Synthesis and Characterization of Manuka Honey Loaded Carboxymethyl Cellulose Hydrogel Prepared by Gamma-ray Irradiation

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Abstract - Manuka honey differs from normal honey because of its additional existence of non-peroxide antibacterial activity which has been used to treat infections in a wide range of wound types. In this study, Manuka honey mixed with carboxymethyl cellulose (CMC) was crosslinked and sterilized by using a γ-irradiation of 50 kGy from a ^{60}Co source to make a new hydrogel wound dressing. The effects of various parameters such as honey concentration (0~20 wt%), gel content, and the absorption ratio in water were investigated in detail. The antibacterial properties were evaluated by paper diffusion test. The antibacterial tests indicated that the manuka/CMC hydrogels have a good antibacterial activity against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*). As effective therapeutic evidence, the rate of vivo wound healing process in db/db mice was investigated based on real-time photographs and wound area contraction.

Key words : Manuka honey, CMC crosslinking, Hydrogels, γ-irradiation, Wound dressing

INTRODUCTION

Manuka honey (MH) containing much higher antibacterial activity levels than regular honey was confirmed by the Unique Manuka Factor (UMF) which is due to the presence of methylglyoxal that non-peroxide activity (Weston *et al*. 1999; Snow *et al*. 2004; Lusby *et al*. 2005; Adams *et al*. 2008; Jose M *et al*. 2014), it is stable so there is no concern about losing its healing properties during storage. When applied to a wound, it is well known that MH not only exhibits anti-bacterial, anti-microbial (Patton *et al*. 2006; Lin *et al*. 2010; Aled *et al*. 2015), anti-septic, anti-viral, anti-inflammatory, anti-oxidant and anti-fungal properties, but also stimulates the growth of new blood capillaries, and white blood cells and supplies nutrients that boost the regeneration of new skin (Visavadia *et al*. 2008; Zerm *et al*. 2010; Huong *et al*. 2019).

Hydrogel wound dressings can absorb wound excreta and protect against excessive loss of bodily fluids due to their unique water absorption and retention properties. In a previous study, carboxymethyl cellulose (CMC) hydrogel not only have a good water absorption and retention ability, but also are environmentally friendly due to their nontoxicity and biocompatibility (Coviello *et al*. 2007; Park *et al*. 2012; Kabir *et al*. 2018). This characterization was proven to be useful as a natural hydrogel substrate by creating a wet environment for wound healing (Liu *et al*. 2005, Nabanita *et al*. 2019). Therefore, in this work, CMC (20 wt%) with different concentrations of Manuka honey (0, 5, 10 wt%) were mixed and crosslinked to fabricate new functional hydrogel dressings, and the advantages of MH and CMC hydrogels were combined and studied for wound healing abilities.

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MATERIALS AND METHODS

1. Materials

CMC was purchased from Sigma-Aldrich Co., (St. Louis, USA). Its degree of substitution (DS) is 1.2. The average molecular weight of CMC is about 2.5×10^5. MH was obtained from Comvita New Zealand Ltd., and shows a UMF2® of 20^+. The UMF relates to the potency of the antibacterial activity of the honey. The UMF numbers derive from a standard laboratory test with 'Active' Manuka which was compared with a standard antiseptic (phenol) to prove its potency. For example, honey with a UMF2® rating of 10+ would be equivalent to the antiseptic potency of a 10% solution of phenol. From these tests, UMF factors are shown to relate to the strength of the antiseptic solution (Badet *et al*. 2011).

All aqueous solutions in this experiment were made by using deionized (DI) water from a water purification system, produced by Young Lin Instrument Co., ltd. All chemicals were used without any further purification.

2. Preparation of CMC-MH hydrogel dressings

MH (0, 5, 10, 15, 20 wt%) and CMC powder (20 wt%) were mixed and dissolved in DI water by using a planetary centrifugal mixer (Thunky company, Japan) at room temperature. The mixture was poured into a mould ($40 \times 20 \times 2$ mm) to form a dressing shape and irradiated using gamma-rays with a total dose of 50 kGy at 10 kGy·hr^{-1} to produce the honey-hydrogel dressings.

