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Accelerated wound healing of physically cross linked gellan gum-virgin coconut oil hydrogel containing Manuka honey

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This study examined the effect of honey in gellan gum (GG) hydrogel containing virgin coconut oil (VCO). Their mechanical, physical, thermal properties and *in-vivo* healing potential on dermal wounds were investigated. The compression performance results show that the inclusion of honey into gellan gum incorporated VCO (GGVCOH) hydrogel improved the compressive stress of the materials by 3-fold and workable to be applied on the different contours of human body. Swelling ratio of GVCO hydrogels increased upon addition of honey, and water transmission rates (WVTRs) values of all hydrogels were in the range of 112-132 g m^{-2} d^{-1}, in which comparable to WVTRs values of commercial wound dressings. Thermal behavior shows the inclusion of honey in GVCO hydrogels improved the thermal stability particularly at high concentration. *In-vivo* healing on dermal wounds exhibits that the inclusion of honey accelerated the wound closure and shows complete neo-epidermal of the wounds. The GVCOH hydrogel has shown promising results to treats acute wound treatments.

Keywords: Gellan gum, Manuka Honey, Biocompatibility, Hydrogel

I. INTRODUCTION

Biopolymers are receiving greater attention than synthetic petrochemical-based polymers due to the environmental concerns. A variety of renewable biopolymer such as gellan gum (GG) derived from bacterium *Pseudomonas elodea* have been studied in the development of wound dressings. GG is approved by the United States Food and Drug Administration (US FDA) and the European Union (EU) for use in the food industry and some emerging scaffold materials for tissue engineering application. For instance, GG has been studied for biomedical application as it has the potential to be used as matrices to repair and regenerate a wide variety of tissue and organ [17]. The development of GG hydrogels re-

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ceives a lot of attentions due to the exceptional properties which meet the quintessential prerequisites of ideal wound dressings. A few studies have observed the good biocompatibility of GG against human skin fibroblasts cells (CRL-2522) [9], human fetal osteoblasts (hFOBs 1.19) [29] and rat bone marrow cells (rBMC) [26].

In wound dressing application, GG has shown promising results to proliferate the cell growth [2]. Although GG has been reported to biocompatible on live cells, researchers are keep working to improve the proliferation rate of GG, for example by forming a complex gel with carboxymethyl chitosan [28] and incorporated with branched polyethyleimine nanoparticles [6].

Honey and virgin coconut oil (VCO) are well-known substances to threat the wounds and burns throughout the ages. Commonly, the healing or other properties of honey and VCO are studied in its pure state, i.e. in liquid form. Numerous studies have been reported the advantages of honey such as the anti-bacterial, anti-inflammatory, anti-viral and anti-oxidant effects [12][27][31]. Meanwhile, VCO offered an antimicrobial, anti-inflammatory and anti-pyretic activities [8][25]. Besides numerous studies of using pure honey and VCO have been used to treat wound and burns, limited studies have been reported to incorporated honey or VCO as a composite with bio-polymer, i.e. transforming honey or VCO with biopolymers to a film or hydrogels. A few studies have been reported to use chitosan and gelatin incorporated with honey as a sheet [30], and some using polyvinylpyrrolidone (PVP) entrapped with honey to produce hydrogels [32]. Other studies using alginate, chitosan and gelatin to produce hydrogel with an addition of honey [11]. To the best of our knowledge, no single study has been conducted to produce gellan gum hydrogels incorporated with honey and virgin coconut oil. This study fabricated and characterized the gellan gum hydrogels incorporating honey and VCO. The mechanical characteristics, water vapor transmission rates, swelling and thermal behaviors of the hydrogels were investigated. The biocompatibility was examined through *in-vivo* healing potential, ultrasound imaging and histological evaluation in the rats.

II. EXPERIMENTAL SECTION

A. Materials

Low-acyl gellan gum (Kelcogel, batch no: 5C1574A) were obtained from CPKelco, Chicago, IL, USA. Glycerin (product number-G2289), anhydrous calcium chloride, $CaCl_2$ (product number-C5670), Tween80 (product number-P1754) and Triton X-100 (product number-T9284) were obtained from Sigma Aldrich, St Louis USA. Virgin coconut oil (product number-VCO0216) are obtained from Phyto Biznet Sdn Bhd, Skudai, Johor, Malaysia. Manuka Honey (Batch-F6B, product code NZ107) were obtained from NZ health

Naturally Ltd. Auckland, New Zealand. All materials were used as received without further purification.

