

RESEARCH ARTICLE

Hydrogel-gauze dressing for moderate-to-severe atopic dermatitis: development and efficacy study on atopic dermatitis-like skin lesions in NC/Nga mice

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Abstract

Topical emollients are known to provide symptomatic relief for atopic dermatitis. In hospitals, wet-wrap therapy has been shown to benefit children with moderate-to-severe atopic dermatitis (AD), but the application of wet-wraps is tedious and time-consuming. Topical emollients have low residence time and often dry out easily. The aim of this work was to develop a hydrogel-gauze dressing that is not only easy to apply but also rehydrates and traps moisture to provide longer relief for AD patients. In this study, a prototype hydrogel-gauze dressing was developed with varying ratios of sodium carboxymethylcellulose (NaCMC) and propylene glycol. The hydrogel-gauze dressings were assessed based on the moisture vapor transmission rate, moisture absorption, mechanical properties and storage stability over three months. Then, the efficacy of the hydrogel-gauze dressing was compared to topical emollients using transgenic NC/Nga mice with AD-like lesions. The NaCMC hydrogel-gauze dressings significantly lowered transepidermal water loss, and the animals displayed a faster recovery, which indicates that hydrogel-gauze dressings can trap moisture more effectively and accelerate AD healing. Hence, we propose that hydrogel-gauze dressings can potentially become an alternative to wet-wrap therapy due to the ease of application and the higher efficacy compared to topical products.

Keywords

Atopic dermatitis, hydrogel-gauze, NaCMC hydrogel, NC/Nga mice, sodium carboxymethylcellulose

History

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Introduction

Atopic dermatitis (AD), a chronic skin disorder that is characterized by inflamed skin, affects from 10% to 20% of children and 1% to 3% of adults¹. It typically occurs in children beginning from infancy or early childhood^{2–4}. In the United Kingdom, the prevalence of atopic dermatitis in children is up to 20%, and the onset of this disease varies in individuals. Approximately 45% of the affected infants and children usually have AD by six months of age, 60% experience the onset of the disease by one year of age and 85% experience it by five years of age^{1,3}.

The etiology of atopic dermatitis is not yet completely understood. Some research studies propose that the development of this disease is closely related to skin barrier defects, as well as to genetic, immunologic and environmental factors^{5,6}. The signs and symptoms of AD are inflammation, redness, pruritus, excoriation and dry skin lesions¹. Dry skin is one of the prominent characteristics of this disease, and most patients generally have dry skin that indirectly causes intense pruritus, which usually worsens during the evening and at night. Pruritus worsens the disease because patients tend to scratch their skin, leading to the formation of excoriation, bleeding and lesions. Severe pruritus

greatly affects the physical, emotional and social well-being of the patient. The severe itchiness that occurs at night can disrupt patients' sleep and results in impairment of quality of life^{7,8}.

The use of non-medicated emollients continues to be the main therapeutic approach for treating dry skin caused by AD⁹. The principle behind the use of emollients is to maintain skin hydration and provide an oily barrier function so that water loss from the epidermis is also reduced. This may also reduce flare-ups of eczema and the need for topical corticosteroids. However, emollients also have their limitations. For example, emollients in general have low residence time; therefore, frequent application is required to maintain skin suppleness. Some emollients, such as Aqueous Cream BP, aggravate skin inflammation in patients. Thick emollient ointments may block the hair follicles in the skin and may cause a mild inflammation or infection of the affected hair follicles, a condition called folliculitis. Thus, topical emollients are only useful for the relief of acute AD, and their use is limited in moderate-to-severe AD patients.

Wet-wrap treatments have been introduced as one of the therapies for moderate-to-severe AD in children¹⁰. It is a treatment used especially for patients whose disease has not been controlled by various topical treatments alone and consists of a combination of topical treatments and a bandaging technique. This therapy involves the use of tubular bandages, an emollient and a mild-potency topical corticosteroid. Several clinical studies showed that there is a vast improvement in patients' conditions after using wet-wrap therapy^{11–13}. Wet-wrap treatment acts as an

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occlusive barrier that promotes skin hydration, enhances the absorption of topical medications and thus increases the efficacy of the medication. It can also act as a barrier between fingers and skin to reduce the damage caused by skin abrasions to indirectly promote the healing of damaged skin^{2,3}. However, this treatment is very time-consuming and requires full cooperation, especially by younger patients. In addition, the dressings can easily become dried out and must be continuously dampened². Hence, there is a need to develop a new type of dressing that is less burdensome and can provide longer relief to patients.

