5 to 50 times on dilution. In this dosage, the hydrogen peroxide is still antiseptic without its damaging effect on tissues. Likewise, most phytochemical factors withstand dilution in wound fluids.

In this study, we use honey combined with hydrogel as the treatment in comparison with hydrogel agar alone.

1.6 HYDROGEL

Hydrogels are important advanced second-generation wound care products. In spite of their high water content (up to 96%), hydrogels can further absorb a mild to moderate amount of wound exudates by swelling (Martineau *et. al*, 2003) They are particularly useful as dressings for many partial-thickness skin defects (e.g: shallow abrasions, superficial wounds), blisters, second-degree burns, and healthy, granulating tissues. However, a distinctive disadvantage of the commercially available hydrogel wound dressings is that they do not provide a barrier against wound infection. It is often recommended clinically that an antimicrobial agent be applied under the hydrogel dressing or blended with the amorphous hydrogel.

Hydrogels are cross linked hydrophilic polymer networks swollen in water. They are mostly biocompatible and mildly mucoadhesive and are able to absorb and release large amounts of aqueous solutions (http://www.csir.co.za). Hydrogels with adequate mechanical integrity may be prepared by physical or chemical crosslinking of polymers or in network forming polymerizations. However, the small molecules usually used as chemical crosslinking agents and the residual unreacted monomers are toxic and are removed with great difficulty and cost. It was therefore decided to consider only physically crosslinked gels for the burn-wound dressing

The honey hydrogel used is a combination of honey and agar hydrogel whereby 6% of the water content was replaced with honey.

2.0 LITERATURE REVIEW

In the name of Allah.....

Then eat of all fruits (all produce of the earth), and follow the ways of thy Lord, made smooth (for thee). There cometh forth from their bellies a drink of hue (varying colors), wherein is healing for mankind. Lo! Herein is indeed portent (sign) for people who reflect.

An-Nahl-16: 69

The paragraph in Al-Quran above had mentioned clearly about the use of honey in healing properties. It is also used topically in the Ayurvedic medicine of 2500 BC, and the Egyptians, Greeks and Romans used it as well. (Kalam *et al*, 2003).

Honey is a mixture of sugars prepared by bees from the natural sugar solutions obtained from flowers (M. Subrahmanyam, 1996). The wound healing properties of honey have been well documented. The beneficial effects of honey include the cleansing of wounds, absorption of edema fluids, antimicrobial activity, and promotion of granulation tissue, epithelialisation and improvement of nutrition. In the prospective study, honey treated wounds were found to have highest rate of healing compared with the other treatment, the least inflammatory reaction, and the most rapid fibroblastic and angioblastic activity in the wound, the fastest laying down of fibrous connective tissue, and the fastest epithelialisation (Miri *et. al*, 2005).

In another study by Ghaderi *et. al*, in evaluating the effects of honey in healing of full thickness skin wound in mice, showed that honey decrease infection, inflammation, edema and dehiscence while increased resilience, ultimate tensile strength and toughness of wound.

The studies of honey did not only compare the effectiveness among the modern medicine but also, there was a study that compared honey with potato peel. Both are naturally occurring substances and can be used as dressing for burns.M. Subrahmanyam in his article entitled ' *Honey dressing versus potato peel in the treatment of burns*' found that honey dressing for burns is non irritating, non toxic, easily available and cheap.

Many more studies have been done and many mores will be published and those are to prove on how honey can be a useful dressing or material in the treatment of burns.

3.0 OBJECTIVES

The objectives of doing this study are:

1) To create full thickness burn wound study model on rats.
2) To evaluate and assess the effectiveness of honey hydrogel dressing compared with hydrogel dressing.

4.0 MATERIALS AND METHODS

4.1 Animal

In this experiment, in order to evaluate the effectiveness of honey when applied in burn wound, animal model is used. Twelve female Sprague-Dawley rats weighing 250-300 grams each were used in this study. They were divided into two groups which received different treatment. Group 1 was treated by hydrogel and group 2 by honey hydrogel. The rats were kept in animal unit at least one week prior to initiation of the study. The rats were given commercial pellet and water *ad libitium* throughout the study. The protocol was approved by faculty's ethics committee.

