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Alginate based bilayer hydrocolloid films as potential slow-release modern wound dressing

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ABSTRACT

The aims of this research were to develop a novel bilayer hydrocolloid film based on alginate and to investigate its potential as slow-release wound healing vehicle. The bilayer is composed of an upper layer impregnated with model drug (ibuprofen) and a drug-free lower layer, which acted as a rate-controlling membrane. The thickness uniformity, solvent loss, moisture vapour transmission rate (MVTR), hydration rate, morphology, rheology, mechanical properties, *in vitro* drug release and *in vivo* wound healing profiles were investigated. A smooth bilayer film with two homogenous distinct layers was produced. The characterisation results showed that bilayer has superior mechanical and rheological properties than the single layer films. The bilayers also showed low MVTR, slower hydration rate and lower drug flux *in vitro* compared to single layer inferring that bilayer may be useful for treating low suppurating wounds and suitable for slow release application on wound surfaces. The bilayers also provided a significant higher healing rate *in vivo*, with well-formed epidermis with faster granulation tissue formation when compared to the controls. In conclusions, a novel alginate-based bilayer hydrocolloid film was developed and results suggested that they can be exploited as slow-release wound dressings.

1. Introduction

Wound healing is defined as body's replacement injured tissues with living tissues. It is a dynamic and intricate process which involve multiple cellular and matrix components act together to restore the integrity of injured tissue. The primary goals of wound care are rapid wound closure and leave minimal or aesthetically acceptable scar. Wound management is important in providing optimum healing milieu for wound healing (Sharman, 2003; Ovington, 2007). Depending on the severity of the wound, the desirable wound dressing may therefore serve among the purposes of (a) to provide moisture and occlusion, (b) protection from infections and contamination, (c) debridement, and (d) easy application and removal avoiding dressing-related trauma (Atiyeh et al., 2005; Singer and Dagum, 2008; Abdelrahman and Newton, 2011). Occasionally, drug-loaded wound dressings are used to treat wound locally such as anti-infections due to secondary infection or for pain control, especially in chronic wounds (Lawrence, 1994; Steffansen and Herping, 2008; Fouda et al., 2009).

Various wound care products are available in the wound care management market and they are targeted towards the treatment of both acute and chronic wounds (Abdelrahman and Newton, 2011). Among the modern wound dressings, dressings cast from hydrogels, sometimes know as hydrocolloid dressings, have been developed and uses as the first major advances in moist wound management. Wound healing is promoted by dressings that maintain a moist environment. Hydrocolloid has the ability to form gels upon contact with wound exudates and the high absorption occurs via strong hydrophilic gel formation (Lanel et al., 1997). The formation of gel allows excess fluid to escape without permitting wound desiccation. However, the fluid handling capacity of hydrocolloid dressings depends on many factors such as the physicochemical properties and the design of the dressing.

Alginate, a natural polymer, is used in the fabrication of hydrocolloid film wound dressings due to its biocompatibility, biodegradation and excellent film forming properties (Thomas, 2000; Balakrishnan et al., 2005). Sodium alginate is a water-soluble salt of alginic acid, a naturally occurring polysaccharide found in the cell wall of brown algae. It contains two uronic acids, β-(1-4)-linked D-mannuronic acid (M) and α-(1-4) linked L-guluronic acid (G), and is composed of homopolymeric blocks M–M or G–G, and blocks with an alternating sequence of M–G blocks. The various degree of crosslinking will reduce significantly the hydrogel swelling in the presence of the water, causing the release of drugs within the alginate matrices will be delayed. As a result, alginate is often being exploited as a drug controlled release vehicle in drug delivery systems (Pepperman et al., 1991; Shu and Zhu, 2002; Dong et al., 2006; Wang et al., 2010b). As wound dressings, alginate hydrogels

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Table 1
(a) Formulations of the single layer films S1–S5 and (b) formulations of bilayer films B1and B2.

(a)

Formulation	Sodium alginate (g)	Ibuprofen (g)	Propylene glycol (mL)	Ethanol (mL)	Glycerol (mL)	Distilled water (mL)
S1	6	5	10	5	6	79
S2	6	5	15	5	6	74
S3	6	5	20	5	6	69
S4	6	5	25	5	6	64
S5	6	5	30	5	6	59

(b)

Formulation	Sodium alginate (g)	Ibuprofen (g)	Gelatin (g)	Propylene glycol (mL)	Ethanol (mL)	Glycerol (mL)	Distilled water (mL)
B1							
Upper layer	6	5	–	15	5	6	74
Lower layer	6	–	–	–	–	6	94
B2							
Upper layer	6	5	4	15	5	6	74
Lower layer	6	–	4	–	–	6	94

can retain and create a moist environment around the wound to promote wound healing (Boateng et al., 2008).

Previously, research has shown that composite films have improved physical, transport and mechanical properties compared to those of single component-based films (López-Caballero et al., 2005; Chiono et al., 2008; Rivero et al., 2009; Gómez-Estaca et al., 2010; Wang et al., 2010a; Pereda et al., 2011). The term 'bilayer film' was described by Rivero et al. (2009) as two hydrocolloid layers, one cast over another. Recently, bilayer composite film systems based on various biopolymers are extensively investigated in food technology and bioengineering applications as edible films due to their better mechanical properties, higher moisture retaining properties and ease of preparation relatively to other film formulation. (Chiono et al., 2008; Rivero et al., 2009; Pereda et al., 2011). However, little is known on bilayers in wound healing and drug delivery applications.