The aim of this work was to develop a new dried hydrogel formulation that is easy to apply, rehydrates dry skin upon application, and simultaneously serves as a moisture-trap to provide longer relief for moderate-to-severe AD patients. In this research, hydrogel-gauze dressings were formulated using a cellulose-based polymer (sodium carboxymethylcellulose), propylene glycol and an elastic conforming bandage. First, a prototype dressing was developed using various ratios of polymers and plasticizers, and then, the dressings were characterized by performing a moisture vapor transmission test, a moisture absorption test and a mechanical test, and by determining the storage stability over three months. The second part of the study compared the efficacy of the hydrogel-gauze dressing to that of commercial topical formulations on transgenic NC/Nga mice with AD-like lesions. Histological studies of the skin of each mouse were conducted to compare the effectiveness of the hydrogel-gauze dressings to commercial formulations in their ability to improve AD lesions.

Materials and methods

Materials

Sodium carboxymethylcellulose (NaCMC), propylene glycol (PG), 80% (v/v) diethylether, 1% 2,4-dinitrofluorobenzene (DNFB), acetone, olive oil and 10% formalin were obtained from Sigma-Aldrich (Munich, Germany). Potassium chloride was obtained from Merck (Darmstadt, Germany), disodium hydrogen orthophosphate, potassium dihydrogen orthophosphate, sodium hydroxide and hydrochloric acid were obtained from R&M Chemicals (Berkshire, UK). Sodium chloride was purchased from Hamburg Chemicals (Hamburg, Germany). Elastic conforming bandages (10 cm × 1.75 m) were purchased from Smith & Nephew (Suzhou, China). Harris hematoxylin & eosin (H&E) were obtained from Microm International (Walldorf, Germany). Distilled water was used throughout.

The commercial topical formulations used were Cetaphil Intensive Moisturizing Cream from Galderma (Malaysia), Sebamed Moisturizing Body Lotion from Sebapharma (Germany), Physiogel Hypoallergenic Cream from Stiefel (Malaysia) and Aqueous Cream from Pharmaniaga (Malaysia).

Hydrogel-gauze dressings

Hydrogel-gauze dressings containing different amounts of NaCMC and PG were prepared and labeled as F1–F6 (Table 1). First, distilled water was added to a beaker containing PG and stirred with a magnetic stirrer. Next, the mixture was heated to

approximately 60 °C. NaCMC powder was then dispersed slowly into the solution and stirred continuously until a homogenous NaCMC hydrogel was formed. The NaCMC hydrogel was left overnight to let the entire polymer fully hydrate and dissolve and to remove bubbles entrapped during the stirring process. The next day, 20 g of the hydrogel was poured onto the upper surface of a piece of elastic conforming bandage inside a dry Petri dish with a diameter of 8.4 cm. The sample was left to dry at 37 ± 2 °C for approximately 48 h or until a constant weight was obtained. Silica gels were placed in a dryer to maintain a relative humidity of 3%. The dressings were then packaged with aluminum foil and stored inside an airtight container at a relative humidity of 3% and a temperature of 28 ± 2 °C.

Evaluation of hydrogel-gauze dressings

Physicochemical characterizations, such as physical assessments, moisture vapor transmission studies, moisture absorption studies, mechanical properties, transepidermal water loss and stability studies, were performed to characterize the NaCMC-based hydrogel-gauze dressings, F1 to F6.

Physical assessments

The thickness and weight uniformity of hydrogel-gauze dressings F1 to F6 were evaluated. The thicknesses of three randomly selected hydrogel-gauze dressings were measured using a digital micrometer with a sensitivity of 0.001 mm (Mitutoyo, Tokyo, Japan). Measurements were repeated at five different places on each of the dressings, and the average value was calculated. For the uniformity of the weight of the dressings, three dressings from each formulation were weighed individually, and the average weight of the dressings was calculated and recorded.