B. Hydrogel Formation

The gellan gum (GG) hydrogels were prepared via evaporative casting method. The GG solution was prepared by dissolving 1.75% (w/v) of GG in 100 mL deionized water ($18M\Omega$), followed by glycerin at 50% w/w with continuous stirring for 2 h at 80 C. Gellan gum-VCO (GVCO) solution were prepared by incorporating 5mL of stabilized VCO microemulsion (consisting VCO: water at 80:20) into GG solution and stirred for 20 min at 80°C. Then, GVCO incorporated with honey (GVCOH) were prepared by adding honey at different concentrations, i.e. 5, 10, 15 and 20 mL and later known as GVCOH5, GVCOH10, GVCO15 and GVCOH20 hydrogels, respectively. The GVCOH solutions were deposited onto petri dishes (90 mm x 15 mm) and dried at room temperature (24°C) for 24 h. Prior to any characterizations, the hydrogels were pre-conditioned and left in room temperature for another 24 hours.

C. Swelling Test

The swelling properties were determined according to the ASTM standard test methods for One-Dimensional Swell (D4546-08). Swelling was measured by weighing dried hydrogel (W_{dry}) with dimensions of 20 mm x 20 mm prior to immersion into 50 mL phosphate buffer solution of pH 7.2 at 37±0.5°C. The hydrogel was removed after 24 hours, gently wiped the hydrogel with tissue to expel the surface water and weighted (W_{wet}). Water uptake (%) was determined according to the equation below:

$$Wateruptake(\%) = ((W_{wet}/W_{dry})/W_{dry})) \times 100 \quad (1)$$

where W_{dry} and W_{wet} are the initial weight and final weight, respectively. A minimum of three independent measurements were obtained per sample.

D. Gel Fraction

The analysis of the gel content of the gellan gum hydrogels was performed by cutting sample into 20 mm x 20 mm and dried at temperature, T = 50°C for 6 hours. After drying, the gellan gum hydrogel was weighed (W_1) and then swelled in 20 mL deionized water at room temperature for 24 h. After removing the wet hydrogel from the solution, it was dried in an oven for another 6 h at 50°C and then weighed again (W_2). Gel content of the hydrogel was calculated using the following equation:

$$Gel\ \ content(\%) = (W_2/W_1) \times 100 \quad (2)$$

E. Compression Test

Mechanical characterization of hydrogels was carried out using Instron Universal Mechanical machine (model 3366) at the cross-speed set at 10 nm/min. Hydrogels were cut into cubes (20 mm x 20 mm x 5 mm) for characterization and were done in triplicate for each sample.

F. Water Vapour Transmission Rate

The water vapour transmission rates (WVTRs) was measured by following a modified ASTM International standard method. The hydrogels were cut into (30 mm x 30 mm) and fixed on the circular opening of a permeation bottle with the effective transfer area (A) of 1.33 cm^2. The permeation bottle was placed in the desiccator containing silica gel at room temperature. The WVTR was then determined by measuring the rate of change of mass (m) in permeation bottles at exposure time of 24 h using equation as follows:

$$WVTR = (m/A \triangle t) \tag{3}$$

where, $m/\triangle$ t is the amount of water gain per unit time of transfer and A is the area exposed to water transfer (m^2).

G. Thermogravimetric Analysis

Thermogravimetric analyses were performed on a Pyris 6, Perkin-Elmer-TGA6. Hydrogel samples were analyzed in platinum pans at a heating rate of 10°C/min to 900°C in an atmosphere of N_2 at flow rate of 50 mL/min. Sample used was approximately 10 mg.

H. Differential Scanning Calorimetry

Differential scanning calorimetry was carried out using Pyris 6, Perkin-Elmer-TGA7. Hydrogel samples were analyzed in platinum pans at a heating rate of 10°C / min to 350°C in an atmosphere of N_2 at a flow rate of 50 mL/min. Sample used was approximately 4 mg.

I. *In vitro* assessment

J. *In vivo* wound healing experiments

K. Animals

In this study, a total number 20 of six-week-old female Sprague-Dawley rats with range of body weight from 200g - 250g were used. They were randomly divided into four experimental groups of 5 rats each. The sample size was designed in order 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. All rats were individually caged with 12-hour light/dark cycle, given adequate commercial pellets and water *ad libitum* throughout the study. All animal experiments were carried out under protocols ap-

proved by the Animal Ethic committee (AEC), Universiti Malaysia Terengganu.

