Influence of Manuka Honey on Mechanical Performance and Swelling Behaviour of Alginate Hydrogel Film

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Abstract. In this study, sodium alginate (SA) hydrogel film incorporated with Manuka honey was successfully developed. Swelling properties and gel fraction of hydrogel films decreased upon addition of Manuka honey in SA film. SA hydrogel film has the highest swelling percentage at 512 ± 21% and decreased to 197 ± 9% in SA hydrogel film containing 10% (v/v) of Manuka honey (SA-H10). In contrast, the SA-H10 hydrogel film has the lowest gel fraction at 12 ± 1% compared to SA hydrogel film at 45 ± 3%. The Young’s Modulus of SA hydrogel films with Manuka honey were increased as the concentrations of Manuka honey increased. The hydrogel films with higher concentration of Manuka honey (SA-H10) were soft with tensile strength, tensile strain and Young’s Modulus at 1.06 ± 3 MPa, 15 ±1% and 34 ± 3 MPa, respectively. The water vapour transmission rates (WVTRs) of SA-H hydrogel films were in the range of 1051 ± 48 – 1428 ± 84 g m⁻² d⁻¹, which comparable to WVTRs values with the commercial wound dressing products. The SA-H hydrogel films can be a potential material to be used as a wound dressing product.

1. Introduction
The biodegradable films based on biopolymers have been widely used in medical application due to its biocompatibility, biodegradability and properties that are similar to the human tissues [1]. Alginate is one of the examples of natural biopolymers that are commonly used in wound healing, drug delivery, soft tissue engineering, cell delivery, and in pharmaceutical industries [2, 3]. Alginates polymers primarily consist of blocks containing two uronic acids consist of two chain-forming heteropolysaccharides made up of blocks of β-(1,4)-linked D-mannuronic (M) and α-(1,4)-linked Lguluronic (G) acids. The latter forms complex with divalent cations to form gels, and their structure varies in dependence on the monomer position in the chain, forming either homopolymeric (MM or GG) or heteropolymeric (MG or GM) blocks [4]. Physical properties of alginates as well as formation of gels depend on the relative proportion of these blocks. The ability of alginates to form gels in the presence of divalent calcium ions is one of their main bio-functional properties and has a great industrial significance [5].

An ideal wound dressing should be able to keep moisture and promote faster healing. Film dressings are designed to stick firmly to the skin surrounding a wound without sticking to the wound itself. Alginates have special properties to offer as an ideal property of dressing materials, which are gelling properties, haemostatic and "moist" dressings [6]. Sodium alginites interact with the wound exudate to form a moist and non-adherent gel. The strength of the materials increased when soaked with exudate...
or blood. The contact with exuding wound will exchange the calcium ions in the dressing with sodium ions in serum or wound fluid and resulting in swelling of fibre and partially dissolves a gel-like mass. Alginate structural match to extracellular matrices of living tissues which are widely used applications in wound healing such as leg ulcers, pressure sores and infected surgical wounds [7].

On other hand, Manuka honey is a natural product that has been widely used for its therapeutic effects [8]. It has been used over thousand years ago in traditional medicine and commonly used to treat wounds [9]. Commonly, the healing or other properties of honey are studied in its pure state, i.e. in liquid form. Numerous studies have been reported the advantages of Manuka honey such as the anti-bacterial, anti-inflammatory, anti-viral and anti-oxidant effects [10-12]. Besides numerous studies of using pure honey have been used to treat wound and burns, limited studies have been reported to incorporated honey as a composite with bio-polymer, i.e. transforming honey with biopolymers to a film or hydrogels. A few studies have been reported to use chitosan and gelatin incorporated with Sunflower honey as a sheet [13], and some using polyvinylpyrrolidone (PVP) entrapped with Gelam honey to produce hydrogels [14]. This study fabricated and characterized the sodium alginate hydrogel films incorporated manuka honey. The mechanical characteristics, water vapor transmission rates, swelling and gel fraction of the hydrogel films were investigated.