No model can act as exact replicate of clinical situations. The predominat form of healing in this animal model is by contractions. In this study, we used rats to measure the wound contraction based on the wound size changes. This is because the biological reactions in rats are almost the same as human especially the skin layer.

Another reason is because the easy handling especially for Muslim workers. The small size also makes it easier to handle compared to other animals.

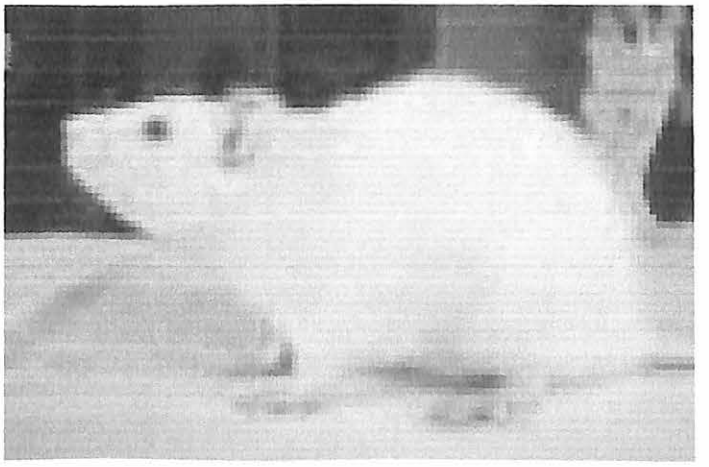


Figure 2: A female Sprague Dawley

4.2 Skin Preparation.

All rats were anesthetized with an intramuscular (IM) injection of ketamine (50mg/kg). Under anesthesia, the back of the body were shaved.

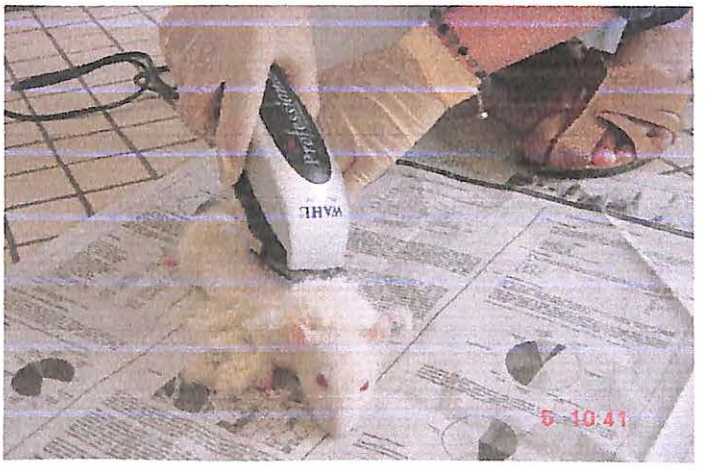


Figure 3: The back area of rats were shaved

4.2.1 Ketamine

Ketamine was used as anesthetize before we apply the hot metal plate at the back of the rats. It can relieve pain and can avoid movement of the rats while applying hot metal plate on their back.

Ketamine may be used in small doses (0.1–0.5 mg/kg/hr) as an analgesic, particularly for the treatment of pain associated with movement and neuropathic pain. It has the added benefit of counter-acting spinal sensitization or wind-up phenomena experienced with chronic pain. At these doses, the psychotropic side

effects are less apparent and well managed with benzodiazepines. Ketamine is a co-analgesic, requiring a concomitant low-dose opioid to be effective.

The effect of ketamine as a depressant on the respiratory and circulatory systems is less than that of other anaesthetics agents. When used at anaesthetic doses, it will sometimes stimulate rather than depress the circulatory system. It is sometimes possible to perform ketamine anaesthesia without protective measures to the airways. Ketamine is also a potent analgesic and can be used in sub-anaesthetic doses to relieve acute pain; however, its psychotropic properties must be taken into account.

