

Honey Hydrogel Dressing to Treat Burn Wound in Rats - A Preliminary Report

Rozaini Mohd. Zohdi¹, Md. Zuki Abu Bakar@Zakaria^{1,3*}, Norimah Yusof⁴,
Noordin Mohamed Mustapha¹, Muhammad Nazrul Hakim Somchit²
and Asnah Hasan³

¹Faculty of Veterinary Medicine,

²Faculty of Medicine and Health Sciences,

³Laboratory of Molecular Biomedicine, Institute of Bioscience,
Universiti Putra Malaysia, 43400 Serdang,
Selangor Darul Ehsan, Malaysia

⁴Division of Agrotechnology & Bioscience,
Malaysian Nuclear Agency, Bangi, 43000 Kajang,
Selangor Darul Ehsan, Malaysia

*E-mail: zuki@vet.upm.edu.my

ABSTRACT

Various studies have shown that honey is effective in healing burns and wounds. In this study, Malaysian honey was incorporated into hydrogel dressing formulation using electron beam irradiation technique and introduced as Honey Hydrogel dressing. The wound healing efficacy of Honey Hydrogel dressing on deep partial thickness burns was monitored on the basis of gross appearances, rate of wound contraction and histopathological changes. Deep partial thickness burns were created by applying an aluminium template preheated to 85°C to the backs of rats for 5 s and randomly treated with Honey Hydrogel or hydrogel while control group received no treatment. Wound appearance was photographed and the rate of wound contraction was calculated at 7, 14, and 21 days post burn. Rats were euthanized after 21 days of treatment and skin samples were taken for histopathological examination. The wounds treated with Honey Hydrogel dressing showed better gross appearances and significantly ($p < 0.05$) enhanced the rate of wound contraction as compared to the control group at 21 days post burn. Faster epithelialization was also seen in the Honey Hydrogel treated group as compared to the other groups, although this was not statistically significant. The results substantiate the potential efficacy of Honey Hydrogel in accelerating burn wound healing.

Keywords: Honey, hydrogel, burn wound healing, wound contraction, rats

INTRODUCTION

Major injuries such as in thermal burns resulting in extensive damage to the skin necessitate immediate coverage to aid repair and regeneration to restore normal skin function (Cuttle *et al.*, 2006). Different therapies that affect burn wound repair have been proposed over the last few decades due to technology and strategic advances in the biomedical field

(Branski *et al.*, 2009). Recently, however, there has been a surge of interest in the use of alternative therapies and natural remedy among modern societies, mainly due to its potential to improve healing and reduce the financial burden at the same time (Salmah & Sidik, 2005; Davis & Perez, 2009).

Honey is the nectar and saccharine exudation of plants gathered, modified and

*Corresponding Author

stored by the honey bee (Molan, 2000). It is one of the oldest and most enduring materials to be used in managing wound (Molan, 1998). It may be used alone or in combination with other substances (Salmah & Sidik, 2005) and has been administered both topically and systemically (Suguna *et al.*, 1992). Much of the effectiveness of honey in many of its medicinal uses is attributed to the antibacterial activity and antioxidant properties (Molan, 1999).

Topical application of Malaysian honey has also been reported to be effective to treat burn and wound in rats (Rozaini *et al.*, 2005; Aljady *et al.*, 2000). However, rapid clearance from the wound site occurred as honey tends to flow out from the wound area, making it difficult to maintain therapeutic concentration over prolonged period of time. In order to further enhance the use of honey in wound management and for easy handling, local honey was incorporated into hydrogel dressing formulation, cross-linked and sterilized using irradiation technique. The present study reports on the preliminary evaluation of Honey Hydrogel dressing in improving the outcome of burn wound by monitoring the morphological changes of the burn wound tissue healing after the treatment with Honey Hydrogel.

MATERIALS AND METHODS

Honey Sample

Local monofloral *Apis mellifera* honey from the floral source of *Melaleuca spp.* (Gelang) trees was used in this study. The honey was supplied by the Department of Agriculture Malaysia through Malaysian Nuclear Agency (Nuclear Malaysia) and was irradiated with 25 kGy gamma irradiation using radioactive source Cobalt 60, with the dose rate of 2 kGy per hour for sterilization purposes at MINTec-Sinagama, Malaysian Nuclear Agency (Model JS 8900). Dosimetry was performed using ceric/cerous sulphate solution and analyzed by potentiometrics.