2. Materials and method

2.1 Materials

Sodium alginate was obtained from Sigma Aldrich, St Louis, MO, USA. Glycerine and anhydrous calcium chloride, CaCl$_2$ were obtained from Sigma Aldrich, St Louis, MO, USA and Manuka honey was obtained from Nature’s Way, New Zealandas. All materials were used as received without any further purification.

2.2 Preparation of sodium alginate hydrogel films

2% of sodium alginate is dissolved in 70ml of deionised water at 80°C at constant stirring. Then 35% of glycerol is dissolved in 30mL of deionised in another beaker and is added into sodium alginate solution. The 5mM CaCl$_2$ solution is added dropwise and solution is heated and stirred for 1 hours and 45 minutes. Then, Manuka honey is added and the solution is stirred for another 15 minutes. These sodium alginate solutions containing 2%, 4%, 6%, 8% and 10% (w/v) Manuka honey are known as SA-H2, SA-H4, SA-H6, SA-H8 and SA-H10 respectively. After 2 hours, sodium alginate solution is poured into 5mm thickness of petri dish and dried in oven at 50°C, 50% fan for 24 hours. Then, the film is cut into 2mm x 2mm and let it immersed into 7mM CaCl$_2$ for 3 minutes. Then after immersion into CaCl$_2$ solution, the film is slightly dried with tissue paper before used for further characterization.

2.3 Characterization of SA-H films

2.3.1 FTIR Characterization.

ATR-FTIR spectra were determined by using Perkin Elmer Spectrum 100 FTIR spectrophotometer with PIKE Miracle ATR accessory with single-bounce beam path, 45° incident angle, and 4 cm$^{-1}$ resolutions. Then all spectra were corrected by using Perkin Elmer Spectrum 100 software. The resulting spectra and functional peaks were recorded.

2.3.2 Swelling Percentage.

The swelling percentage test was carried out by immersing SA-H (20 mm x 20 mm) in phosphate buffer solution at pH 7.2 in water bath (37 ± 0.5 °C). The samples were removed after 24 hours and lightly wipe with wet filter paper to expel surface solution. The swelling degree was determined from equilibrium swelling ratio as in Equation 2.1:

$$ \text{Swelling percentage (\%)} = \frac{M_f - M_i}{M_i} \times 100 $$  \hspace{1cm} (1)

Where, $M_f$ = final weight of sample and $M_i$ = initial weight of sample.
2.3.3 Gel fraction.
The film sample was cut into 20 mm x 20 mm and let dried for 24 hours. Then the film was weighed \(W_i\) and the film was immersed in 10 ml deionised water at room temperature for 24 hours. After removing film from solution, the film was dried in oven at 50 °C for 24 hours and weighed again \(W_f\) and gel content was calculated based on Equation 2.2:

\[
\text{Gel fraction (\%)} = \frac{W_f - W_i}{W_i} \times 100
\]  

(2)

Where, \(W_f\) = final weight of sample and \(W_i\) = initial weight of sample.

2.3.4 Water Vapour Transmission Rate.
The film was cut into 30 mm x 30 mm and put as a cap on a vial with diameter of 16 mm that contain 10 ml deionised water. Then it was weight \(W_i\) and left in the desiccator for 24 hours. After 24 hours, it was weighed again \(W_f\). The value of WVTR \((\text{g m}^{-2} \text{d}^{-1})\) was calculated based on Equation 2.3:

\[
\text{WVTR} = \frac{(W_i - W_f)}{A}
\]  

(3)

Where, \(W_i\) = initial weight, \(W_f\) = final weight and \(A\) = area of vial opening

2.3.5 Tensile Strength.
The tensile strength measurement was obtained by using Instron universal testing machine, model 3366 with cross-speed set at 10 mm/min. All films were cut into 20 mm x 60 mm for tensile stress-strain measurement. The tests were repeated triplicates per sample for defined ratio.