Moisture vapor transmission rate study

Moisture vapor transmission tests were carried out to test the moisture permeability of the hydrogel-gauze dressings over defined periods of time. This experimental method was modified from the study conducted by Kulkarni et al.¹⁴. Plastic cups of equal diameter (2.8 cm) were used as transmitting cells. Before running the study, both plastic cups and the silica gels were dried in a dryer at 40 °C to remove any moisture and to ensure that the cup was completely free from moisture. The dressings from each formulation were cut into round pieces and were fixed over the top of the plastic cup containing 2 g of dried silica gels. An open plastic cup with no dressing was used as a control in this study. The initial weight of each transmitting cell was taken, and then, the cell was placed into a closed desiccator containing 500 mL of a saturated potassium chloride solution, which maintains a relative humidity of 84% in a desiccator at 28 ± 2 °C. The amount of moisture vapor transmitted through the dressings was calculated from the increase in weight of the transmitting cells after 24 h using an analytical balance (Mettler Toledo, Zürich, Switzerland). The moisture vapor transmission rate was determined using Equation (1):

$$\text{MVTR} \left(\frac{\text{g}}{\text{m}^2} \text{ per day} \right) = \frac{(W^2 - W^1)}{A} \quad (1)$$

where W^1 and W^2 are the weights of the whole cup at the initial hour and after 24 h, respectively, for each formulation and A is the transmitting area of the dressings in m^2 . These experiments were performed in triplicate and the average value was calculated.

Moisture absorption study

The moisture absorption study of the dressings was modified from Ramli and Wong¹⁵. The moisture absorption of the dressings was

Table 1. Composition for NaCMC hydrogel-gauze dressing formulations.

Constituents (%w/w)	Formulations					
	F1	F2	F3	F4	F5	F6
NaCMC	3	3	4	4	4	4
Propylene glycol	40	50	20	30	40	50
Distilled water	57	47	76	66	56	46

determined at a relative humidity of 84% and a temperature of $28 \pm 2^\circ\text{C}$. Hydrogel-gauze dressings from each formulation were cut into a round shape with a diameter of 2.8 cm and then weighed using an analytical balance (Mettler Toledo, Switzerland) with a sensitivity of 0.0001 g. Then, each dressing was placed on a petri dish and put in a closed desiccator containing 500 mL of a saturated potassium chloride solution for 24 h. At predetermined time intervals (2, 4, 6, 8, 10 and 24 h), the change in weight of each dressing was measured and recorded. The amount of moisture gained from the environment by each dressing was defined by Equation (2):

$$\text{Amount of moisture gained by dressings} = W_2 - W_1 \quad (2)$$

where W_1 and W_2 represent the weight of the dressing at the initial time (W_1) and at a predetermined (W_2) time interval. The moisture absorption of each dressing after 24 h was also determined and compared to moisture absorption after 12 weeks. This study was conducted in triplicate and the average value was calculated.

Mechanical tests

The mechanical properties of the hydrogel-gauze dressings were evaluated using a Universal Testing Machine (Instron 5567). Hydrogel-gauze dressings from each formulation were cut into a dog-bone shape (length 75 mm, neck length 29 mm, width 4 mm) using an ASTM D-412-C mold (Mecomb Sdn Bhd, Malaysia). The thickness of each dressing was measured with a digital micrometer (Mitutoyo, Japan) prior to mechanical analysis. Then, each hydrogel-gauze dressing was held between two grips at a constant distance. The hydrogel-gauze dressing was pulled at a rate of 20 mm/min with a load cell of 500 N¹⁶. The tensile strength (N/mm²) and percentage of elongation at break (%) of the dressings were measured and calculated using Equations (3) and (4), respectively. Three samples from each formulation were subjected to the mechanical test, and the average value was calculated.

$$\text{Tensile strength} = \frac{\text{load at break (N)}}{\text{cross sectional Area (mm}^2\text{)}} \quad (3)$$

$$\% \text{ Elongation at break} = \frac{\text{increase in length (mm)}}{\text{initial length (mm)}} \times 100\% \quad (4)$$

Stability studies

The hydrogel-gauze dressings (F1 to F6) were kept at room temperature ($28 \pm 2^\circ\text{C}$) and at a relative humidity of 74% for a span of 12 weeks. At week 12, a vapor transmission study (MVTR) and a moisture absorption study were carried out. The differences between the dressings at week one and at week 12 were plotted and compared using statistical analysis.