In this study, an alginate-based bilayer film formulation was developed and investigated for its potential as slow-release wound healing vehicle. A model drug (ibuprofen) was loaded onto the upper layer while the drug-free lower layer was acted as a rate-controlling membrane. Ibuprofen was chosen because it is used as an effective adjunct in wound pain management for reducing pain during dressing changes. Jørgensen et al. (2006) evaluated the efficacy and pain reduction of Biatain®-Ibu, a new commercial wound dressing containing ibuprofen on patients with chronic leg ulcer wound pain. They concluded that ibuprofen could reduce persistent and temporary chronic leg ulcer wound pain, thus increase patients' quality of life. The aims of this research were to characterise alginate–gelatin bilayer film in terms of thickness, solvent loss, moisture vapour transmission rate, expansion rate, rheological and mechanical properties. The surface and cross-sectional morphology was examined using scanning electron microscopy and the *in vitro* drug release was conducted using Franz diffusion cells. The *in vivo* animal study was also undertaken to evaluate the effect of bilayer film formulations on full-thickness wound healing. All of the bilayer properties were compared to that of the single layer films.

2. Materials and methods

2.1. Materials

Sodium alginate (SA), ibuprofen and gelatin powder (from bovine skin, Type B) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Propylene glycol, 99% ethanol and glycerol were obtained from Merck (Germany). Ketamine hydrochloride, xylazine hydrochloride and formaldehyde were procured from Troy Laboratories (Australia). Haeris haematoxylin and eosin were obtained from Microm International (Germany). Distilled water was used throughout.

2.2. Pre-formulation

SA single layer films were first dried cast from the gel formulations in Table 1a, S1–S5, with varying amount of propylene glycol and ethanol. All films were prepared in a plastic petri dish with an area of 58.05 cm^2. To this end, 6 g of SA was stirred in distilled water until an uniform gel slurry was obtained at 40 °C. 5 g of ibuprofen was dissolved in 20 mL of propylene glycol and ethanol co-solvent of varying ratio shown in Table 1a. The ibuprofen solution was then added to the SA gel and stirred homogenously. Then, 6 mL of glycerol was added into the SA gel while stirred continuously. The prepared SA film was cast by pouring approximately 12.5 g of SA gel into the plastic petri dish. The SA gel was dried in incubator at 37 °C and RH 50% for at least 24 h. The dried SA film was stored at room temperature.

2.3. Preparation of bilayer hydrocolloid films

The most satisfactory film of S1–S5 based on appearance and physical characteristics was selected to formulate bilayer films. For the bilayer films, only the upper layer was loaded with model drug (ibuprofen). Here, S2 was chosen to proceed for bilayer film formulation. Two bilayer formulations, namely B1 and B2 were prepared (Table 1b). For B1 lower layer, 6 g SA powder was dissolved in distilled water and 6 mL glycerol was added. Instead of 12.5 g, 45 g of the SA gel was poured into petri dish and was dried as lower layer in an incubator at 37 °C and RH 50% for 72 h. As for B1 upper layer, the preparation method was similar to that of single layer S2. After the gel was prepared, 12.5 g of the gel containing ibuprofen was poured above the dried lower layer and the composite was then placed at 37 °C and RH 50% for further 72 h. To prepare the B2 lower layer, 4 g of gelatin powder was stirred in 94 mL distilled water at 90 °C. 6 mL of glycerol was added and stirred until a uniform gel was obtained. The SA/gelatin gel was cast as by pouring approximately 45 g onto the plastic petri dish. The lower layer film was dried in an incubator at 37 °C and RH 50% for 72 h. For the upper layer, the SA/gelatin gel mixture was prepared according to lower layer method above. Ibuprofen 5 g was dissolved in 20 mL of co-solvent of propylene glycol and ethanol of 15:5 ratios. The ibuprofen solution was then added into the SA/gelatin gel. Then, the 12.5 g prepared SA/gelatine gel containing ibuprofen was poured above the dried lower layer in the petri dish. The composite was then placed at 37 °C and RH

50% for 72 h. The dried bilayer films were kept at room temperature prior to further studies.

From all single and bilayer film formulations, only B2 and S2, which showed satisfactory film physical characteristics, were continued to further studies.

2.4. Film thickness and solvent loss

The average thickness of the S2 and B2 films was determined by using digital caliper (Messzeuge, Germany). Five random measurements were taken on the bilayers as well as single layer films. The average of the five values and their standard deviation (S.D.) of individual films were calculated. Thickness measurements were performed in triplicate.

The solvent loss, *i.e.* difference between the weights of each layer before and after drying was calculated using Eq. (1).

$$\text{Solvent loss (g)} = \text{weight of hydrogel before drying} - \text{weight of dried film after drying} \quad (1)$$

2.5. Moisture vapour transmission rate

The determination of moisture vapour transmission rate (MVTR) of polymeric films was described by Rao and Diwan (1997). The B2 and S2 were cut using a 30 mm diameter circular template. The cut films were fixed over the brim of a 6 mL glass vial (30 mm diameter), containing 3 g of fused calcium chloride as dessicant. The vial was weighed and kept in dessicator at RH of 84% controlled with saturated solution of potassium chloride at 25 °C. The vial was removed from dessicator and weighed at every 1 h interval for a period of 48 h. The MVTR was calculated from the slope of amount of water gain at each time interval versus time. The experiment was run in triplicate and the average values were calculated. MVTR was defined in Eq. (2).

$$\text{MVTR} = \frac{WT}{S} \quad (2)$$

where W is g of water, T is number of hours of experiment, S is exposed surface area of the film.