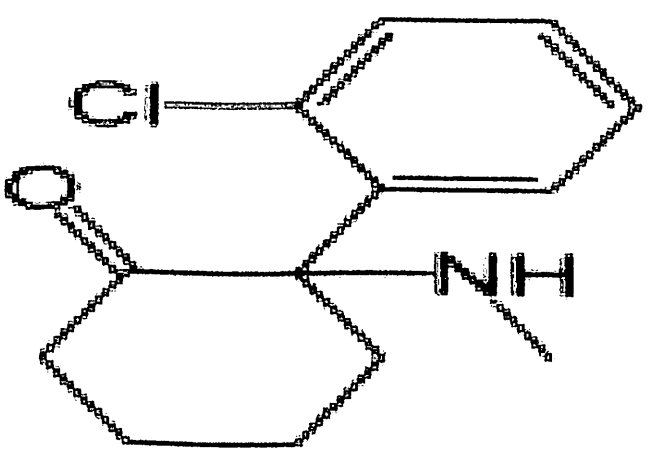


Figure 4: Structure of ketamine

4.3 Thermal Source

A method from the Rozaini et al. was used with modifications. A square metal plate (1.5cmx1.0cm) were heated in boiling water by hot plate at a constant temperature at 85°C for 3 hours prior to inflicting burn areas on the skin of the rats.

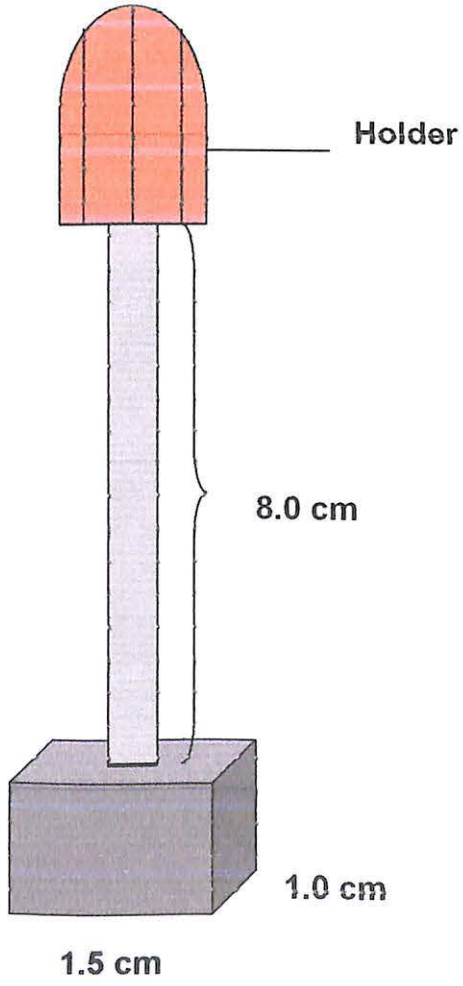


Figure 5: Metal plate for burn creation

4.4 Burn lesions

Rats were again anesthetized with an IM injection of ketamine (50mg/kg). The shaved area was applied with alcohol and iodine to avoid contamination. The procedure was performed in a sterile room like an operating theatre with sterile instruments. The location of burn was marked. Then the hot metal plate was applied to the marked site for 10 seconds to produce third degree burn.

Figure 6: Burn lesion

4.5 Treatment

Subsequently the rats were treated. Group 1 was treated with hydrogel and group 2 received honey hydrogel treatment. Both type of hydrogel were supplied by Malaysian Institute of Nuclear Technology (MINT). Both hydrogel were cut approximately larger than the burn lesions. Then the treated wounds were covered by bandage.