Animal study

The hydrogel-gauze that displayed the most desirable physico-chemical properties (F4) was further tested in an animal study. All animal tests were performed with the approval of the Universiti Kebangsaan Malaysia Animal Ethics Committee (UKMAEC) (FF/2012/FERN/23-MAY/430-MAY-2012-JAN-2013). Ten female and 30 male 6-week old NC/Nga mice (20–25 g each) were obtained from the Animal Care Laboratory of the Universiti Kebangsaan Malaysia (UKM), Bangi. The mice were fed a standard diet and received water *ad libitum*. During the experiment, mice were housed in a controlled environment with a 12-h light and dark cycle. The animals were divided into eight groups (with five mice per group): normal, DNFB-control, saline-control,

hydrogel-treated, Cetaphil-treated, Sebamed-treated, Physiogel-treated and Aqua cream-treated.

Induction of AD-like skin lesions and treatment

The method for inducing AD-like skin was adopted from Karki and co-workers¹⁷. Forty NC/Nga mice had their dorsal hair removed using an electric shaver and hair removing cream containing 80% thioglycolate one day prior to sensitization. The next day, 35 mice were anesthetized with diethylether, and 200 mL of 1% DNFB (w/v) in acetone/olive oil (3:1) was applied to the dorsal skin for six consecutive days. The remaining five mice were not induced and reserved as the normal control group. After six consecutive days of induction, the DNFB-control group was not treated, the saline-control group was treated with normal saline drops, the hydrogel-treated group was treated by applying a hydrogel-gauze, the Cetaphil-treated group was treated by applying Cetaphil Intensive Moisturizing Cream, the Sebamed-treated group was treated by applying Sebamed Moisturizing Body Lotion, the Physiogel-treated group was treated by applying Physiogel Hypoallergenic Cream and the Aqua cream-treated group was treated by applying aqueous cream. The dressing was wetted with distilled water prior to application. The creams were applied as a thin layer, just enough to cover the area of the AD-like skin lesion. The treatments were continued for eight consecutive days, once daily.

Measurement of transepidermal water loss

Transepidermal water loss (TEWL) on the dorsal skin of mice was measured using a skin evaporative water recorder (Tewameter TM310; Courage and Khazaka, Cologne, Germany). The resulting data were analyzed using a microprocessor and were expressed in g/m²/h. TEWL was measured before and after induction to confirm that an AD-like condition had developed. Then, TEWL was measured before and after eight days of treatment to compare the performance of different types of topical formulations to that of the hydrogel-gauze. Measurements were recorded when the TEWL readings had stabilized, approximately 30 s after the probe was placed on the skin. The percent reduction in TEWL was determined using Equation (5):

$$\begin{aligned} \text{Percentage of reduction in TEWL (\%)} \\ = \frac{\left\{ \begin{array}{l} \text{TEWL before treatment} \\ - \text{TEWL after treatment} \end{array} \right\}}{\text{TEWL before treatment}} \times 100\% \end{aligned} \quad (5)$$

Histological examination

The mice were sacrificed on the 15th day after induction by an over-dose inhalation of 80% (v/v) diethylether. The induced and treated dorsal skin was removed and fixed in a 10% formalin solution for 24 h. The tissues were embedded into paraffin, and 5 µm thick sections were prepared. The sections were stained with hematoxylin and eosin (H&E) and were observed under a light microscope to compare leukocyte infiltration, keratin deposition and stratum corneum integrity in the control and treatment groups.

Statistical analysis

All the data are expressed as the average $\pm$ standard deviation. All results were statistically evaluated using one-way ANOVA and the paired *t*-test. For both the one-way ANOVA and the paired *t* test, $p < 0.05$ was considered as a statistically significant difference.