3. Result and discussion
3.1 FTIR Characterization
The ATR-FTIR spectra of SA films incorporated with Manuka honey were shown in Figure 1. There are a few functional peaks were appeared in SA-H films compared to blank SA film. Shifting and appearance of peak indicates that there was interaction occur between sodium alginate and honey [15]. Blank SA film shows a few main peaks at 3363.38 cm\(^{-1}\) was due to stretching vibration of O-H group [16]. A peak was shown at wavelength 2873.44 cm\(^{-1}\) which is saturated C-H stretch due to sp3 hybridization of C-H branched alkane, and a few peaks at 1609.88 cm\(^{-1}\) and 1412.60 cm\(^{-1}\) due to C=O bond stretching, bending or stretching mode of -COOH of sodium alginate. The peak shown around 1230.76 cm\(^{-1}\) could be attributed to the C-OH stretching vibration, while 1032.10 cm\(^{-1}\) to the O-H bending vibration of alginate [17].

Inclusion of Manuka honey in SA films show shifting vibration in SA film at 3363.38 cm\(^{-1}\) to SAH10 at 3354.21 cm\(^{-1}\). This indicates there is a O-H vibration of H-bonded between sodium alginate and Manuka honey. The C=O bond stretching of blank SA at 1609.88 cm\(^{-1}\) and 1412.60 cm\(^{-1}\) were shifted to 1608.43 cm\(^{-1}\) and 1415.83 cm\(^{-1}\) in SA-H10 films which indicates the stretching band of carbonyl groups C=O and C=C stretching related to phenolic molecules present in Manuka honey. This shifting indicates that there was interaction occurred between sodium alginate films and Manuka honey [18].
3.2 Swelling percentage
The swelling percentages of SA-H films were recorded in Table 1. Swelling percentage of SA films decreases with addition of Manuka due to the formation of rigid structure of SA films. Blank SA absorbed 512 ± 21% of phosphate-buffered saline (PBS) solution (pH 7.4) and decreased to 430 ± 10% in SA-H2 film and further decreased to 197 ± 9% in SA-H10 film. This might due to hydrogen bond that occurred between SA and Manuka honey limits the availability of free-hydroxyl group (OH) to bind with water molecules. The higher of Manuka honey content incorporated in SA, the higher water resistance was exhibited.

Table 1. Swelling percentage, gel fraction and water vapour transmission rate (WVTR) of sodium alginate (SA) SA-H films

| Sample   | Swelling percentage (%) | Gel fraction (%) | WVTR (g m⁻² d⁻¹) |
|----------|-------------------------|------------------|---------------------|
| Blank SA | 512 ± 21                | 45 ± 3           | 1428 ± 84           |
| SA-H2    | 430 ± 10                | 41 ± 1           | 1345 ± 61           |
| SA-H4    | 345 ± 16                | 38 ± 2           | 1297 ± 46           |
| SA-H6    | 316 ± 8                 | 29 ± 1           | 1204 ± 35           |
| SA-H8    | 257 ± 13                | 18 ± 2           | 1139 ± 25           |
| SA-H10   | 197 ± 9                 | 12 ± 1           | 1051 ± 48           |

3.3 Gel Fraction and Water Vapour Transmission Rates
Gel fraction is measured to examine the crosslinking degree of polymer chains within the SA films which can indicates the crosslinking behaviour of films. Table 1 shows the gel fraction of SA films depending to the Manuka honey contents. Higher percentage of Manuka honey incorporated in SA films caused the gel fraction values were decreased. Blank SA has the highest gel fraction at 46 ± 3%, and SA-H10 film has the lowest value at 13 ± 1%. It shows that increases the Manuka honey content strengthen the crosslinking with sodium alginate. This result is in agreement with swelling percentage.
of SA-H films, in which strong crosslinking behaviour decreased the swelling values of the films. Similar observations have been reported by Sirousazar et al. for swelling behaviour and structural characteristics of polyvinyl alcohol/montmorillonite nanocomposite hydrogels[18].