Preparation of Honey Hydrogel Dressing

Polyvinyl pyrrolidone (PVP) with a molecular weight of 1.2×10^6 (Kollidon 90) and Polyethylene glycol (PEG) with molecular weight of 400 were obtained from BASF, Ludwigshafen, Germany. Technical grade agar was supplied by Oxoid. The mixture, containing 15% PVP (Kollidon 90), 1% Protein Free Agar solution, and 1% PEG, was added with 6% honey (Yusof *et al.*, 2007). The mixture was poured into plastic molds (5 cm in diameter; 3-4 mm in thickness), and left to set at room temperature (37°C) before it was covered with polyethylene sheet and individually packed. The gels were cross-linked and sterilized by electron beam at 25 kGy at Alutron Irradiation Facility, Malaysian Nuclear Agency (Model EPS-3000, conveyer speed of 4.4m/minute, beam current of 10mA and energy of 3MeV).

Study Design

A complete randomized design was used to determine the efficacy of Honey Hydrogel dressing to treat deep partial thickness burns in rats. The experimental protocol was approved by the Animal Care and Use Committee (ACUC) at the Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM) (Reference No: 08R36/ July 08-Jun09).

Animals

In this study, a total of 18 male Sprague-Dawley rats (weight 200-300 g) were used and they were randomly divided into three experimental groups of 6 rats each. The sample size was designed to minimize the number of animals required, which was still adequate to generate statistical analysis. The animals were acclimatized to the laboratory conditions for one week prior to the onset of experiment. The rats were individually caged and given commercial pellet and water *ad libitum* throughout the study.

Skin Preparation

Rats were anaesthetized with an intramuscular (IM) injection of ketamine (50 mg/kg) and xylazine (5 mg/kg) into the caudal thigh muscle. Under anaesthesia, the back and flank of both sides of the body were shaved. Following this procedure, rats were returned to their cages for 24 hours to allow any oedema caused by the shaving procedure to recede.

Thermal Source

A method described by Kaufman *et al.* (1990) was used with modification. Cylindrical aluminium templates (2.5 cm diameter × 3 cm length, a handle measuring 24 cm, and total weight 400 g) were heated in a water bath at a constant temperature of 85°C for 3 hours prior to inflicting burn areas on the skin of the rats. Five templates were heated simultaneously, used alternately, and then returned to the water bath to ensure maintenance of the desired temperature of the template surface. There was approximately 5 minutes elapsed between each use of a template.

Burn Lesions

Rats were again anaesthetized with an IM injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). The anaesthetized rat was positioned in sternal recumbency, restrained and stretched on a metal stage. The location of the burn was marked between the last ribs and the horizontal line of the sacroiliac joints. Deep partial thickness burn was inflicted on the dorsal part of the rat between the last thoracic vertebra and the first sacrum by placing the heated and moistened template at the right angles perpendicular to the dorsum of the rat on the pre-marked location for 5 seconds, using an analogue stopwatch. Minimal and constant pressure was applied to ensure a perfect contact between the template surface and the skin. The shaved skin was smoothened to ensure sufficient contact and uniform pressure over the entire lesion.

Treatment Protocol

Approximately 15 minutes after wound creation, all wounds in the treatment groups were dressed with Honey Hydrogel or hydrogel followed by OpSite® film dressing (Smith and Nephew, Hull, England) as secondary dressing. The dressings were held in place by wrapping the whole trunk with sterile gauze and plastered with Leukoplast® (BSN Medical, Pinetown, SA). Leukoplast® was applied to firmly affix the dressings to the animal's skin. Every 7 days, wounds were redressed with fresh hydrogel or Honey Hydrogel, while the rats were under anaesthesia. The secondary dressing and the hydrogels were removed and the wounds were flushed with sterile saline to remove debris and to clean the wound area. Sterile techniques were utilized when changing the dressings to minimize infection by pathogens to the wound site. Once the wounds have been analyzed, fresh dressings were placed on the wounds. Control group did not receive any treatment.

Assessment of the Wound

The progress of burn wound healing was recorded at 0, 7, 14 and 21 days post-burn. All the wounds were digitally photographed in the presence of a standard reference ruler. The wound area was measured immediately by placing a transparent tracing paper over the wound and tracing it. The tracing paper was placed on a 1 mm² graph sheet and traced accordingly. The squares were counted and the area was recorded. The wound area was assessed by the same blinded observer.