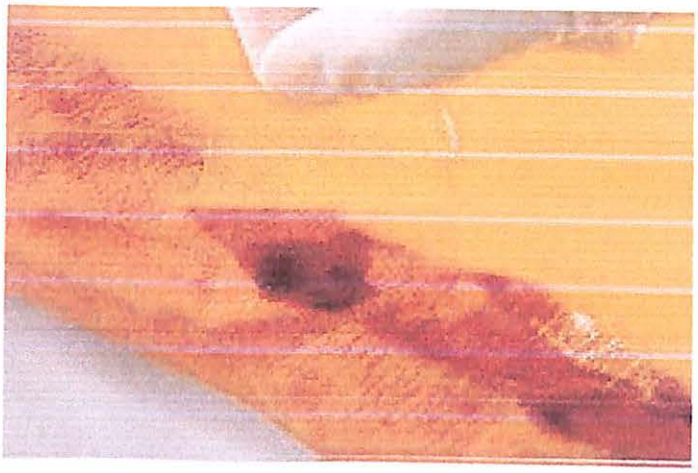


Figure 7: Honey hydrogel

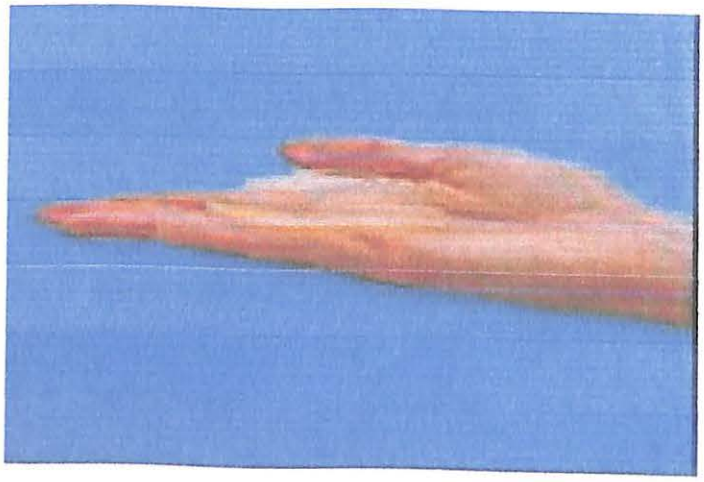


Figure 8: Hydrogel

4.6 Measurement

The burn lesions were measured on the 7th and 14th day using a ruler and the surface area was calculated

4.7 Standardization

In order to assure that there were no external factors interfering our experiment, all the procedures in creating burn was done in a sterile room. All instruments were autoclaved a day before experiment. The rats were also disinfected with alcohol and iodine to avoid contamination that could affect the result.

Figure 9: Rats with burn lesion, ready to be measured

4.8 Statistical analysis

Statistical significance was determined by Repeated Measure (RM) ANOVA employing SPSS Statistical Packages. A repeated measure analysis of variances was applied when the same variables were measured on several occasions for each subject. Types of data required for this study were within subject variable and between subject variable.

5.0 RESULT

Results of burn wound size measurement is shown in Table 1 and 2 and expressed graphically in figure 10 and figure 11

TREATMENT	Surface area (cm^2)		
	Day 0	Day 7	Day 14
GROUP 1 HYDROGEL (CONTROL)	1.5	1.50	0.78
	1.5	0.60	0.30
	1.5	1.50	1.50
	1.5	0.90	0.30
	1.5	1.50	0.45
	1.5	1.50	0.52
GROUP 2 HONEY-HYDROGEL	1.5	1.50	0.39
	1.5	0.50	0.15
	1.5	0.60	0.04
	1.5	0.70	0.12
	1.5	1.50	0.75
	1.5	1.00	0.01

Table 1: Wound size measurement of healing burn wounds

TREATMENT	DAY 7		DAY 14	
	Length (cm)	Width (cm)	Length (cm)	Width (cm)
HYDROGEL (Control)	1.5	1.0	1.3	0.6
	1.2	0.5	1.0	0.3
	1.5	1.0	1.5	0.5
	1.5	0.6	1.0	0.3
	1.5	1.0	1.5	0.3
	0.5	1.0	1.3	0.4
HONEY HYDROGEL	1.5	1.0	1.3	0.6
	1.0	0.7	0.5	0.5
	0.6	0.1	0.1	0.1
	1.1	1.0	0.4	0.3
	1.5	1.0	1.5	0.5
	1.0	0.7	0.1	0.1