L. Establishment of wound skin

Rats were anaesthetized using an intraperitoneal (i.p) injection of ketamine (90mg/kg) and xylazine (10 mg/kg). The dorsal skin was prepared by removing the hair with razor blade and the surgical area was disinfected with 70% ethanol. Since shaving procedure produced marked oedema of the skin, the prepared rats were left for 24 hours before the wound was inflicted. After the rats were anaesthetized with combination of ketamine and xylazine via i.p., a full-thickness wound was created by using an 8-mm sterile skin biopsy punch. Each rat will have two full-thickness wound at their back dorsal. This method for wound model in murine is according to [5][18] with a slight modification.

M. Treatment of hydrogel

All wounds in the treatment groups were dressed up with either control GG, GVCO and GVCOH (dimensions of 20 mm x 20 mm) followed by Opsite film dressing [26] as the secondary dressing. The hydrogel with secondary dressing of Opsite films were then held in place on wounds and covered with gauze to give pysical protection to the wound and dressing. The changing of dressing was done every 3 days to minimize the infection to the wound site. The Opsite film dressing acted as positive control for comparison to the other treatments. The treated wound with GG dressing consider as negative control. The rats wound was photographed by using 13.1-megapixel Sony camera for evaluation of wound closure with the actual measurement.

N. Macroscopic observation of wound

The wound measurement of size taken at the time of biopsy was used to calculate the percent of wound contraction using equation [1];

$$\%Wound\ \ contraction = \frac{W_0 - W_t}{W_0} \times 100 \quad (4)$$

where W_0 is the original wound area and W_t is the wound area on the selected day after biopsy. The measurements of wound size were taken on day 2, 4, 7, 11, and 14 consecutively throughout the study. The wound area was measured by placing the 1 mm^2 graph over the wound picture. The squares were counted and the area was recorded. The wound area was accessed by the same blinded observer.

O. Ultrasound imaging

The wound area also was analyzed by using real-time high-resolution 20 MHz ultrasound imaging equipment (Dermalab Combo, Cortex, Denmark) skin analyzer to produce images representing cross-section of the wound skin. A standard echo graphic gel was applied and used

as a medium between probe and wound skin surface. The images produced were recorded.

P. Histological examination

Rats were euthanized at day 14 and skin samples that contained the wound area were taken for histological study. The skin samples were fixed with 10% buffered formalin for 24 hours. The samples were embedded in paraffin and cut into 6 mm-thick sections for middle part of the wounds. The sections were subsequently stained with haematoxylin and eosin (H E) staining procedure. The H E slides were visualized using light microscope at 20x magnification and digital micrograph were captured using an image analyzer.

Q. Statistical analysis

All data are presented as the mean±standard deviation (SD). The data are processed by two-way ANOVA using statistical software analysis SPSS (version 20). The p value <0.05 were considered statistically significant. Multiple comparison post-hoc tests were applied when necessary.

III. RESULTS AND DISCUSSION

A. ATR Spectroscopy

ATR spectra of GVCOH hydrogels showed the presence of characteristic peaks of gellan gum (GG), VCO and honey Figure 1. VCO has 6 prominent peaks dominated by its fats and oils content. The peaks at 2947 cm^{-1} and 2892 cm^{-1} were related to the saturated alkyl and the carbonyl groups of the fatty acids, respectively. Other peaks appearing at 1759 cm^{-1}, 1471 cm^{-1}, 1395 cm^{-1} and 1185 cm^{-1} were from the stretching of carboxylic (C=O), bending of methylene (CH_2), bending of methyl (CH_3), and stretching of esters (C-O), respectively [19].

Whilst, honey shows 6 prominent peaks at 3358 cm^{-1}, 2884 cm^{-1}, 1671 cm^{-1}, 1447 cm^{-1}, 1227 cm^{-1} and 1085 cm^{-1} which represent O-H (H-bonded), C-H stretching, O-H (water) bending, $-CH2$ bending (strong), $-CH2$ bending (medium) and C-O stretching [20]. The peak of honey at 1085 cm^{-1} is due to the C-O-C symmetric bending (C-O-C) and bending vibrations (C-O-H) of protein. Meanwhile, GG hydrogel exhibits a prominent transmittance bands due to stretching of O-H group at 3476 cm^{-1}, which does not appear in pure VCO nor honey [13]. By combining the VCO and honey, the spectra of GVCOH hydrogels show the enhancement of O-H group in every GVCOH hydrogels (different concentrations of honey) and confirming the interaction of the GG, VCO and honey occurred in these hydrogels [21].