3. Measurement

The gel content of the CMC-MH hydrogels was measured by extraction in DI water at 37°C for 48 hr and dried in an oven at 37°C for 48 hr until reaching a constant weight. The gel content (G_c) was defined by

$$G_c\,(\%) = (W_d/W_i) \times 100.$$

where W_d is the oven-dried gel weight after swelling for 48 hr, and W_i is the initial weight of the dried hydrogels, respectively (Gwon *et al*. 2010).

The degree of swelling can be described as the water absorptivity of the hydrogels. The hydrogels were immersed in distilled water for different intervals at room temperature until an equilibrium state of swelling was reached. After the excess surface fluids were removed with filter paper, the weight of the swollen gels was measured at various times. The procedure was repeated until no further weight increases were observed. The degree of swelling (S_w) can be calculated as follows:

$$S_w\,(\%) = [(W_s - W_i)/W_i] \times 100$$

where, W_s is the weight of the swollen gels at various time intervals, and W_i is the initial weight of the dried gel.

The compressive strength of the hydrogels was measured using an INSTRON 5569 (Instron Co., USA). The strength was measured through 50% compression and decompression of the hydrogels between the plates of the test machine with a crosshead speed of 10 mm/min.

4. Antibacterial test

The antibacterial activities of CMC-MH hydrogels were studied against *S. aureus* ATCC 6538P and *E. coli* ATCC 25922 on solid growth media. *S. aureus*/*E. coli* bacteria were grown on tryptic soy agar (TSA) plates after evenly smearing with 100 μL of freshly grown bacterial inoculums (10^6 cells ml^{-1}) and incubation at 37°C in an oven for 3 hr. Afterwards, the plates were supplemented with cylindrical CMC hydrogel stabilized Ag NP samples (Diameter: 8 mm, Height: 10 mm) and incubated at 37°C for 24 hr; the clear ratios were obtained by measuring the inhibition zones and calculated as follows:

$$\text{Clear ratio}\,(\%) = (d_i^2 - d_0^2)/d_0^2 \times 100$$

where, d_0 is the diameter of samples (8 mm) and d_i is the diameter of the clear zone after 24 hr.

5. Wound healing experiment

The female db/db mice (C57BLKS/J Iar$^-$ + Lepr^{db}/+ Lepr^{db}, weight between 18 and 24 g, age at 5 weeks) were experimented in vivo wound healing assessment. Three experimental groups, namely, an untreated control, and those with CMC and CMC-MH hydrogel (Manuka honey of 10 wt%) dressings, were composed of 10 mice each. Deep round skin traumas were inflicted on the dorsum of each body. The wound dressings were changed every 2 days, real-time photograph observations were followed and the healing rates of the wound area contractions were calculated.

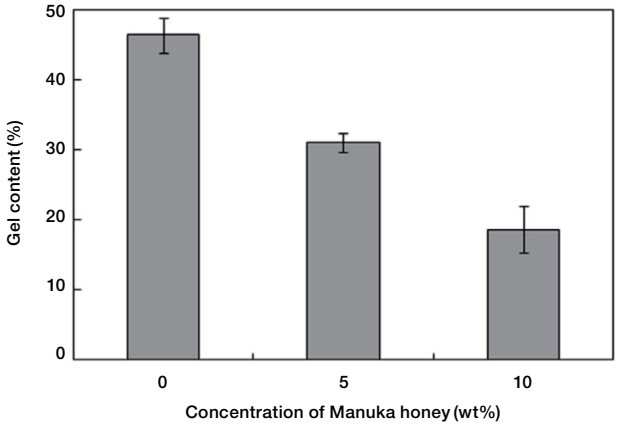

Fig. 1. Variation of gel content with different content of honey in CMC-MH hydrogels.