2.6. Expansion study

The procedure for the expansion study was adapted from previous studies (Matthews et al., 2005). The expansion ratio was performed on a gelatin medium to imitate wound surfaces. To prepare the gelatin medium, 4 g of gelatine powder was dissolved in 100 mL of distilled water at 90 °C and stirred until the clear solution was formed. Then, 25 g of clear gelatin solution were poured into the glass petri dishes and allowed to cool to room temperature (25 °C) overnight. Then, 3 cm diameter of B2 or S2 films were trimmed and placed on the gelatin surface. The change in diameter at time intervals of every 1 h (D_t) for a period of 48 h was measured. The following formula (Eq. (3)) was used to calculate the expansion ratios. Each film was carried out in triplicate.

$$\text{Expansion} = \frac{\text{diameter of sample at time } t\,(D_t)}{\text{diameter of sample at time } 0\,(D_o)} \quad (3)$$

2.7. Rheological measurements of rehydrated films

The dried B2 and S2 films were rehydrated into gel by adding the amount of solvent loss calculated from Eq. (1). Afterward, the rheological profile of the respective rehydrated films were determined using a cone-and-plate rheometer, 25 mm diameter, angle 1° (Anton PaarPhysica Rheometer, Japan) at a constant shear rate of 500 s^{-1}. The shear rate was increased from zero to 500 s^{-1} in 3 min followed by a constant rate decrease to zero in the same time interval. Typically, three independent measurements were taken for each sample. The average apparent viscosity at the flow curve apex (*i.e.* at 500 s^{-1}) of each sample was obtained.

2.8. Mechanical properties

Tensile strength (TS) and percentage elongation at break were evaluated using a Universal Testing Machine (Texturometer TA XT2i, Stable Microsystems, UK). The B2 and S2 hydrocolloid films under investigation were cut using the ASTM standard dumbbell shape template. The tensile properties of the films were examined by stretching the dumbbell shaped specimens (30 mm in length and 5 mm in width) to break at a crosshead speed of 5 mm/min. TS (MPa) was calculated by dividing the required maximum load (N) for breaking film by transverse sectional area of the film (thickness × width) (ASTM, 2001). Percentage elongation at break (E%) was calculated by dividing the initial gage length of the sample (30 mm) by difference in the length at the moment of rupture and multiplying by 100. At least 5 repeats were carried out for each hydrocolloid film formulation and the average values were calculated.

2.9. Morphology cross-section studies using scanning electron microscopy

Morphology cross-section study of B2 and S2 films was performed on a SEM (Philips model SEM 505, Fuji, Japan) at 12 kV. Film samples were examined for cross-section characteristics, which were affixed to aluminium stubs with double-sided cellophane adhesive tape and sputter-coated with a layer of gold prior to imaging at magnifications: 100× and 1000×.

2.10. Franz cell drug permeation studies

Drug permeation studies of ibuprofen liberated from dried single layer and bilayer films were investigated using Franz diffusion cell (Permegar. Inc., USA). A clean, dried receptor cell was filled with phosphate buffer solution (PBS) pH 7.4 and allowed to equilibrate at 37 °C. The cellulose acetate (pore size 0.45 μm) membrane was mounted between receptor and donor compartment. Then, S2 or B2 film was placed above the cellulose acetate membrane and sandwiched between receptor and donor compartments. The temperature of the receptor compartment was maintained at 37 °C with circulating water jackets throughout the entire experiment. All openings including donor top and receptor arm were occluded with parafilms to prevent evaporation. Using a glass syringe, the volume of 0.5 mL samples were withdrawn from the receptor medium at regular time intervals for 8 h and receptor volume was kept constant by replacing equal volume of fresh PBS solution of 37 °C. The samples were measured by using UV spectrophotometer (Shimadzu 1800, Japan) at 264 nm. The cumulative amount of ibuprofen drug diffusion was plotted against time. The ibuprofen drug flux was obtained from the steady state slope of each plot. The average values were calculated from Franz cell experiments (n = 6).

2.11. In vivo wound healing studies

2.11.1. Animal model

The wound healing characteristics of the films were evaluated using a rat model. All experiments were performed with the approval of the Universiti Kebangsaan Malaysia Animal Ethics Committees (UKMAEC) (FF/2010/FERN/14-JULY/306-JULY-2010-APRIL-2011). In total, twelve healthy male Sprague–Dawley rats, weighing approximately 250 g were obtained from the laboratory

Table 2
Thickness values, solvent loss, elongation and tensile strength of sodium alginate (SA) single and bilayer films (data expressed as mean ± S.D.).

Films	Thickness (mm)	Solvent loss (g)	Elongation (%)	Tensile strength (MPa)
Single layer	0.69 ± 0.02	9.48 ± 0.51	23.78 ± 3.30	20.82 ± 2.29
Bilayer	3.14 ± 0.04	38.29 ± 0.75	59.05 ± 2.54*	27.22 ± 0.95*

* $p < 0.05$ when compared with single layer films.

animal resource unit of Universiti Kebangsaan Malaysia (UKM). The rats were fed with standard pallet diet and water *ad libitum*. During the experiment, the rats were maintained under a controlled environmental condition with 12 h of light and dark cycle. The animals were divided into two groups: group 1 and group 2, with each group containing six rats.

2.12. Wounds creation and treatment

The previous protocol for wound creation in animals (Teoh et al., 2009) was adapted. On the day of the surgery (day 0), the rats were anaesthetised by intramuscular injection of ketamine and xylazine at a dose of 40 and 5 mg/kg body weight, respectively. The skin of the animal was shaved and disinfected using 70% (v/v) ethanol. Two full-thickness skin excision wounds of 6 mm in diameter were created using a punch-biopsy needle (Stiefel Laborateries, Sligo, Ireland), one on each side of the median line, approximately 20 mm from each other, and a depth of about 1 mm on the dorsal aspect of the thoracolumbar region of the rats. Then the wounds were treated by applying 1 cm^2 area film dressings. In both group 1 and 2, the left wounds were treated with bilayer films (B2). In group 1, the right wound was covered with single layer film (S2). In group 2, the right wound was treated with normal saline solution and remained uncovered throughout the experiment. The dressings were changed every 24 h.