B. Compression Properties

Compression test was examined to determine the strength and strain of the fabricated hydro-

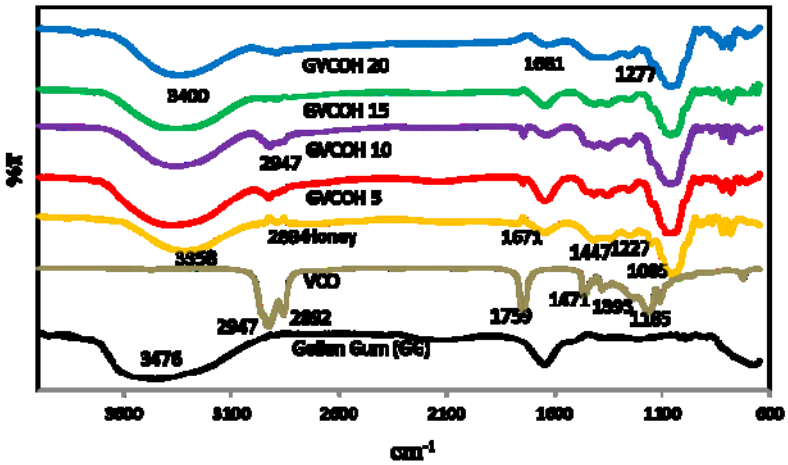

Figure 1. ATR spectra of gellan gum (GG), virgin coconut oil (VCO), Honey, and GVCOH at different concentrations of honey.

gels. Figure 2 shows the stress-strain curves of the GVCOH hydrogels at different loadings of honey and summarized the tensile stress (kPa), tensile strain (%) and Youngs Modulus (kPa) of the hydrogels in Table 1. Free standing gellan gum (GG) hydrogels were brittle (easy to break apart) and almost impossible to be used in pharmaceutical applications or as a wound dressing material. To elucidate this behavior, VCO and honey was added into the GG hydrogels to improve the strength of the materials. The inclusion of virgin coconut oil (VCO) and honey (5% w/w) into GG increased the stress and Youngs Modulus of the hydrogels. The stress value increased to 12.5±0.8 kPa for GVCOH5 hydrogel film compared to GG hydrogel film at 4.0±0.2 kPa, an increment of 3-fold. The Youngs Modulus of GVCOH5 was also increased to 309±39 kPa compared at 85±7 kPa (GG hydrogel film). The addition of higher loading of honey further increased the stress to 13.4±0.8 kPa for GVCOH20 hydrogel film, meanwhile the Youngs Modulus of GVCOH20 was further increased to 3-fold than the GG hydrogel film.

In contrast, the strain of the GVCOH hydrogels was decreased to 1-fold after addition of honey and maintain the value even after addition of honey at highest concentration (GVCOH20≈ 8.3±0.1%). The improvement of stress value and the Youngs Modulus of the hydrogels is a result of an increased in hydrogen bonding interaction between gellan gum-VCO-honey [13]. Without VCO and honey, the interaction occurs between gellan gum-gellan gum and therefore contribute to the brittle property of the hydrogels [2]. The addition of VCO and honey was successfully improved the strength of the hydrogels and make it possible to be used on different contours of our body as dressing materials.

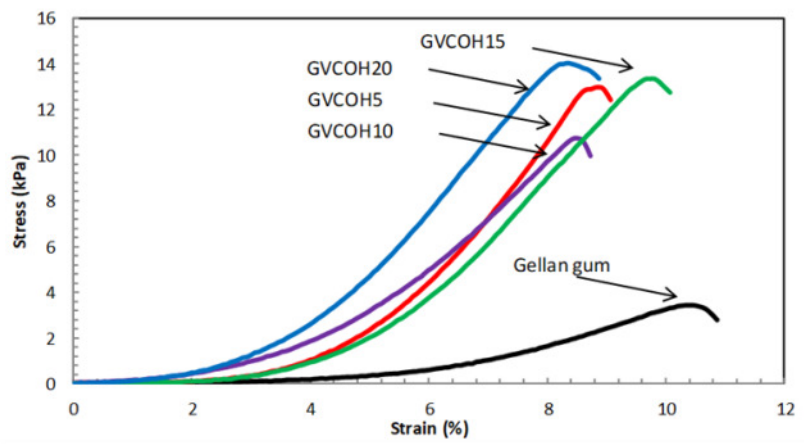

Figure 2. Typical stress-strain compression curves of GG and GVCOH hydrogels at different loadings of honey.

Table 1. Compression stress, Young′s Modulus (YM) and compression strain of GVCOH hydrogels at different loadings of honey.