Table 2: Length and width of treatments at 7^{th} and 14^{th} day

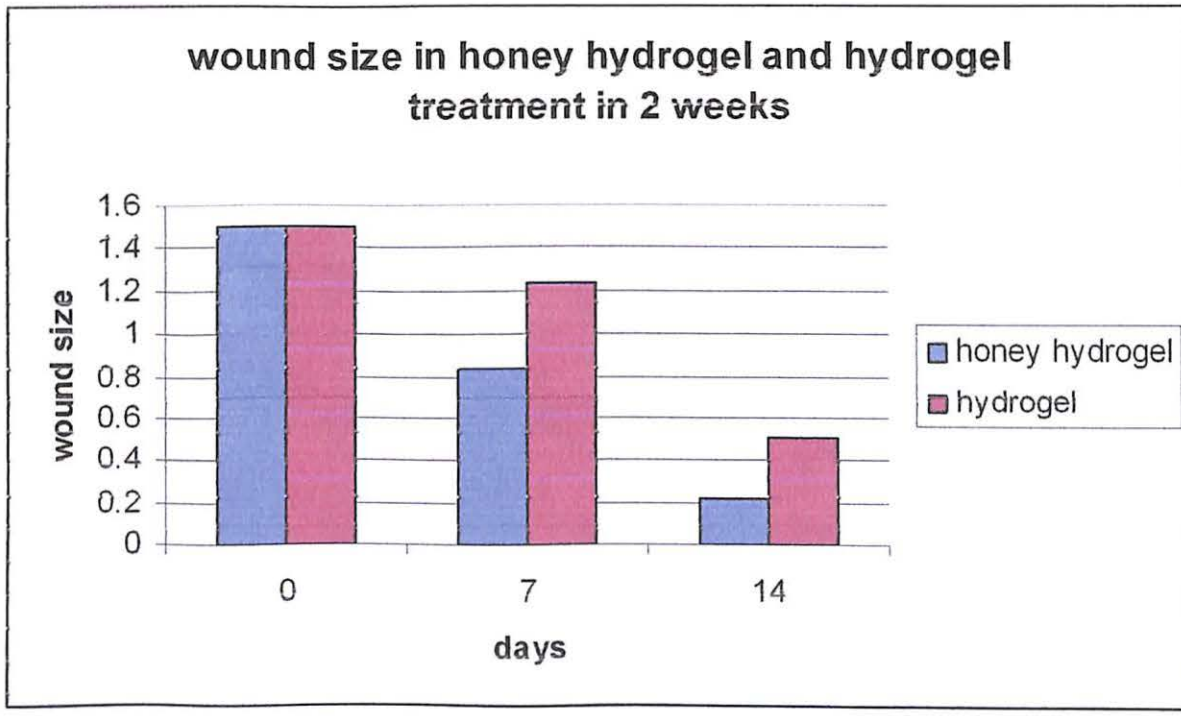


Figure 10: Bar chart of wound healing.