2.12.1. The appearance and the percentage of the remaining wound areas

On the 0, 4, 6, 8 and 10 days after wound creation, the wounds diameter were serially measured and the wounds appearance were examined visually. The diameter of unhealed area was measured by normal ruler. The percentage of the remaining wound areas of days 0, 4, 6, 8 and 10 was calculated as area before treatment minus area contracted after treatment, divided by area before treatment, multiplied by 100% (Eq. (4)).

$$\%\ \text{of remaining wound area} = \frac{\text{wound area day 0} - \text{contracted wound area day}(n)}{\text{wound area day 0}} \times 100 \quad (4)$$

2.12.2. Histological examination

The rats were sacrificed on the 10th day after wound creation by an overdose inhalation of 80% (v/v) diethyl ether. The skin wounds were excised using punch-biopsy needle having 6 mm diameter. The tissues excised from the site of the wound were fixed in 10% (v/v) formalin solution, dehydrated through a graded series of alcohol (50–100% (v/v)), cleared in xylene and embedded in paraffin wax (melting point 5 °C). Serial thin sections of 7 μm thickness were made by using a microtome, stained with haematoxylin and eosin and examined under light microscope using image analysis software (VideoTesT-Master Morphology: Video TesT, St. Petersburg, Russia). For each wounded skin sample of each rat at least 10 sections were taken and examined.

2.13. Statistical analysis

All data were expressed as mean ± standard deviation. Student's *t*-test was applied for the calculation of *p*-values to examine the significance differences between experimental data. The $p < 0.05$ were considered statistically different. Statistics on a completely randomised design for *in vivo* animal studies were also determined using a Student's *t*-test and $p < 0.05$ was considered significant. All statistical analysis was performed using SPSS software (Version 19; SPSS Institute Inc., IBM.2010).

3. Results and discussion

3.1. Pre-formulation

An ideal film dressing is required to be supple, possess homogeneous and smooth surfaces (Boateng et al., 2009). Since ibuprofen is a poorly water soluble drug, cosolvents PG and ethanol were used to increase ibuprofen solubility in the film formulation. It was observed that single layer film formulations S1, S3, S4 and S5 which formed on addition of PG up to 20 mL showed inhomogeneous with ibuprofen crystals appearing on the surface of the films. Smooth, flexible and homogenous films were produced from single layer film formulations of S2 only. The non-homogeneity could be due to the breakage of temporary bonds (*e.g.* hydrogen bonds) that formed between the propylene glycol and surrounding water molecules during drying. The bilayer film formulations (B1 and B2) produced from S2 composition produced the films with smooth, flexible and homogenous surface. However, based on the physical appearance of the film, B1 formulation could not be able to form a bilayer film and appeared as a single layer film visually. On the other side, for B2 appeared as two distinct layers visually (later confirmed with SEM) and this could be due to the addition of gelatin as an adhesive in such formulation which improved the physical appearance of the bilayer films.

From the pre-formulation studies, only S2 and B2 were satisfactory and they were continued for characterisation studies. Hereinafter, the term 'single layer film' and 'bilayer film' were referred instead of S2 and B2.

3.2. Thickness and solvent loss

The thickness values and solvent loss are shown in Table 2. The bilayer films were approximately 4.5 times thicker than the single layer films. The thickness uniformity of both single layer and bilayer films was evidenced by low S.D. values, which also showed the high reproducibility of the film preparation method. The solvent loss from the film could be of water and ethanol because only these solvents are likely to evaporate at 37 °C, which was the drying temperature for film formation.

3.3. Moisture vapour transmission rate

The moisture gain per area increased steadily over 8 h and plateau after 24 h. The MVTR over 8 h of SA single layer films was 0.056 ± 0.001 g/cm^2/h and 0.022 ± 0.001 g/cm^2/h for bilayer films. MVTR values showed that both single layer and bilayer films were permeable to water vapour. The results showed that MVTR of the

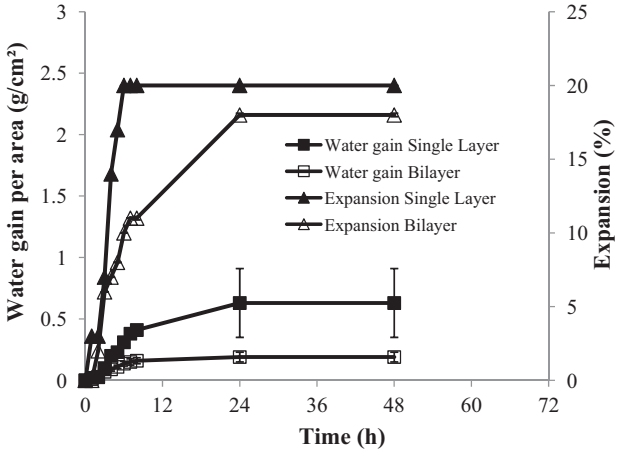

Fig. 1. The moisture water weight gain and expansion of single and bilayer films over 48 h ($n = 3$).