	Compressive Stress (kPa)	Compressive Strain (%)	Modulus (kPa)
GG	4.0±0.2	10.7±0.4	85±7
GVCOH5	12.5±0.8	8.9±0.3	309±13
GVCOH10	11.1±0.3	8.4±0.4	254±9
GVCOH15	12.8±0.5	9.3±0.3	276±11
GVCOH20	13.4±0.8	8.3±0.1	266±10

C. Swelling ratio, Gel Fraction and Water Vapor Transmission Rate (WVTR)

Swelling properties of the hydrogels are important characteristics to determine the ability of the sample to absorb any exudates from any wound. In this study, due to the characteristic of the hydrogel sample, low swelling ratio was recorded for all samples (Table 2). The swelling ratio was increased upon addition of honey (GVCOH5) to 4±0.7% and further increased to 13±2.3% for GVCOH20, an increment of 4-fold than GG hydrogels. The increased values of swelling of GVCOH hydrogels with content of honey could be due to the presence of number of hydroxyl functional (OH) groups of GVCOH available to bind with the hydroxyl group (OH) from water. For gel fraction, GG hydrogels have the highest gel frac-

tion at 32±1.7% (Table 2). The addition of honey decreased the gel fraction to 18±0.7% for GVCO20 hydrogel film. This behavior could be attributed to the addition of honey in hydrogel disrupted the crosslinking properties of the hydrogels, and consequently affected the gelation process. This result correlate with the swelling results, in which the decreasing gel fraction (GVCOH20) contributed to the increasing of swelling behaviors of the hydrogels.

Water vapor transmission rates (WVTRs) is an important parameter to be determine in order to control the loss of body fluid due to the evaporation process. The loss of huge amount of body fluid could cause the decrease in body temperature, and the low or disrupted process of evaporation could build up the pressure around wound and give pain to the patient [23]. Because of that, the WVTRs values are crucial to be examined and confirm the ability of the hydrogels to allow the transmission of body fluid. The WVTRs values of the GVCOH hydrogels are shown in Table 2. WVTRs values of GVCOH hydrogel significantly decreased to 112±7 g $m^{-2}d^{-1}$ compared to 964±47 g $m^{-2}d^{-1}$ of free-standing GG hydrogel. Lowest WVTR values of GVCOH20 is due to huge amount of honey included into the GG hydrogel and disturbed the diffusion of water through the hydrogels. On other hand, GVCOH5 hydrogel with lower amount of honey possess higher WVTR value at 132±10 g $m^{-2}d^{-1}$. Nevertheless, all samples show the acceptable WVTRs values of commercial wound dressing products in the range of 90-2893 g $m^{-2}d^{-1}$ [16].

D. Thermal Studies

The thermal stability of GG hydrogel with VCO and honey was characterized by using thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). The heating process of TGA was started at 25°C, and then further increased up to 725°C along with a temperature rate change of 10°C/min. The thermogram and derivative thermogram of pure GG and GVCOH hydrogels were presented in Figure 3. The TGA/DTA traces show two main regions at 1) 25°C to 160°C, which related to the evaporation of the water, and 2) 160°C to 250°C due to decomposition of the aromatic bonds of honey [7]. The degradation of GG hydrogel started approximately at temperature onset, T_o = 54°C and the temperature completion, T_c at 130C (Table 2). Addition of honey did not significantly affect the temperature onset (T_o in range of 51°C - 58°C), but increased the temperature completion, T_c (T_c = 430°C - 470°C) depending to the concentration of honey. For example, the temperature completion of GVCOH20 hydrogel recorded at T_c = 433°C, with an increment of 3-fold than GG hydrogel. The weight loss (%) of GVCOH20 hydrogels also the lowest value at 89%, or in other word shows the highest residue values at 11% compared to other samples. This suggesting that the honey with sugar composi-

Table 2. Swelling, gel fraction, and water vapor transmission rates (WVTR) values of GVCOH hydrogels.

Hydrogen Sample	Swelling Ration (%)	Gel Fraction (%)	WVTR (g $m^{-2}d^{-1}$)
Control	-	-	1547±12
GG	3±0.6	32±1.7	964±11
GVCOH5	4±0.7	23±0.4	132±10
GVCOH10	9±0.5	18±0.5	138±13
GVCOH15	10±1.3	16±0.5	121±7
GVCOH20	13±2.3	18±0.7	112±7

tions provide better thermal stability to the GG hydrogels and the results are in agreement with other studies [3].

DSC results showed that the addition of honey improved the thermal behavior of the GVCOH hydrogels (Figure 4 and Table 4). The glass transition, T_g is increased depending to the honey content, in the range of 29°C to 39°C. GVCOH20 hydrogel offered the highest T_g value ($T_g = 39$) than GG hydrogels ($T_g = 29$). Broad exothermic peaks observed for GVCOH hydrogels with increased the range of T_c-T_o at higher content of honey showing that the inclusion of honey has improved the thermal stability of the samples. The range of T_c-T_o of GG hydrogels is at 83C, and GVCOH20 hydrogel at 128°C. It can be concluded that the enhancement of the thermal stability of the GVCOH hydrogels was attributed to the presence of honey which improved the crystallinity of the hydrogel samples and thus increased the thermal stability of the materials.