Water vapor transmission rates (WVTRs) is an important test to be determine in examine the loss of body fluid due to the evaporation process. The loss of huge quantity of exudates could causes the decrease in body temperature, disrupted the process of evaporation and could build up the pressure around wound and thus give pain to the patient [19]. Because of that, the WVTRs values are crucial to be examined and confirm the ability of the films to allow the transmission of body fluid. Water vapour transmission rates (WVTRs) of blank SA and SA-H films were in the range of 1428-1051 g m$^{-2}$ d$^{-1}$ (Table 1). SA-H10 film has the lowest WVTR values which 1051 ± 49 g m$^{-2}$ d$^{-1}$ that has highest incorporation of Manuka honey. The higher amount of Manuka honey in SA-H10 resist the water uptake in SA film thus resulting in lowest WVTR values. As compared to other, SA-H2 with low concentration of Manuka honey has the higher WVTR values at 1345 ± 61 g m$^{-2}$ d$^{-1}$ than SA-H10. The WVTR values of SA films is comparable to commercial wound dressing product such as OpSite at 792 g m$^{-2}$ d$^{-1}$ and Metalline at 1272 g m$^{-2}$ d$^{-1}$ that are suitable and safe as wound dressing materials [20].

3.4 Tensile test

The tensile test is conducted to study the mechanical and to measure the strength and flexibility of the polymers films. The characterization of tensile is important for wound dressing materials to be examined and make sure it can function properly and protect wounds. Figure 2 shows the stress-strain curves of the blank SA and SA-H films at different loadings of Manuka honey and the tensile stress ($\sigma$), tensile strain ($\varepsilon$) and Young’s modulus (YM) were summarized in Table 2. In general, addition of Manuka honey into SA films increased the YM values of the hydrogel films. YM of the SA-H10 was observed at 34 ± 3 MPa compared to SA-H2 at 28 ± 3 MPa.

![Figure 2. Tensile stress-strain curve of blank SA and SA-H films.](image)

In contrast, the tensile stress and tensile strain of the films were decreased upon addition of Manuka honey. SA-H10 film has the lowest tensile strength and tensile strain at 1.0 ± 3 MPa and 15 ± 1%, respectively, meanwhile SA-H2 film exhibited the highest tensile strength and strain at 3.9 ± 4 MPa and 37 ± 2%, respectively. The behaviour of honey, i.e. soft and stickiness contributed to the decreased of tensile stress and tensile strain of the films at higher concentration of honey. The slight improvement of the Young’s Modulus of the hydrogel films is a result of an increased in hydrogen bonding interaction between biopolymer-honey. Even though the film has an improved hydrogen bond, higher
The content of honey caused the stickiness to the films and reflected to low tensile stress and strain values. Without honey, the interaction occurs between SA-SA and therefore contribute to the brittle property of the hydrogel films and could not be measured [21]. The addition of honey was successfully improved the YM of the hydrogel films and make it possible to be used on different contours of our body as dressing materials.

Table 2. Tensile strength (σ), Young’s Modulus (YM) and tensile strain (ε) of sodium alginate films incorporated with Manuka honey.

| Sample | σ (MPa) | YM (MPa) | ε (%) |
|--------|---------|----------|-------|
| SA-H2  | 3.9 ± 4 | 28 ± 3   | 37 ± 2|
| SA-H4  | 3.9 ± 3 | 21 ± 2   | 21 ± 1|
| SA-H6  | 2.9 ± 9 | 26 ± 2   | 18 ± 3|
| SA-H8  | 2.4 ± 5 | 29 ± 5   | 16 ± 5|
| SA-H10 | 1.0 ± 3 | 34 ± 3   | 15 ± 1|

4. Conclusion
The sodium alginate (SA) film incorporated with Manuka honey (SA-H) was successfully prepared. The incorporation of Manuka honey in sodium alginate films (SA-H) caused the swelling percentage and gel fraction were decreased. The water vapour transmission rate of SA-H films was in the range of commercial dressings such as OpSite and Metalline, meanwhile tensile test of SA-H film shown improved Young’s Modulus with insertion of Manuka honey. The SA-H film exhibit good properties to be used as a wound dressing application.