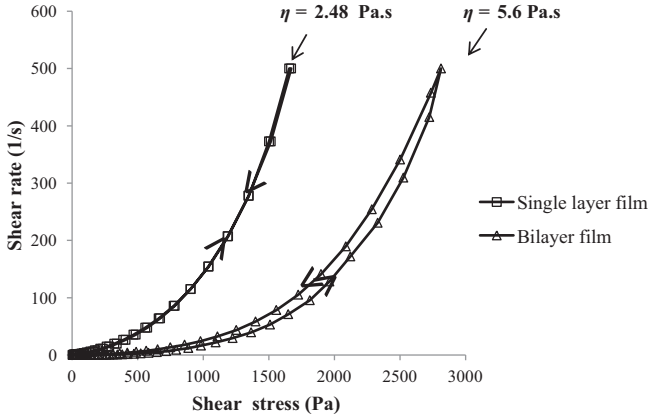

Fig. 2. Flow curves of SA single layer and bilayer rehydrated films (η = apparent viscosity at 500 s^{-1}).

bilayer films was significantly lower ($p < 0.05$) than that of single layer films (Fig. 1). This was due to the thicker bilayer films (3.14 mm) since MVTR of the hydrophilic polymeric films dependent on the length of pathway of which the moisture traverse (McHugh et al., 1993; Parris et al., 1995). Thus in the present study, the time required for diffusion of the water molecules across the bilayer films (3.14 ± 0.04 mm) were longer in comparison to single layer films with lower thickness (0.69 ± 0.02 mm). It had been mentioned that the dressings having low MVTR, which fall in the range of 0.19–0.58 g/day/cm^2, are suitable for wounds with low exudates and those having MVTR of 0.58–0.78 g/day/cm^2 and 0.78–0.98 g/day/cm^2 would be suitable to apply on the moderate and heavily exudating wounds, respectively (Thomas et al., 1996; Thomas, 1997). So, we suggest that single layer film having too high MVTR (1.36 g/day/cm^2) is not suitable to apply on the moderate to heavily exudating wounds since dressings with too high MVTR could not be able to create humid environment (Thomas et al., 1996). On the other hand, the bilayer film dressings with low MVTR (0.53 g/day/cm^2) may be useful for treating low suppurating wounds since maceration of the skin surrounding a wound may occur if a dressing with a low fluid handling capacity is used on a heavily suppurating wound.

3.4. Expansion study

The gelatin medium provides water producing substrate and resemblance to a suppurating wound. A gelatin model was used as a quick and reliable quality control method for quantifying the differences in flow properties, hydration and the expansion of the dried formulations in an exudating environment (Matthews et al., 2006). The percentage of expansion ratios for the films as a function of time was shown in Fig. 1. The single layer film quickly absorbed fluid and transformed from dried film to highly viscous gel upon the uptake of water compared to the bilayer film. It expanded 14% of its original sizes within 4 h. It was observed that single layer film only retained their shape in the first 6 h. After 6 h, single layer film could not expand further and maintain at 20 ± 0.01%, and also loss its disc shape. On the other hand, the bilayer film took longer time to hydrate and retained its spherical shape longer (up to 24 h) than single layer film. For bilayer film, it only expanded up to 18 ± 0.12% at 24 h. After 24 h, bilayer films did not expand further. The expansion values of both single layer and bilayer films showed significantly different ($p < 0.05$) at 24 h. The rate of which the film expanded was thought to be related to the rheological properties of the film formulation. Since, the apparent viscosity of the rehydrated bilayer films measured was higher than that of single layer films. Thus, lower rate of expansion of bilayer films was observed.

From this study, it was postulated that monolayer film is not suitable for medium to heavy suppurating wound because the film quickly revert to gel form when in contact with biological fluid. Darkovich et al. (1990) noted that the transforming of a texture of the materials to the gel may result in a gummy residue deposited in and around the wound. This will cause the dressing to be difficult to remove from the wound area and the residue of dressings will need to be rinsed out with normal saline, making the changing procedure laborious and time-consuming. The bilayer film which maintain its texture up to 24 h will eliminate any difficulties associated with clean up and removal of the dressing since of the convenience of removing the dressing in one piece.

3.5. Rheological measurements

Generally, both types of formulations displayed shear thinning with pseudoplastic flow properties (Fig. 2). This inferred that the films were able to revert back into hydrogel state without losing the rheological behaviour. At the shear rate of 500 s^{-1}, the bilayer rehydrated films showed higher apparent viscosities (5.6 Pa s) than that of the single layer rehydrated films (2.48 Pa s). This was expected due to the presence of gelatin, which is also a viscosity modifier, in the bilayer films. Gelatin and SA are hydrophilic miscible biopolymers. This miscibility is attributed to specific interactions between polymeric components such as electrostatic attraction, covalent unions, hydrogen bonds and dipole–dipole interactions between others, thus attributed to a higher in the viscosity in the formulation. It is possible to suggest the residence time of the dressing on the skin surface by measuring the viscosity of the formulation (Matthews et al., 2005, 2006; Boateng et al., 2008). In wound healing, one of the requirements for wound dressings is the dressings should be able to retain an adequately high viscosity in order to inhibit flow for longer period of time when applying on the suppurating wound surfaces (Matthews et al., 2005). The bilayer film formulation with higher viscosity has an advantage in inhibit viscous flow and also can remain on the target area for longer periods of time compared to single layer film.