E. In-vivo assessment

F. In-vivo wound healing

The wound contraction results show that the GVCOH20 hydrogels accelerated significantly the wound closure after seven days of wound operation by more than 50% (Figure 5). It is further enhanced the wound contraction to 67±4% and 92±4% at day 7^{th} and 11^{th} subsequently. Meanwhile the wound contraction of GVCO enhanced to 28±11% and 46±3%, a step below than to a commercial dressing, Opsite at 30±3% and 49±11% at day 7^{th} and 11^{th} respectively. At day 14th, the GVCOH20 hydrogel showed the healing by 98±1%, followed by GVCO, and Opsite with 95±2% and 94±2%, respectively. The GG hydrogel shows the lowest percentage of wound closure compared to other treatment.

Along this study, no single died rat was

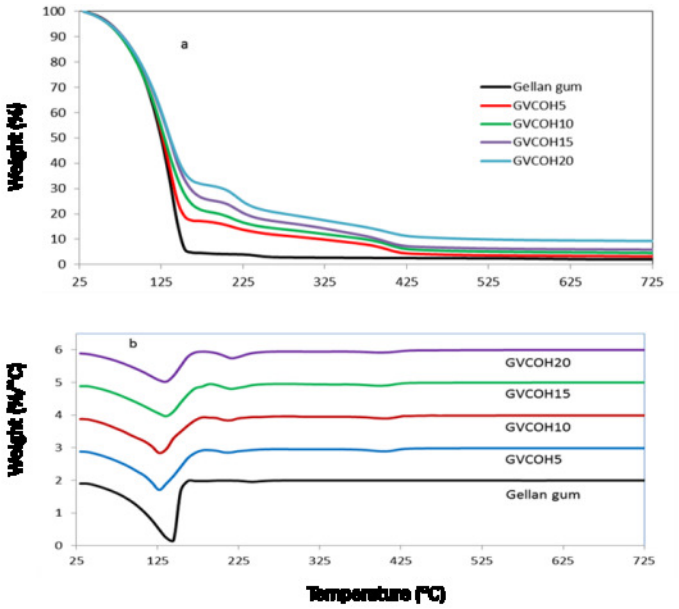

Figure 3. (a) Thermogravimetric thermograms and (b) derivative thermograms of GG and GVCOH at different concentrations of honey.

Table 3. Thermal gravimetry properties of GG and GVCOH hydrogels.

Sample	Temperature Onset, T_o (°C)	Temperature Completion, T_c (°C)	Weight loss (%)
GG	54	130	95
GVCOH5	56	476	96
GVCOH10	58	434	94
GVCOH15	56	430	93
GVCOH20	51	433	89

recorded. The reaction of skin irritation was observed, and all of the treatment did not cause irritation to skin and wound. It indicated that the GG is a safe material and suitable for cutaneous wound dressing. The general appearances and size of wound were monitored on day 7^{th} and 14^{th} post-wound by capturing images of each of animals (Figure 6). The data indicated the treatment of GVCOH20 hydrogels accelerated the wound closure and wound gradually disappeared by time-course. The ultrasound images of the thickness growing on the wound skin is shown in Figure 7. It shows that the skin formation of wound treated with GVCOH20 hydrogel exhibited the optimum recovery compared to other samples. The intensity of white/yellowish

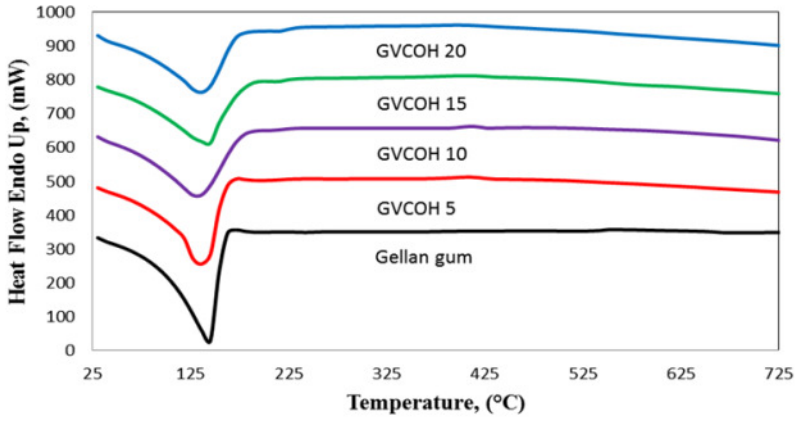

Figure 4. (Differential scanning calorimetry thermograms of GG and GVCOH hydrogels.