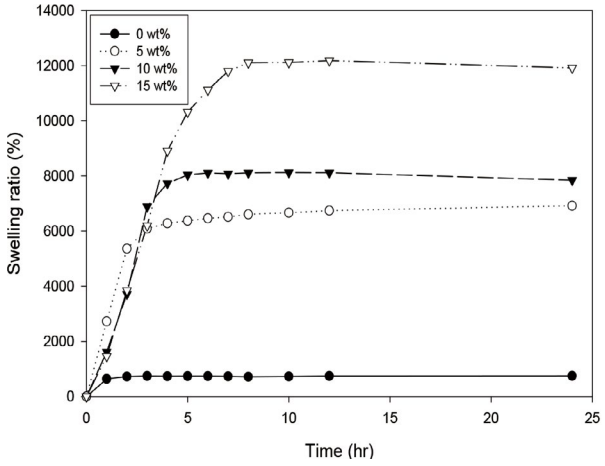

Fig. 2. The absorption ratio of CMC-MH hydrogels with different content of honey.

RESULTS AND DISCUSSION

As shown in Fig. 1, the varied gelation content as a function of the content of Manuka honey (0, 5, 10 wt%) of the CMC-MH hydrogel samples. The gel content decreased as the concentration of honey increased. In our previous study, it was found that the gelation of CMC (with a DS of 1.2) increased when increasing the irradiation dose and concentration of CMC. However, when the honey, which is a complex mixture of highly concentrated sugars, is added (Kamal *et al*. 2011), this complex including sugars in MH discouraged the formation of the crosslinking structure of CMC.

Fig. 2 shows the dynamic swelling behavior of different CMC-MH hydrogel samples. As the concentration of Manuka honey increased, the equilibrium swelling ratio of the hydrogels significantly increased. It is reasonable that the degree of crosslinking in the polymer networks restricted the chain relaxation process, thus causing a highly crosslinked gel content, leading to a low equilibrium swelling ratio. The results indicate that CMC-MH could be a better hydrogel wound dressing material than pure CMC hydrogel, and the CMC-MH may have a lower mechanical property due to fewer crosslinked chains, but it would supply a moist wound healing environment and absorb much more wound exudates, making the wound heal faster.

Fig. 3 shows the compressive strength for the CMC-MH hydrogel with the contents of MH. The compressive strength decreased when increasing the contents. In general, the compressive strength of the hydrogel was proportional to the gelation. In fact, the compressive strength of CMC

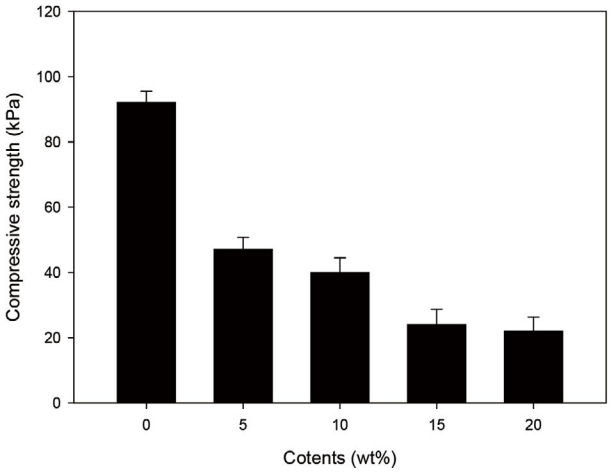

Fig. 3. Compressive strength of CMC-MH hydrogels with different content of honey.

hydrogel substantially decreased due to the presence of MH as compared with neat CMC hydrogel.

The antibacterial activities of CMC-MH hydrogel against *S. aureus* and *E. coli* were proven and recorded through photographs. Fig. 4 and Table 1 shows the antibacterial image and clear ratios of the samples, which were calculated by measuring the inhibition zones on the tryptic soy agar (TSA) plates. As shown in Fig. 4 and Table 1, the antibacterial effects of CMC-MH hydrogel increased when increasing the MH content. The CMC-MH hydrogel exhibited good antibacterial activity both *S. aureus* and *E. coli* compared to the blank (neat CMC hydrogel). It is possible that the MH in CMC hydrogel chemically reacted with cell of the bacteria. Hydrogen peroxide, organic acids, flavonoids, mehtlylglyoxal in MH are important chemical factors that