3.6. Mechanical properties of hydrocolloid films

Table 2 compiles tensile strength and elongation values of single layer and bilayer films. Between the two types of film, the bilayer films were mechanically stronger as indicated by high values of

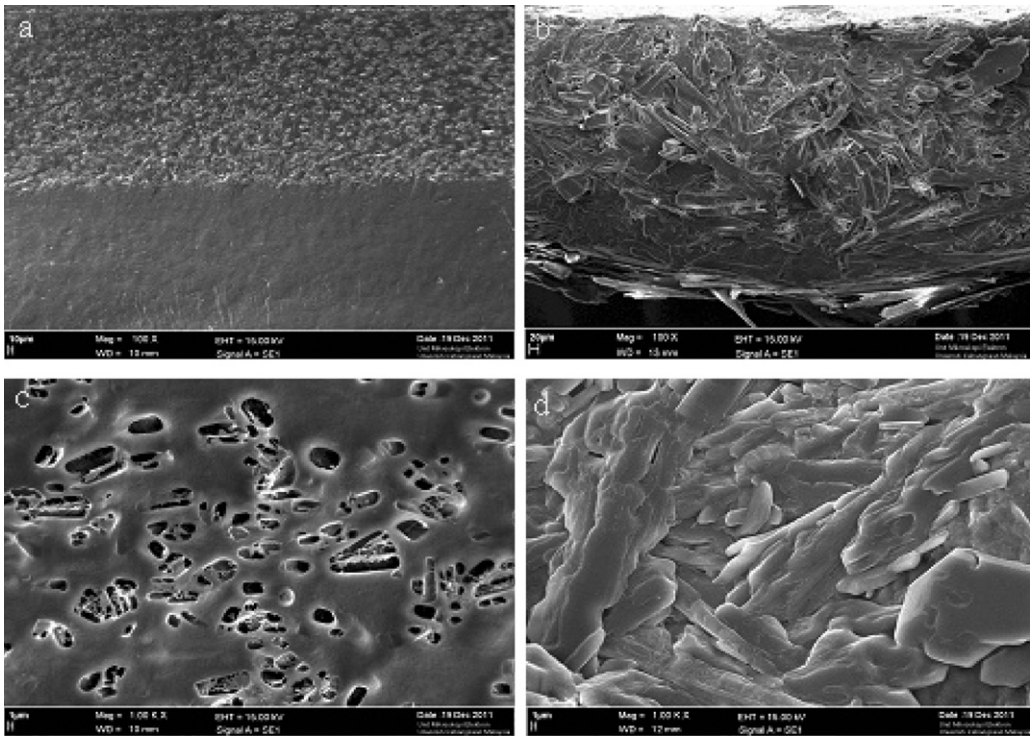

Fig. 3. SEM cross-sectional morphology of bilayer films at (a) 100×, and (c) 1000× (top layer only), and single layer films at (b) 100×, and (d) 1000×.

TS which was 27.22 ± 0.95 MPa and high values of *E*% which was 59.05 ± 2.54%. On the other hand, the single layer films exhibited average TS and *E*% values of 20.82 ± 2.29 MPa and 23.78 ± 3.30%, respectively. The tougher texture of bilayer film was thought to be due to the thickness as well as the presence of gelatin in bilayer film formulations. Rivero et al. (2009), who worked with composite films mixing gelatin and SA polymers had reported that the addition of gelatin produced stronger films with TS ranges between 27 and 53 MPa. Films for wound dressings should possess high tensile strength because wound dressings are required to be durable and stress resistant for their application and handling purposes (Khan et al., 2000; Boateng et al., 2008; Rivero et al., 2009).

By examining the elongation of the films at breaking point, it is also possible to predict the other mechanical properties such as brittleness, elasticity and ductility. The increase in percent elongation at break for film formulation occurs when the film become rubbery that is films changed from ductile to elastic state (Debeaufort and Voilley, 1997; Turhan and Sahbaz, 2004). The results showed that the *E*% for the bilayer films significantly higher ($p < 0.05$) compared to single layer films and it was obtained at higher ductility. This suggested that flexible films could be produced by a mixture of sodium and gelatin. Gelatin was able to form a maximum number of contacts with SA due to its random coil conformation (Wang et al., 2010a). However, if films are made from pure SA, such films will tend to be very brittle, shown by Wang et al. (2007). A study was conducted on a biodegradable film dressings composed of lactic acid and caproic acid copolymer and the highlighted that dressings exhibited high elongation (>50% of its original length) which were ideal for applying on the wounds as they degrade readily thereby avoiding painful removal (Jurgens et al., 1995). The *E*% of the films in this study must be further improved to achieve the desirable elongation. But between the two formulations, bilayer film dressings with higher *E*% also showed that the bilayers films are able to retain their original shape for a longer period of time and to withstand the stresses encountered during their application.

3.7. Cross-sectional morphology

The cross-sectional micrographs of bilayer and single layer films are shown in Fig. 3. It is clear from the images that distinctive film structures were formed (Fig. 3a). The bilayer revealed smooth matrix without any pores with good integrity. Aggregates of grain-like substance dispersed throughout the upper layer matrices were deduced to be the ibuprofen precipitates during the film-formation drying process (Fig. 3c). The ibuprofen would expect to re-dissolve when in contact with solvent. The cross-section of single layer film appeared coarse, uneven and fibrous (Fig. 3b). The fibrous structures may be the result of alginate alone failed to produce homogenous polymeric network (Fig. 3d). The fibrous network also attributed the poor mechanical properties of the single layer film, *i.e.* less flexible, more brittle and less *E*% compared to the bilayers.