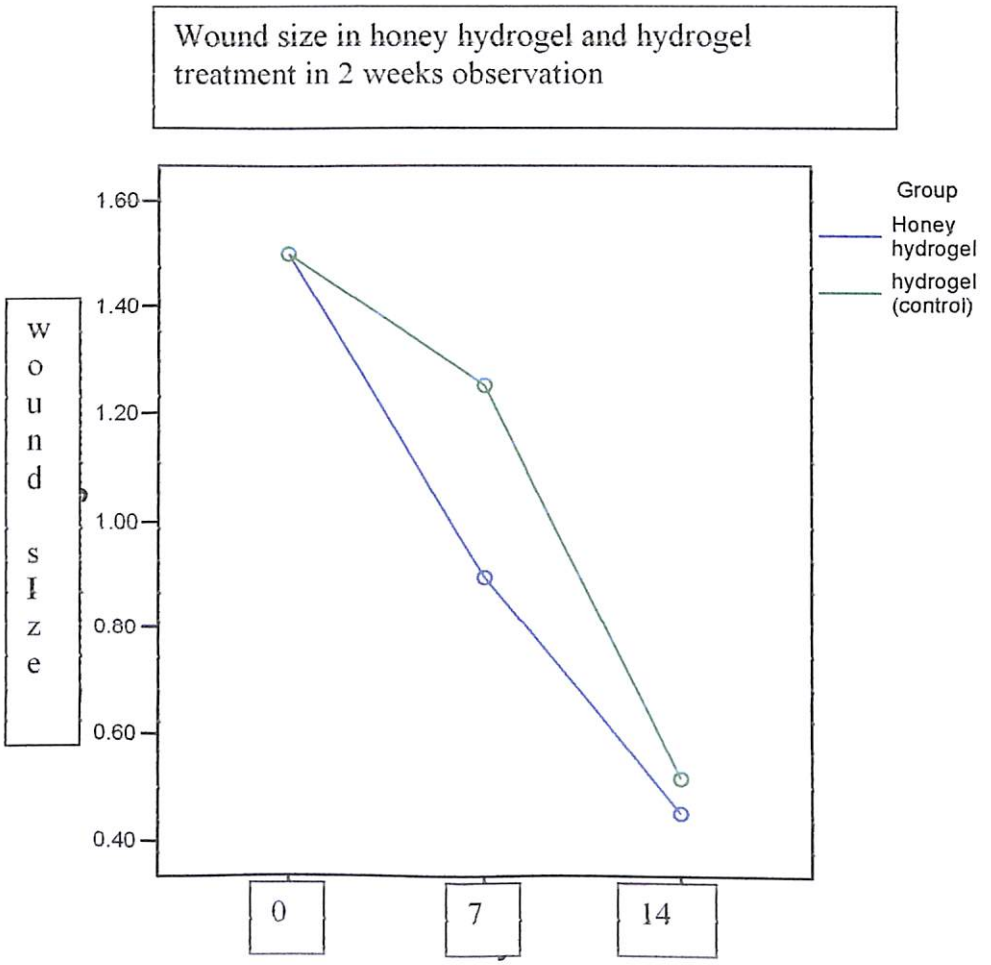


Figure 11: Line graph of wound healing

5.1 STATISTICAL ANALYSIS

Group	Mean (SD)		
Day	0	7	14
Honey Hydrogel	1.500(0.000)	0.8933(0.5771)	0.4517(0.3504)
Hydrogel (control)	1.500(0.000)	1.2500(0.3988)	0.5167(0.2108)

Table 3: Mean and Standard Deviation (SD) of wound healing

Estimated Marginal Margin (confidence interval=95%)						
Group/Day	0		7		14	
	Upper bound	Lower bound	Upper bound	Lower bound	Upper bound	Lower bound
Honey hydrogel	1.500(0.000)	1.500(0.000)	0.442	1.345	0.189	0.715
Hydrogel (control)	1.500(0.000)	1.500(0.000)0	0.799	1.701	0.254	0.780

Table 4: Estimated Marginal Margin (confidence interval=95%)

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.
day	.696	3.255	2	.196

Table 5 : Mauchly's Test of Sphericity

	F stat	F value
Day and Group	1.175	0.329

Table 6: Sphericity Assumed

5.2 INTERPRETATION

This experiment required two weeks observation in determining the effectiveness of honey hydrogel treatment compared to hydrogel as a control. In the first week, which is on day 7, the average length and width for honey hydrogel treatment showed more reduction; 1.12cm length and 0.75 width compared to reduction in hydrogel treated wound that resulted in 1.45 lengths and 0.85 widths. The wound size on day 0 was 1.5cm^2. After group treatment with honey hydrogel, the average size was 0.84cm^2 while in hydrogel treatment, the wound size was 1.23 cm^2. This indicates that, during the first week honey heals the wound faster.

The wound size was measured again one week later. On day 14, the wound size was 0.22cm^2 (0.65cm x 0.34cm) in honey hydrogel treated group and 0.51cm^2.in hydrogel treatment.

The difference between wound size after treatment with honey hydrogel and hydrogel showed clearly that honey can be an effective treatment because of its contents that support wound healing.