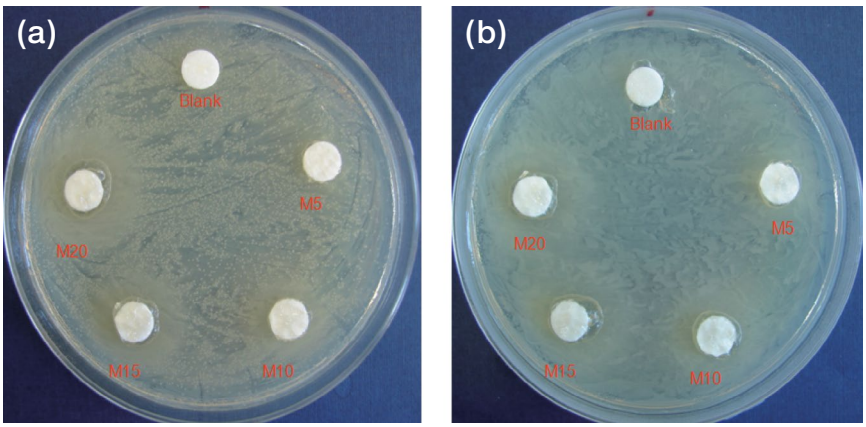

Fig. 4. Antibacterial image of CMC-MH hydrogels with different content of honey against *S. aureus* (a) and *E. coli*.

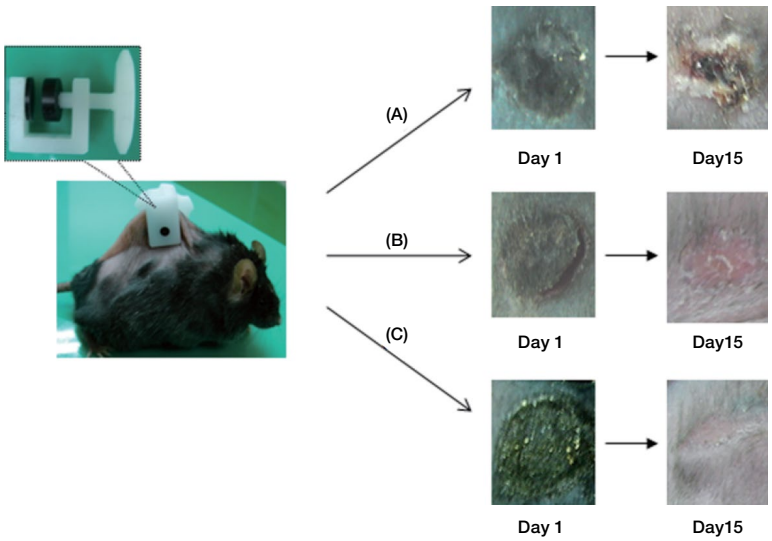

Fig. 5. Photographs of wound healing observation at day 1 and day 15: (A) untreated control wound, (B) CMC hydrogels treated wound, (C) CMC-MH treated wound.

provide antibacterial activity (Atrott and Henle 2009; Wang *et al*. 2012). In addition, the CMC-MH hydrogel exhibits more good antibacterial activity against *E. coli* than *S. aureus*. It is possible due to the difference in the structure of the cell wall between *E. coli* he Gram-positive and *S. aureus* (Shrivastava *et al*. 2007; Park *et al*. 2013).

The clinical effect of CMC-MH for wound healing was assessed in db/db mice. Wound area contraction and real-time healing photographs have been followed.

Figs. 5 and 6 show that the wound healing state of the mice at days 1 and 15. The mice were wounded by using a regular squeezing mold that made a deep round repeatable skin trauma on the dorsum of the body. These wounds were covered by (A) normal gauze, (B) pure CMC hydrogel dressings and (C) CMC-MH hydrogel dressings. The photographs clearly represent that different therapeutic effects appeared after 15 days. The necrotic and inflammatory tissues could still be observed in the untreated control wound, while the wound treated only using CMC hydrogels had

Table 1. Clear ratio of CMC-MH hydrogels with different content of honey against *S. aureus* and *E. coli*.