Histopathological Analysis

Rats were euthanized at day 21 post-burn by halothane inhalation and the skin samples were taken for histopathological examination. The skin samples were fixed in 10% formalin solution and embedded in paraffin. Tissue sections of 4-5 µm thickness were cut, stained with haematoxylin and eosin (H&E), and examined under light microscope. Digital photomicrographs were

captured at representative locations using a digital camera attached to a Nikon Eclipse FX-35DX microscope.

Statistical Analysis

Data are expressed as mean ± Standard Deviation (S.D). The statistical analysis of data was performed using two-way ANOVA using the SPSS® Statistical package (SPSS, Version 10.0, Chicago, Illinois, USA). The effects with P<0.05 were considered statistically significant.

RESULTS

Measurement of the Wound Size

On days 7 and 14 post-burn, there were no significant differences (p>0.05) observed in the wound area measurement between all the experimental groups (Table 1). On day 21 post-burn, nevertheless, the wound area measurement showed a significant (p<0.05) reduction in the wound size of the treated group as compared to the control with Honey Hydrogel dressing, showing the lowest mean of the wound size (60.8±2.2), followed by the hydrogel treated wound (80.8±2.6). In addition, the wound size of the untreated control group was significantly



Fig. 1: General appearance of the wound sites in all the experimental groups at day 7 post-burn. Burned skin started to exhibit moist, intact, soft and supple yellow-brown discoloration in the treated groups, while the control group exhibited dry, intact, light-dark brown discoloration.

TABLE 1
Measurement of the wound sizes (mm²) of the control and the treated groups at 0, 7, 14 and 21 days post-burn. Results are shown in mean ± standard deviation (S.D). *P<0.05 compared with the control group.

Groups	Measurement of the wound area (mm ²) (mean±S.D)			
	0	7	14	21
Control	430.4±3.26	400.4±3.2	285.6±1.3	149.6±3.6
Honey Hydrogel	427.2±1.03	388±1.33	258.8±5.4	60.8±2.2*
Honey	423.3±2.23	388.0±3.1	272.3±1.7	80.8±2.6*

($p < 0.05$) higher at day 21 post-burn with $149.6 \pm 3.6 \text{ mm}^2$. No significant difference was observed in the treated groups at 21 days post-burn, although there was a trend towards improvement in the Honey Hydrogel treated group.

General Appearance of the Wound

Gross changes in the general appearance and the size of burn wounds were monitored at 7, 14 and 21 days post-burn by capturing digital images of each animal. Towards the end of the first week (day 7 post-burn), the wounds in both the treated groups exhibited moist, soft and supple, yellow-brown discoloration, with red rim around the lesion, while the control group was covered with dry scab (*Fig. 2*). After 14 days, the treated groups still exhibited moist appearance with soft, yellow-brown lesions, while the control wounds were covered with dry, intact, dark brown-coloured scab (*Fig. 3*). The best healing was seen in Honey Hydrogel treated wounds after 21 days of post-burn with the wounds getting considerably smaller, while the control wound still exhibited dry appearance with dark brown scab (*Fig. 4*).

Histopathological Study

After 21 days of injury, epidermal regeneration was observed in all experimental wounds. Histopathologic comparisons showed that on day 21, Honey Hydrogel treated wounds resulted in better re-epithelialization as compared to the control and hydrogel treated rats. In addition, the inflammatory cells were absent in both treated wounds. In the untreated control wounds, though new epithelium was noted to regenerate, inflammatory cells particularly neutrophils and macrophages were still present on the upper dermis. Less scab formation was seen in the wounds treated with Honey Hydrogel as compared to the hydrogel and untreated control wounds (*Fig. 5*).