The bar chart (figure 10) shows the different of wound size in honey hydrogel treatment and hydrogel treatment. The wound size of both groups was 1.5 cm^2. On day 7, honey hydrogel showed contraction to an average size of 0.9cm^2 and in hydrogel group, the average wound size was 1.3cm^2. On day 14, the size in honey hydrogel group contracted into 0.24cm^2 and hydrogel group was 0.64cm^2.

The line graph on figures 11 also showed the differences of wound size in the honey hydrogel group and hydrogel treatment. On day 0, the wound size was the

same, 1.5cm^2, so that both treatment lies on the same dot. The pattern begin to change on day 7 when honey hydrogel caused contraction that lead to the decrease of wound size more than the hydrogel .On day 14, both group showed rapid contraction but honey hydrogel resulted in faster healing.

Another method to demonstrate the difference is by statistical analysis using repeated measure (RM) ANOVA. The statistical analysis is shown in Table 4, 5 and 6. From the Mauchly's the value of 0.196 is not significance so we proceeded to the sphericity assumed test. Basically the assumed of sphericity means that the correlation of treatment levels is the same. If the Mauchly's test is less than 0.05, the test is violated but if the value is more than 0.05, the sphericity is met. In this study, according to RM ANOVA, there was no difference between treatment by honey hydrogel or hydrogel alone; however this is not the final result in view of other limitations of the study.

6.0 DISCUSSION

The purposes of this study were to create full thickness burn wound model on rats and to evaluate honey hydrogel as a dressing in comparison with hydrogel. There have been many types of burn models that are used for examining wound healing and the systemic response to thermal injury. Both partial thickness and full thickness models have been developed. The determination of thickness depends on the usual determinants of burn depth; temperature of contacting agent, duration of contact thickness of skin and blood supply. The partial thickness burn model must be of lower temperature, shorter duration and on thicker skin while the full thickness needs high temperature approximately at boiling point, longer duration (approximately 10 seconds) and applied on less thick skin. The burning agent can be scald, flame or heated piece of metal (usually placed in boiling water) to produce a contact burn. The burn wound heals by tissue repair.

There are several in vitro model of tissue repair. It is extremely important to remember that no one model is an exact replicate of the clinical situation. A key factor in choosing a model is to determine which form of healing is to be investigated. Tissue repair is divided into three main components; creation of a scar, contraction and re-epithelialization. Literally thousands of publications using mice for wound healing experiments. There are many differences in the healing of these small rodents when compared to humans. The predominant form of healing in mice is by contraction. In vitro model are designed to answer specific questions involving one or two cell types in response to one or few stimuli. There are many

factors that influence tissue repair such as extracellular matrix, multiple cytokines and growth factors, pH, oxygenation, temperature, nutritional status and overall health.

Animal models are essential to examine factors that influence healing in this complex environment. Mice heal burn wounds by contracting the wound and laying down granulation tissue while mouse and rats model is used to investigate the enhancement of contraction in a burn wound. There are many aspects of healing such as the formation of granulation, collagen deposition, re-epithelialisation and contraction. Different depths of an open wound as burn wound examine different types of healing. Partial thickness wounds focus on re-epithelialization. These wounds need to be created in thick skin animal. The porcine model is used most often. The re-epithelialization was measured subjectively or by measuring the wound surface electrical capitance or rate of water evaporation to give a functional measurement of creation of a new epithelium.

In this study, rats were used and the measurement was by contraction. Although contact burns create small burns, larger burns usually are created by scalding or flame injury. In this model, a metal plate was placed to limit the size of injury and lead to relatively standard sized burns.