Table 4. Differential scanning calorimetry (DSC) properties of gellan gum (GG) and GVCOH hydrogels at different concentrations of honey.

	Temperature (°C)				Range (°C)
Sample	T_g	T_o	T_m	T_c	$T_c - T_o$
GG	29	67	125	150	83
GVCOH5	36	62	125	159	63
GVCOH10	34	58	121	174	116
GVCOH15	36	58	129	187	129
GVCOH20	39	41	125	169	128

color indicates the well formation of epidermis and dermis of GVCOH20 hydrogel followed by Opsite and GVCO hydrogel. The GG hydrogel as a control shows less intensity among the others.

After 14^{th} days of wound and treatment, the epidermal regeneration was clearly observed in all experimental groups. Hematoxylin and eosin (H E) staining was performed to evaluate the quality of the wound tissue. Histological evaluation results show that the GVCOH20 hydrogel resulted in better re-epithelization as compared to other samples (Figure 8). The inflammatory cells were not observed under the defects, and surprisingly the complete neo-epidermal replaced the defect was accomplished. In group of Opsite and GVCO hydrogel, it was clearly observed that the formation of epithelial growth, and the number of inflammatory cells reduced. Meanwhile in control group (GG hydrogel), the new epithelium was noted to regenerate and a little scab was spotted and the necrotic tissue

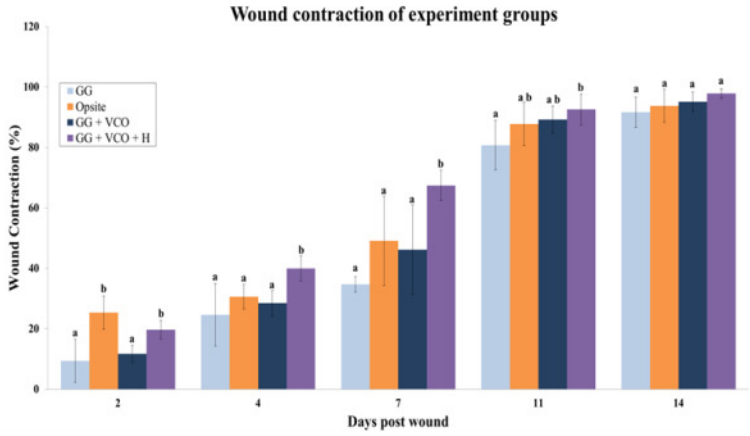

Figure 5. The percentage of wound contraction of experiment groups on day 2, 4, 7, 11, and 14. The bars are expressed as mean±standard deviation. Bars followed by the same letter (a, b) on the graph on the same day post wound are not significantly different at 5% level using Least Significant Different (LSD).

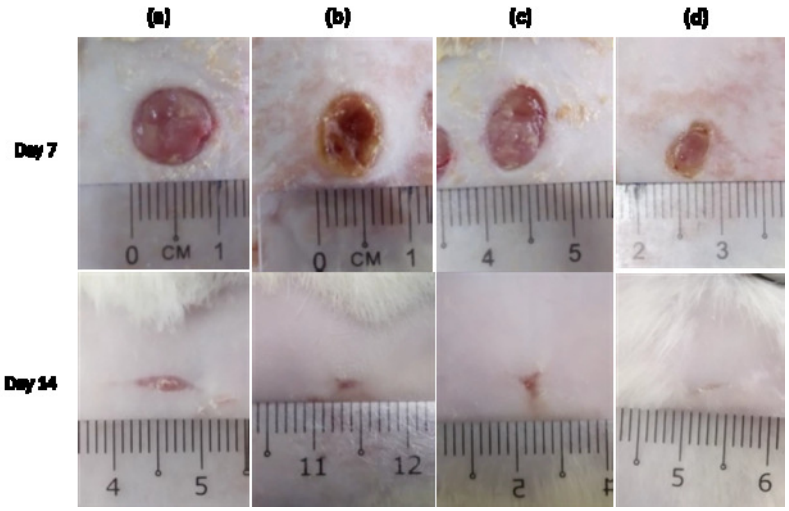

Figure 6. General appearance of wound skin on day 7^{th} and 14^{th} of (a) GG hydrogel, (b) Opsite, (c) GVCO hydrogel and (d) GVCOH20 hydrogel.

was found under defect. The skin treated with GVCO hydrogel and Opsite presented a better result than the control.