3.8. Franz cell drug release studies

Franz cell drug release studies were also performed to compare drug release profiles from single layer and bilayer films. Drug release profile was shown in terms of cumulative amount over 8 h in Fig. 4. The results showed that the flux of ibuprofen liberated from bilayer film was (0.021 ± 0.05 mg/cm^2/h) significantly ($p < 0.05$) slower than those of single layer films (0.036 ± 0.005 mg/cm^2/h). This was due to the presence of an extra layer in bilayer film formulations which acted as a controlling membrane. The receptor fluid first penetrates the lower layer (drug free) of the bilayer film. The lower layer hydrates and swells to form gel. Then the receptor fluid begins to penetrate the upper layer. This in turn causes hydration and subsequent swelling of the upper layer films to form a gel.

It was proposed that lower layer plays a part in slowed drug release from wound dressings when they come into contact with wound exudates. As the lower layer had fully swollen, the fluid continued to penetrate the upper layer which containing drug. As a consequence, chain relaxation takes place and the incorporated

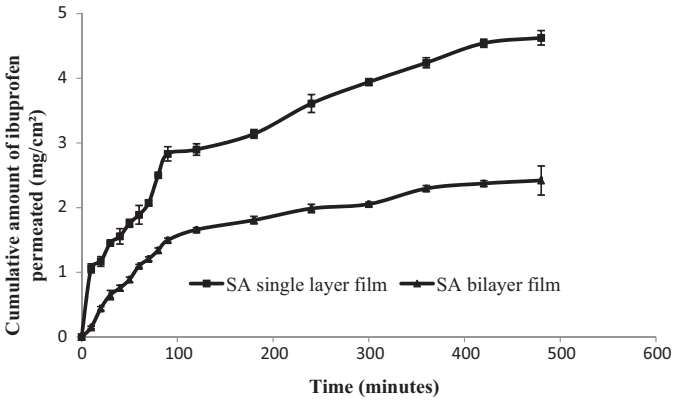

Fig. 4. Cumulative amount of ibuprofen release from sodium alginate (SA) single layer and bilayer films.

drug such as ibuprofen begins to diffuse from the swollen upper layer. It had been revealed that slow release of drugs from polymeric medicated dressings offer some potential advantages which generally includes prolonging the action of the active drug over longer periods of time by allowing continual release from such dosage form. It had been also cited that the wound care products which release a therapeutic substance to a wound interface in a slow release manner for a prolong period of time could improve the patient compliance by reducing the problem encountered with frequent dressing changes. Thus the present *in vitro* drug release study implied that by using the bilayer films which retain the drug for a longer period of time, thus minimise the dressing changing frequency.

3.9. In vivo wound healing studies

3.9.1. The appearance and percentages of remaining wound area

The wound healing of rats were characterised by wound appearance, percentages of remaining wound area and wound histology. The 1 mm depth skin punch biopsy had removed full thickness skin and subcutaneous fat as well as created a light suppurating wound. The percentages of the remaining wound areas on day 0, 4, 6, 8 and 10 were measured as a percentage reduction from the original wound area, shown in Fig. 5. Generally, the wounds treated with bilayer films were healed faster than those treated with SA single layer film and normal saline. On day 10, The remaining percentages of the wound areas treated with single layer films was recorded at 10% and normal saline at 20%. The size of the wound areas treated with bilayer films also attained full wound closure and the propensity of re-epithelialisation over the 10 days was significantly higher compared to single layer films and normal saline ($p < 0.05$). The skin of the control groups also appeared haemorrhagic and scabs were present on the wound bed.

It had been reported that the matrix formed from the blending of SA and gelatin showed the haemostatic effects, thus that makes it a potential wound-dressing material (Coviello et al., 2007). The blending of SA with gelatin polymer in one vehicle could enhance epidermal regeneration and accelerate the wound closure, compared to the single layer film which composed of SA polymer alone (Rivero et al., 2009). Our finding also was in agreement with previous reported studies that alginate film dressings increased re-epithelialisation rates by 10–50% and healing 2–6 times faster than open wounds treated with normal saline (Cho and Lo, 1998). It was generally known that dressings which create a moist environment that helps to maintain an electrical gradient between the wound and surrounding the skin, which may stimulate epidermal migration (Ramos-e-Silva and Ribeiro-de Castro, 2002). The lower layer of bilayer film retained moist in the wound and epithelialisation can be accelerated if the wound is kept moist (Madden et al., 1989). Keratinocytes migrated more easily over a moist wound surface than underneath a scab as epidermal cells can migrate at a speed of twice as fast as under a scab in dry wounds (Winter, 1962).

3.9.2. Histological examination

The histology of healing effects of bilayer film compared to single layer and normal saline after 10 days of post-wounding was studied. On day 10, the wounds treated with bilayer films were completely covered with epidermis and with the newly formed dermis (Fig. 6d). It showed that the epidermis was composed of keratinocytes which form the outer protective keratinised layer attached to the below intact epidermal skin layer. Underlying the developing epidermis showed the granulation tissue, including neatly arranged collagen fibres and active fibroblasts, which is responsible for the synthesis of extracellular matrix and collagen. The dermis had almost the same appearance as the normal skin. The collagen fibres in the dermis layer appeared in a more dense and organised form. Besides, the junction (interdigitation) was also present between the dermal and epidermal layer shown in Fig. 6a. The wounds treated with single layer films showed re-epithelialised epidermis. However, epidermal layer formed was not perfect in terms of differentiating keratinocytes and it was composed of less keratinocytes on the surface (Fig. 6b). The junction (interdigitation) present between the dermal and epidermal layer was also apparent. According to Fig. 6c, to which normal saline were