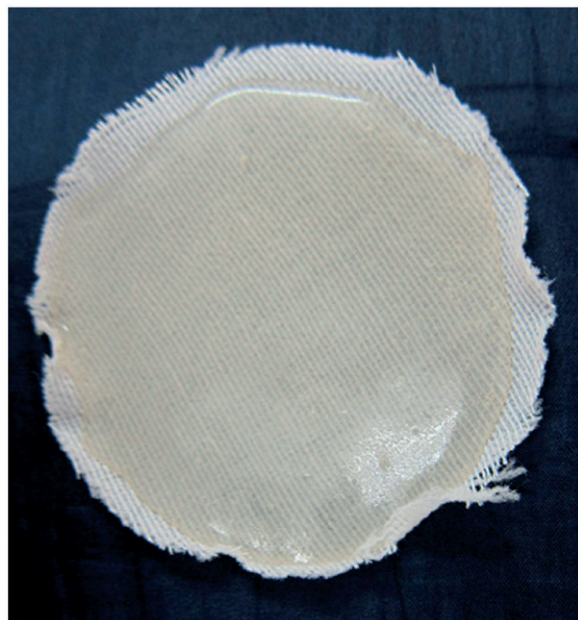


Figure 1. Hydrogel-gauze dressing is a layer of dried hydrogel cast over elastic conforming gauze bandage.

Results and discussion

The hydrogel-gauze dressing is a dried, transparent hydrogel layer cast over an elastic conforming gauze bandage. The dried gel and gauze are not separable. Figure 1 shows a representative photograph of a hydrogel-gauze dressing formulation.

Physical characterization

Based on the results, the NaCMC hydrogel-gauze dressings were homogenous, flexible, transparent, non-sticky films that were easily removed from the petri dish, except dressings F2 and F6, which have a sticky texture. The weight of the hydrogel-gauze dressings ranges between 5.62 ± 0.09 and 12.04 ± 0.11 g, and the thickness ranges between 0.578 ± 0.004 and 1.39 ± 0.012 mm (Figure 2). The low S.D. indicates that the preparation method is reproducible. At week 12, there was no change in the texture of the dressings. There was no change in the thickness or weight of the hydrogel-gauze dressings after 12 weeks of storage at room temperature ($28 \pm 2^\circ\text{C}$), except for in those of dressing F5. The texture of dressings F2 and F6 also changed and became oilier.

The physical characteristics of a dosage form depend on the tolerability by the patients¹⁸. Flexible dressings are desirable because they can be easily applied onto various parts of the body¹⁹. One of the important features of an ideal dressing is that it is easy to apply and remove from the skin without causing discomfort or pain²⁰. The F2 and F6 dressings are not preferable because they are sticky compared to the rest. The weight and thickness of the hydrogel-gauze dressing had decreased after three months of storage. This result may be due to the instability of PG in the formulation²¹, so the decrease in weight was due to the loss of plasticizer by volatilization or degradation during storage. According to a previous study²², PG has the ability to retain water in the formulation. When PG is lost by volatilization or degradation, the ability to bind to water decreases and this results in a decrease in thickness and weight of the dressings.

Moisture vapor transmission rate study

Moisture vapor transmission rate (MVTR) reflects the moisture retentiveness of a formulation. A MVTR of less than $840 \text{ g/m}^2/\text{day}$ indicates that the dressings have moisture retention capability²³.