DISCUSSION

Burn wounds lead to a loss of integrity of the skin and have complex healing process. Wound healing proceeds through an overlapping pattern of events consisting of inflammation, proliferation and tissue remodelling. A number of studies have indicated that honey is a potential agent for wound healing, mainly due to its antibacterial, anti-inflammatory and antioxidant

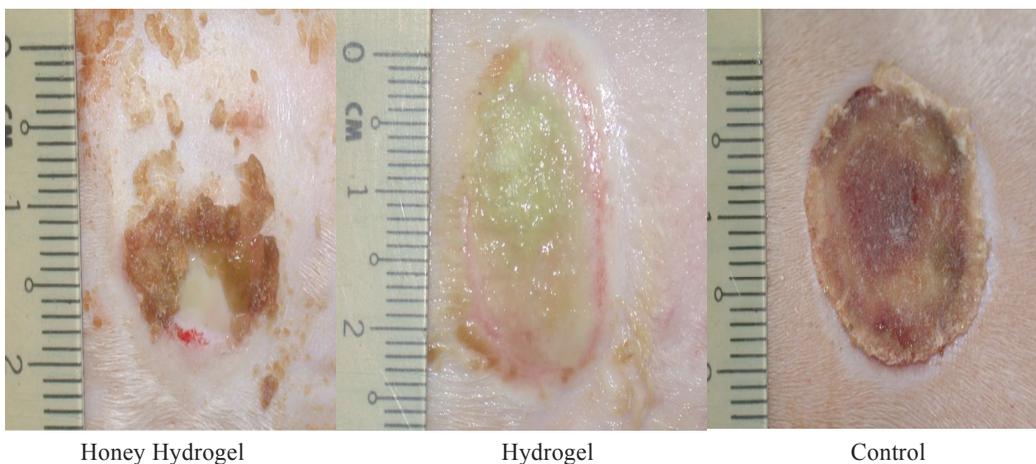


Fig. 2: General appearances of the wound sites in all the experimental groups at day 14 of post-burn. The wound treated with Honey Hydrogel showed better healing with scab started to slough off while hydrogel treated wound exhibited moist appearance with soft, yellow-brown discoloration. However, the untreated control wound was covered with dry, intact, dark brown discoloured scab.

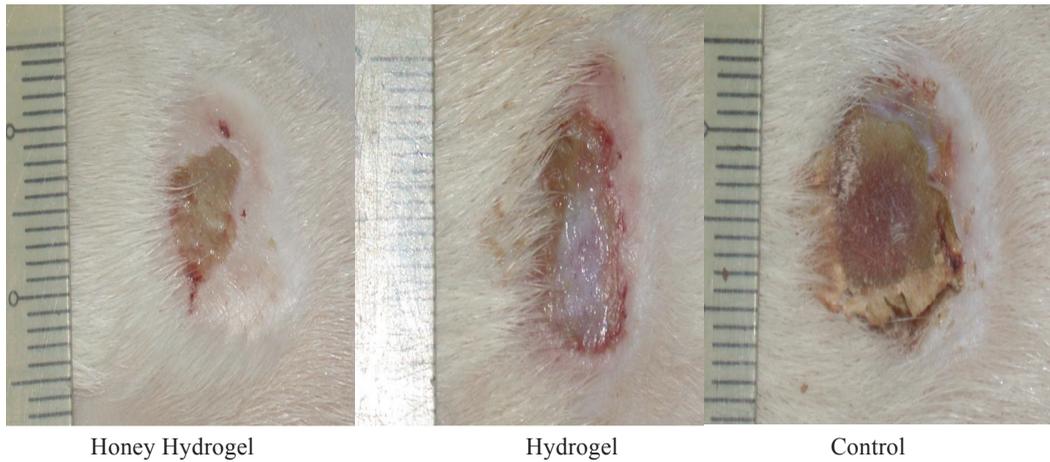
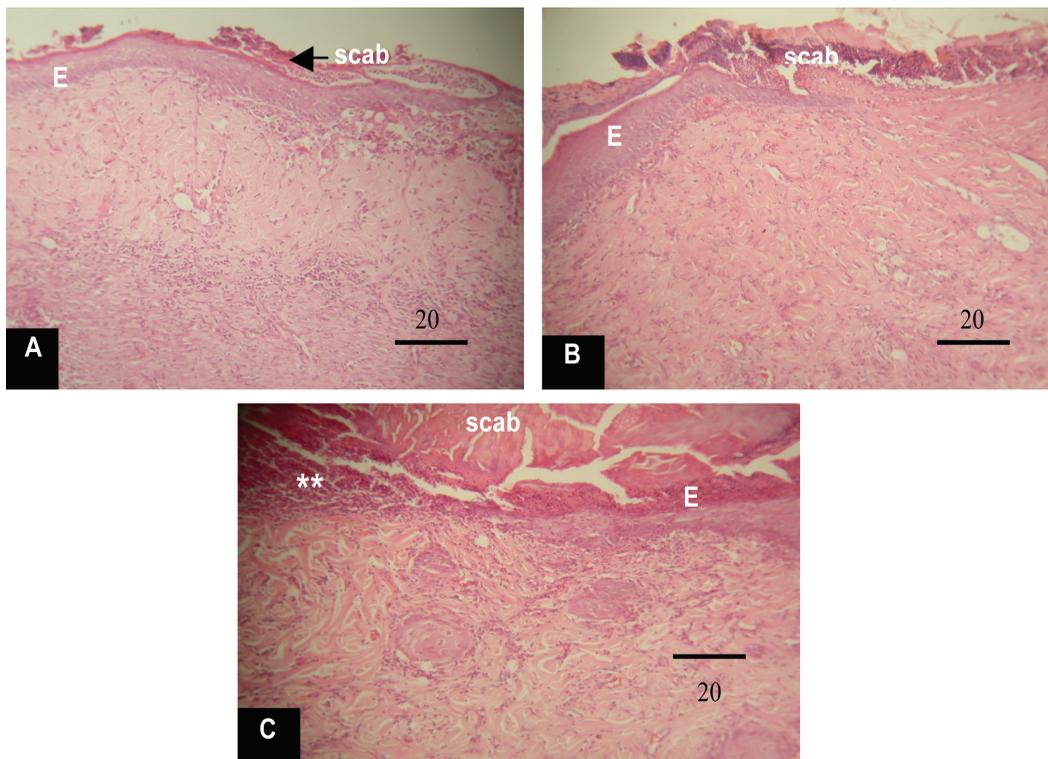