5. References
[1] Pereira R, Mendes A and Bártolo P 2013 Alginate/Aloe vera hydrogel films for biomedical applications Procedia CIRP 5 210-5
[2] Hay I D, Wang Y, Moradali M F, Rehman Z U and Rehm B H 2014 Genetics and regulation of bacterial alginate production Environmental microbiology 16 2997-3011
[3] Soni M L, Kumar M and Namdeo K 2010 Sodium alginate microspheres for extending drug release: formulation and in vitro evaluation International Journal of Drug Delivery 2
[4] Castro L S E P W, de Sousa Pinheiro T, Castro A J G, Santos M d S N, Soriano E M and Leite E L 2015 Potential anti-angiogenic, antiproliferative, antioxidant, and anticoagulant activity of anionic polysaccharides, fucans, extracted from brown algae Lobophora variegata Journal of applied phycology 27 1315-25
[5] Ruvinov E and Cohen S 2016 Alginate biomaterial for the treatment of myocardial infarction: progress, translational strategies, and clinical outlook: from ocean algae to patient bedside Advanced drug delivery reviews 96 54-76
[6] Pawar S N and Edgar K J 2012 Alginate derivatization: a review of chemistry, properties and applications Biomaterials 33 3279-305
[7] Lee K Y and Mooney D J 2012 Alginate: properties and biomedical applications Progress in polymer science 37 106-26
[8] Robson V, Dodd S and Thomas S 2009 Standardized antibacterial honey (Medihoney™) with standard therapy in wound care: randomized clinical trial Journal of advanced nursing 65 565-75
[9] Vandamme L, Heyneman A, Hoeksema H, Verbelen J and Monstrey S 2013 Honey in modern wound care: a systematic review Burns 39 1514-25
[10] Lee H, Churey J J and Worobo R W 2008 Antimicrobial activity of bacterial isolates from different floral sources of honey Int. J. Food Microbiol. 126 240-4
[11] Tan H T, Rahman R A, Gan S H, Halim A S, Hassan S A, Sulaiman S A and Kirnpal-Kaur B 2009 The antibacterial properties of Malaysian tualang honey against wound and enteric microorganisms in comparison to manuka honey BMC complementary and alternative medicine 9 34
[12] Yaghoobi R and Kazerouni A 2013 Evidence for clinical use of honey in wound healing as an anti-bacterial, anti-inflammatory anti-oxidant and anti-viral agent: A review Jundishapur journal of natural pharmaceutical products 8 100-4
[13] Wang T, Zhu X-K, Xue X-T and Wu D-Y 2012 Hydrogel sheets of chitosan, honey and gelatin as burn wound dressings Carbohydrate polymers 88 75-83
[14] Yusof N, Hafiza A A, Zohdi R M and Bakar M Z A 2007 Development of honey hydrogel dressing for enhanced wound healing Radiation Physics and Chemistry 76 1767-70
[15] Azam N A N M and Amin K A M 2017 The Physical and Mechanical Properties of Gellan Gum Films Incorporated Manuka Honey as Wound Dressing Materials IOP Conference Series: Materials Science and Engineering 209 012027
[16] Shalumon K, Anulekha K, Nair S V, Nair S, Chennazhi K and Jayakumar R 2011 Sodium alginate/poly (vinyl alcohol)/nano ZnO composite nanofibers for antibacterial wound dressings Int. J. Biol. Macromol. 49 247-54
[17] Xiao Q, Gu X and Tan S 2014 Drying process of sodium alginate films studied by twodimensional correlation ATR-FTIR spectroscopy Food Chem. 164 179-84
[18] Sirousazar M, Kokabi M and Hassan Z 2012 Swelling behavior and structural characteristics of polyvinyl alcohol/montmorillonite nanocomposite hydrogels Journal of Applied Polymer Science 123 50-8
[19] Augustine R, Kalarikkal N and Thomas S 2014 Role of wound dressings in the management of chronic and acute diabetic wounds Diabetes Mellit Hum Health Care Holist Approach Diagn Treat 273-314
[20] Wu P, Fisher A, Foo P, Queen D and Gaylor J 1995 In vitro assessment of water vapour transmission of synthetic wound dressings Biomaterials 16 171-5
[21] Amin M, Anuar K, Gilmore K J, Matić J, Poon S, Walker M J and Wilson M R 2012 Polyelectrolyte Complex Materials Consisting of Antibacterial and Cell Supporting Layers Macromolecular bioscience 12 374-82

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