	Contents of Manuka honey	Inhibition zone	
		S. aureus	*E. coli*
Blank	0 wt%	–	–
M5	5 wt%	8%	11%
M10	10 wt%	27%	35%
M15	15 wt%	41%	47%
M20	20 wt%	62%	65%

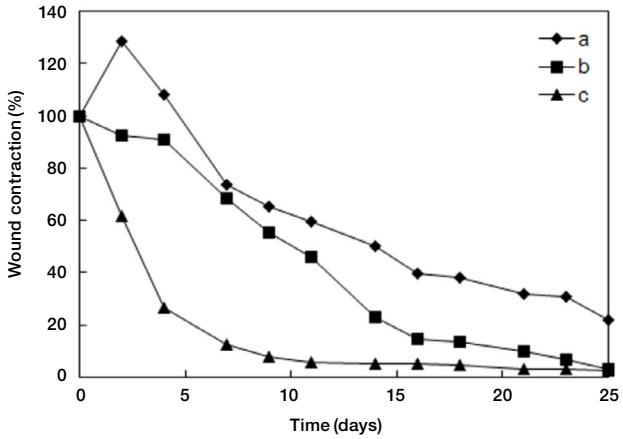

Fig. 6. Wound area contraction of db/db mice at different days as percentage of original wound size: (a) untreated control wound, (b) CMC hydrogels treated wound, (c) CMC-MH treated wound.

already closed but still had a fresh red scar. However, in the CMC-MH treated wound, significant epithelial regeneration and tissue repair had already appeared, and the wounds were healed and clean.

The wound contractions (W) of the db/db mice were measured every 2 days and calculated by using the following equation:

$$W(\%) = S_t/S_0 \times 100.$$

Where S_t is the wound area of mouse at time intervals, S_0 is the wound area of mouse at the initial time.

As shown in Fig. 6, both CMC and CMC-MH (10 wt% of Manuka honey) hydrogel dressings significantly stimulated the rate of wound healing. The untreated wound exhibited an inflammation phenomenon at days 2 through 5 (wound contraction: 128.57% to 108.49%), accompanied with excreta and a bad odor. On the contrary, the hydrogel dressings absorbed wound excreta, moved the dirty and septic tissues, and formed an efficient barrier against wound contamination. Naturally, the wound treated by CMC-MH healed faster and did not cause any inflammation, and it is thus reasonable to conclude Manuka honey exerted antibacterial and antimicrobial properties. The microscopic actions of CMC-MH on wounds may be multiple. Hydrogel appears to draw fluid from the underlying circulation, and Manuka honey may provide both a moist environment and topical nutrition that may enhance tissue growth and improve epithelialization.

CONCLUSION

In this study, a new hydrogel dressing with MH was synthesized by using a "one-step" process, which can be simply manufactured and sterilized though gamma irradiation technology. The effects of various parameters such as honey concentration, gel content, the absorption ratio in water and compressive strength were investigated in detail. The antibacterial tests indicated that the CMC-MH hydrogels have a good antibacterial activity against *E. coli* and *S. aureus*. In addition, the CMC-MH hydrogel dressing stimulated the rate of wound healing through animal experiments.

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REFERENCES

Adams CJ, Boult CH, Deadman BJ, Farr JM, Grainger MNC, Manley-Harris M and Snow MJ. 2008. Isolation by HPLC and characterisation of the bioactive fraction of New Zealand manuka (*Leptospermum scoparium*) honey. *Carbohyd. Res.* **343**:651-659.

Aled ELR, Helen LB and Rowena EJ. 2015. On the antibacterial effects of manuka honey: mechanistic insights. *Res. Rep. Biology* **6**:215-224.

Atrott A and Henle T. 2009. Methylglyoxal in manuka honey - correlation with antibacterial properties. *Czech J. Food Sci.* **27**:S163-S165.

Badet C and Quero F. 2011. The in vitro effect of manuka honeys on growth and adherence of oral bacteria. *Anaerobe* **17**:19-22.