After creating burn wound, we need to cover the wound with the material called a dressing. There are many types of dressing; biological, synthetic, conventional and alternatives dressing. Honey acts as alternative to modern therapy for burns. The honey used in this study was prepared in hydrogel form whereby 6% water content of hydrogel was replaced by honey. Many studies had compared the effectiveness

of honey in wound healing with other modern treatment. The beneficial effect of honey include the cleansing of wounds, absorption of edema fluids, antimicrobial activity, promotion of granulation tissue, epithelialisation and improvement of nutrition. Hydrogel acts as control and is important advanced second generation wound care products that have high water contents (up to 96%) which are a useful wound dressing. When honey combine with hydrogel, honey hydrogel is produced as a most potential wound dressing especially for burn wound. Twelve rats were divided into two groups that received honey hydrogel treatment and hydrogel respectively. The wound size was used as parameters to measure the effectiveness in healing process of both honey hydrogel and hydrogel treatment. The first reading taken was immediately after the burn lesions created and it formed the baseline of wound size which was 1.5cm x 1.0 cm. The wound size was measured again on day 7 and day 14. On day 7, there was not much different between the contractions for both treatment and average size was 1.1cm x 0.9 cm in honey hydrogel treatment and 1.45cm x .0.9 cm in hydrogel treatment. But on the 14^{th} day, there was evidence that honey hydrogel has superior healing properties. In the honey hydrogel treatment group, the average wound size was 0.65cm x 0.32 cm while in hydrogel was 1.3cm x 0.5 cm. Based on the wound contraction of both group, there was clear indication that honey hydrogel with its unique ingredients can heal the wound especially the burn wound faster compared to hydrogel alone.

Burn wound healing is the new research in Hospital Universiti Sains Malaysia (HUSM). Since this is a new research, there are some limitations that could affect the result. These include instrument and the methods that are used.

The main limitations are the instrument in creating burn. As the study model involves full thickness burn, the first issue need to be addressed was the technique in creating a consistent actual third degree burn. Different study used different methods but the most applicable methods was using plate that can be boiled in water at high temperature.

After discussion, it was decided to use hot metal plate but we have to design it first. The 1.5 cm x 1.0 cm square plate is a newly designed plate with a holder and the effectiveness in producing a consistent burn through the layer skin of rats need to be verified.

The other limitation is when creating the burns at the back of rats. According to the procedure, in order to standardize the experiment, the hot plate must be applied on rats without pressure. However since the design of the plate that cannot standby itself, we need to hold it and sometimes the rat pressed itself against the plate particularly when the rats moved. There is also difficulty in using honey in its conventional form, but when incorporated in hydrogel, was more effective application. The honey hydrogel proved to be an effective mean of delivering honey as a moist wound dressing.

7.0 SUMMARY AND CONCLUSIONS

Burn wound healing is essential for any organism to survive injury. The increased number of burn wound causes leads to research and development in the effectiveness of wound dressing. One of most interesting dressing today is the ancient medicine namely the honey. Honey has a long history of traditional use in healing of burns and other wounds. In vitro studies demonstrate that honey possess antibacterial properties by virtue of their physiochemical properties. A number of clinical studies have shown that honey can reduce infection, accelerate overall healing, and also may reduce wound inflammation in a variety of wound. Our findings conclude that the burn wound healing in term of wound contraction is more effective using honey hydrogel treatment compared to hydrogel. More research is required to determine the types of wounds that response the best to honey.

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8.0 APPENDICES

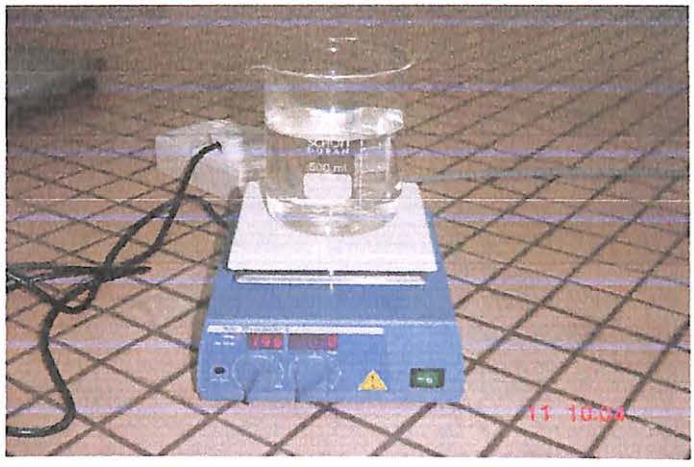

Figure 12: Hot plate

Figure 13: Burn Lesion