The results revealed the significant effect of GVCOH20 hydrogel after 11 days during cutaneous wound healing on animals. Migration a number of keratinocytes to the wound area and integration into neo-epidermis at early stage of healing could initiate the re-epithelization of skin tissue [4]. The moist environment characteristic of hydrogel is the most suited for re-epithelization and enhancing wound healing

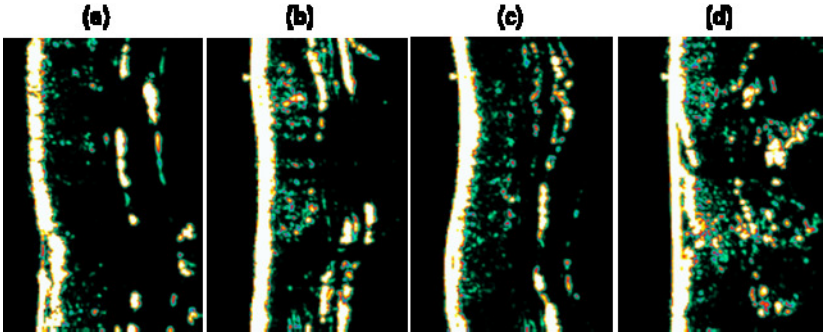

Figure 7. Typical ultrasound images of the wound skin on day 14^{th} of post-wound of (a) GG hydrogel, (b) Opsite, (c) GVCO hydrogel and (d) GVCOH20 hydrogel.

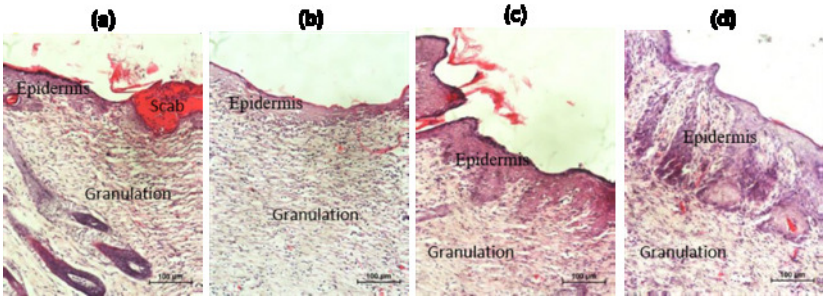

Figure 8. Representative of the histological evaluation section on day 14^{th} post-wound stained with hematoxylin and eosin on (a) GG hydrogel, (b) Opsite, (c) GVCO hydrogel and (d) GVCOH20 hydrogel. The epidermis, scab and granulation can be seen in images. The bar on micrograph represents 100μm.

mechanism in skin. Honey is a well-known and a powerful substance to treat the wounds since a long time ago, mainly due to its anti-bacterial, anti-inflammatory, and anti-oxidant properties [14]. The action of honey to the wound margin can stimulate the angiogenesis and growth of fibroblast under the skin. In order to address the excessive reactive oxygen species (ROS) during wound healing activity, the antioxidant capacity is crucial which specifically act as secondary messenger to promote proliferation and differentiation of the wound skin [15]. In the meantime, VCO also has been actively studied to utilize in wound healing treatment. Due to higher antioxidant profile [24], it can be a suitable candidate in wound healing treatment. An in-vivo study conducted by [16], wound healing on rats addressed the successful result using VCO as wound treatment. VCO is also exhibited anti-inflammatory properties and moderate analgesic effect in rats [8]. Overall, the emerging of VCO and honey in the form of hydrogel gives a significant effect of cutaneous wound contraction compared to the others.

IV. CONCLUSION

This study revealed the effect of virgin coconut oil and honey into gellan gum (GG) hydrogels to fasten the healing process. In turn, the chemical and physical properties of the materials were examined. The ATR-FTIR confirmed the chemically interaction between the GG and honey of the hydrogels. Inclusion of higher concentration of honey (GVCOH20) has increased the compression stress (durability of the hydrogels) and swelling ratios to offer a moist environment to the wound and less pain to the patients. Water vapor transmission rates (WVTRs) values are comparable to the commercial dressing in the range of 90-2893 g $m^{-2}d^{-1}$. The thermal behavior of GG hydrogel was also improved with addition of honey. *In-vivo* assessment results, i.e. wound contraction, ultrasound images and histological evaluation further supported the benefit of honey into GVCO hydrogels. To conclude, this study exhibited that the GVCOH hydrogels comprise a suitable material for effective treatments for acute wound treatment.

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