Coviello T, Matricardi P, Marianecci C and Alhaique F. 2007. Polysaccharide hydrogel for modified release formulations. *J. Control. Release* **119**:5-24.

Gwon HJ, Lim YM, Nho YC and Baik SH. 2010. Humectants effect on aqueous fluids absorption of γ-irradiated PVA hydrogel followed by freeze thawing. *Radiat. Phys. Chem.* **79**:650-653.

Huong TLN, Naksit P, Stefan KEP and Nitin M. 2019. Honey and Its Role in Relieving Multiple Facets of Atherosclerosis. *Nutrients* **11**(67):1-22.

Jose MAS, Massimiliano G, Tamara YFH, Luca M and Francesca G. 2014. The Composition and Biological Activity of Honey: A Focus on Manuka Honey. *Foods* **3**:420-432.

Kabir SMF, Sikdar PP, Haque B, Rahman-Bhuiyan MA, Ali A and Islam MN. 2018. Cellulose-based hydrogel materials: chemistry, properties and their prospective applications. *Prog Biomater*. **7**:153-174.

Kamal MA and Klein P. 2011. Determination of sugars in honey by liquid chromatography. *Saudi J. Biol. Sci*. **18**:17-21.

Lin SM, Molan PC and Cursons RT. 2010. The post-antibiotic effect of manuka honey on gastrointestinal pathogens. *Int. J. Antimicrob. Ag*. **36**:467-468.

Liu P, Peng J, Li J and Wu J. 2005. Radiation crosslinking of CMC-Na at low dose and its application as substitute for hydrogel. *Radiat. Phys. Chem*. **72**:635-638.

Lusby PE, Coombes AL and Wilkinson JM. 2005. Bactericidal Activity of Different Honeys against Pathogenic Bacteria. *Arch. Med. Res*. **36**:464-467.

Nabanita S, Rushita S, Prerak G, Biman BM, Radostina A, Maja DS and Petr S. 2019. PVP-CMC hydrogel: An excellent bioinspired and biocompatible scaffold for osseointegration. *Mater. Sci. Eng. C* **95**:440-449.

Park JS, Kuang J, Lim YM, Gwon HJ and Nho YC. 2012. Characterization of silver nanoparticle in the carboxymethyl cellulose hydrogel prepared by a gamma ray irradiation. *J. Nanosci. Nanotechn*. **12**:743-747.

Park JS, Kuang J, Gwon HJ, Lim YM, Jeong SI, Shin YM, Khil MS and Nho YC. 2013. Synthesis and characterization of zinc chloride containing poly (acrylic acid) hydrogel by gamma irradiation. *Radiat. Phys. Chem*. **88**:60-64.

Patton T, Barrett J, Brennan J and Moran N. 2006. Use of a spectrophotometric bioassay for determination of microbial sensitivity to manuka honey. *J. Microbiol. Meth*. **64**:84-95.

Shrivastava S, Bera T, Roy A, Singh G, Ramachandrarao P and Dash D. 2007. Charaterization of enhanced antibacterial effects of novel silver nanoparticles. *Nanotechnology* **18**: 225103-225111.

Snow MJ and Manley-Harris M. 2004. On the nature of non-peroxide antibacterial activity in New Zealand manuka honey. *Food Chem*. **84**:145-147.

Visavadia BG, Honeysett J and Danford MH. 2008. Manuka honey dressing: An effective treatment for chronic wound infections. *British J. Oral Max. Surg*. **46**:55-56.

Wang T, Zhu XK, Xue XT and Wu DY. 2012. Hydrogel sheets of chitosan and gelatin as burn wound dressings. *Carbohyd. Polym*. **88**:75-83.

Weston RJ, Mitchell KR and Allen KL. 1999. Antibacterial phenolic components of New Zealand manuka honey. *Food Chem*. **64**:295-301.

Zerm R, Jecht M, De-Malter P, Friedrich M, Girke M and Kr M. 2010. Treatment of diabetic foot syndrome with Manuka honey. *Eur. J. Intern. Med*. **2**:258-259.

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