Fig. 3: General appearance of the wound sites in all the experimental groups at day 21 post burn. Better sign of healing in the wound treated with Hydrogel Honey with wound getting considerably smaller. However, the control wound still exhibited dry appearance with dark brown scab.



*Fig. 4: Representative micrographs of the histological sections at 21 days of post-burn stained with H&E. Note the advanced epidermal regeneration in the wound treated with (A) Honey Hydrogel. Marked inflammatory response still persists in (B) hydrogel (C) control group. (E=epidermis, **=inflammatory cells; 40x mag.). Bars on the photomicrograph represent 20 μm.*

properties (Molan, 2000; Subrahmanyam, 1998). Recent advances in radiation have resulted in the ability to incorporate honey into the hydrogel matrix for better handling. The present study showed that topical application of Honey Hydrogel accelerated the rate of wound healing as demonstrated by the increased rate of wound closure and better cosmetic appearances. Meanwhile, the histopathological evaluation of the wound site also provided evidence that Honey Hydrogel stimulated the healing process by reducing the inflammatory response and enhancing re-epithelialization.

One of the goals of wound therapy is to reduce excess inflammatory responses (Cho *et al.*, 2003). Honey has been reported to reduce inflammation when it is applied to wounds (Subrahmanyam, 1998); its anti-inflammatory activity may be associated with the antioxidant property of honey which is responsible to scavenge free radicals involved in various aspects of inflammation. Aljadi and Kamaruddin (2004) reported that honey has antioxidative and radical scavenging properties, which are mainly due to its flavonoids and phenolic constituents.

Re-epithelialization was also found to have remarkably advanced in the Honey Hydrogel treated wounds as compared to other groups. Meanwhile, the nutrient contents of honey, such as laevulose and fructose, improved the local substrate supply and helped in promoting epithelialization and tissue growth (Subrahmanyam, 1998). The application of Honey Hydrogel also kept the wounds moist and it has been proven that keratinocytes migrated more easily over a moist wound surface than that underneath a dry scab (Winter and Scales 1963). Furthermore, Honey Hydrogel dressings may be clinically easier to use, maintaining shape and consistency on or within the wound cavity.

CONCLUSION

The current study has shown that Honey Hydrogel is effective in healing burns, although not statistically more effective than hydrogel. Nonetheless, perhaps statistical significance would be reached if the study period was

prolonged and the wounds had been followed longer, as there was clearly a trend to greater improvement in the Honey Hydrogel treatment compared to hydrogel. Further studies on the cellular and molecular mechanism of Honey Hydrogel were carried out.

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