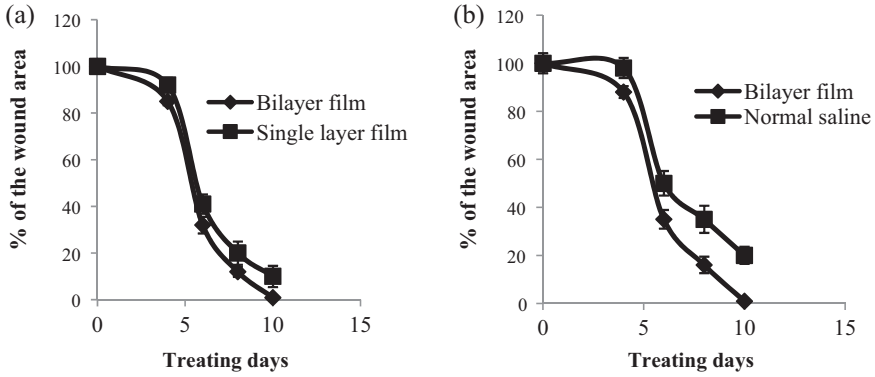

Fig. 5. Average percentages of the remaining area of the wounds treated with (a) bilayer and single layer films and (b) bilayer film and normal saline. *$p < 0.05$ compared with single layer film and normal saline.

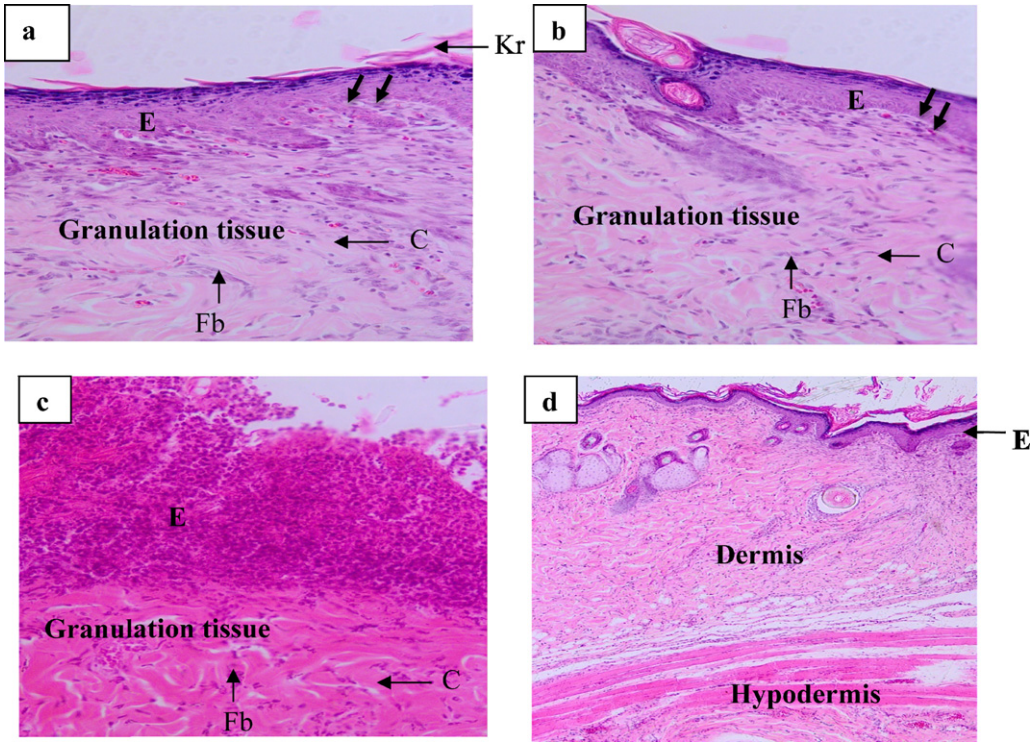

Fig. 6. Photomicrographs showing section of skin tissues with H&E staining at day 10 for (a) bilayer film dressings' (b) single layer film dressings; (c) normal saline at 200×, where arrows indicated in showed the interdigitation; E: epidermis, Kr: keratinocytes, Fb: fibroblasts, and C: collagen fibres at: (d) bilayer film dressings at 25×.

applied, an incomplete re-epithelialisation process was observed with no interdigitation between the dermal and epidermal layer. In addition, the skin surface was not repaired and did not gain the normal appearance. There were no keratinocytes with keratin like epidermis in the intact skin. This is in agreement with the literature where the wounds healed at slower rate than when remained air-exposed (Ramos-e-Silva and Ribeiro-de Castro, 2002). These findings supported that both film dressings are able to provide suitable condition for granulation tissue formation with epithelialised epidermis. This was because films were converted to gel which provide a moist microenvironment which is essential for wound repair. The wounds treated with bilayer films showed increased granulation tissue compared to the single layer and normal saline. Previous study showed that re-epithelialisation rate is higher and the deposition of collagen in the dermis is well organised when SA and gelatin blended film are used (Balakrishnan et al., 2005). In this study, the bilayer film dressings (blending of SA and gelatin in one vehicle) contributed to a desirable feature that likely promoted the faster rate of wound healing than those of the single layer film and normal saline. In addition, the histological micrographs of the wounds treated with bilayer films showed a well-formed epidermis with normal epithelium, thereby indicating the accelerated healing effect of the bilayer films.

4. Conclusions

Smooth, flexible with two distinct uniform layers of alginate bilayer hydrocolloid films were produced. The bilayer film with a low MVTR may be useful for treating low suppurating wounds. The mechanically strong bilayer films which hydrate slowly and low drug flux infer that they are potentially better suited for slow release application on wound surfaces. The faster rate of wound closure and histological data that show well-formed epidermis with faster granulation tissue formation for the bilayer film dressing suggest that bilayer hydrocolloid film systems can be potentially exploited as a slow-release wound dressing for low to medium suppurating wound.

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