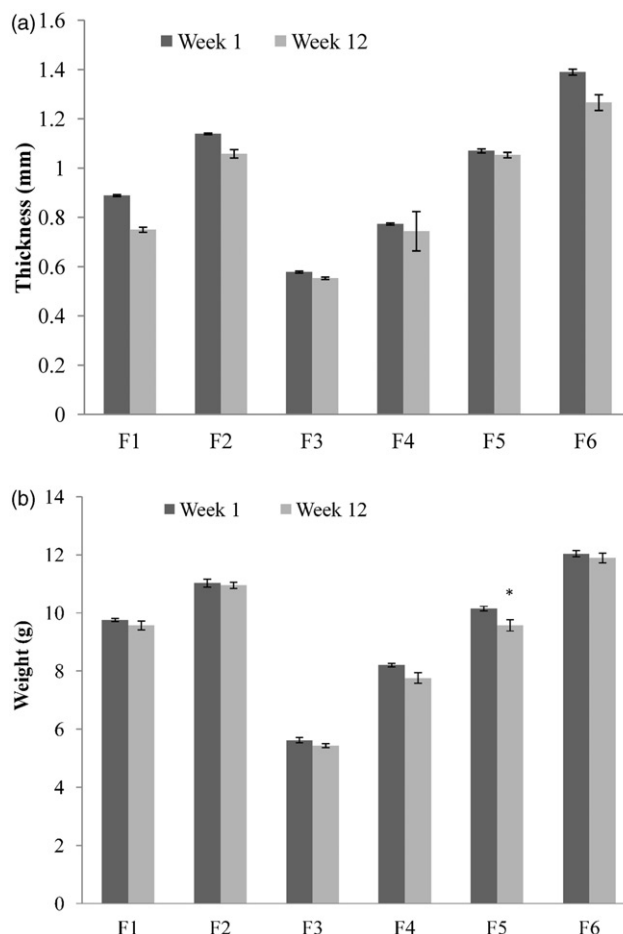


Figure 2. (a) Thickness and (b) weight of wet wrap-hydrogel dressings at week 1 and week 12, $n = 3$.

The results of the MVTR study are presented in Figure 3. In this study, all hydrogel-gauze dressings have MVTRs in the range of $400\text{--}640 \text{ g/m}^2/\text{day}$, showing that they all have moisture retention capabilities. Dressings with low MVTRs will be able to promote skin hydration as they can retain the surface moisture from being lost to the environment²⁴. This is ideal for patients with atopic dermatitis because these patients typically have dry skin due to a higher transepidermal water loss compared to patients with normal skin²⁵. There was a direct correlation between the MVTR and the amount of plasticizer. The MVTR increased when the amount of PG in the hydrogel-gauze dressings increased ($F6 > F5 > F4 > F3$). PG has a relatively small molecular size ($MW = 76.1$); thus, it can disperse between the NaCMC polymeric chains in the gel and form intermolecular forces with the polymers. This intercalates with the polymeric chain structure and leads to an increase in the porosity of the dressing, resulting in a higher MVTR²⁶. These findings are supported by a study by Wittaya-areekul and Prahsarn²⁷ that showed an increase in PG will increase the MVTR of a film. When comparing dressings that contain the same amount of PG but different amount of NaCMC polymer (F1 and F5, F2 and F6), the dressings with a high percentage of polymer had a higher MVTR than the dressings with a lower percentage of polymer. The MVTR also increased when the percentage of polymer increased in the formulation due to the hydrophilic nature of the polymer. This finding is supported by previous studies that show an increase in polymer will increase the MVTR^{28,29}.

After 12 weeks of storage, the MVTR of all the formulations had decreased significantly, except for that of F3. This result may be due to the loss of PG after prolonged storage at room temperature ($28 \pm 2^\circ\text{C}$). PG is an external plasticizer as it forms

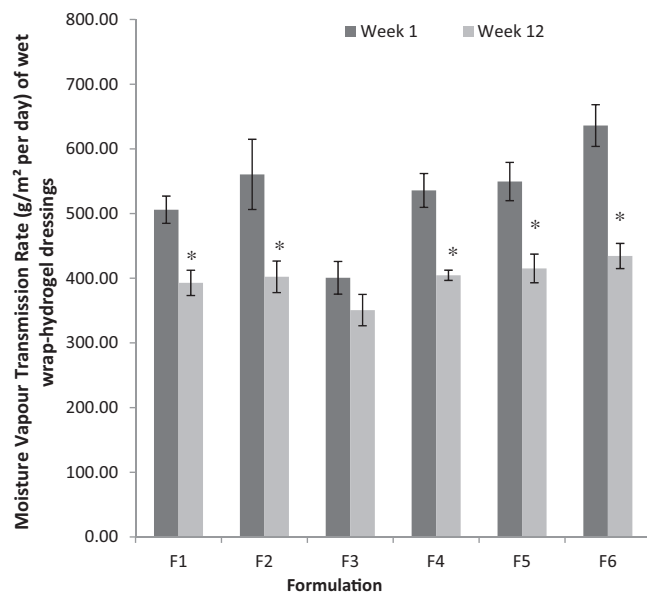


Figure 3. Moisture vapour transmission rate (MVTR) of hydrogel-gauze dressings (g/m^2 per day) at week 1 and week 12, $n=3$. *Denotes significant difference compared to week 1 ($p < 0.05$).

temporary intermolecular hydrogen bonds with polymer chains when it is added into polymer chains²⁶. This plasticizer can be lost by evaporation or migration because it is not chemically attached to the polymer chain by a covalent bond³⁰. When plasticizer is lost, the polymer structure realigns, resulting in a more compact structure of polymer chains. This structure is less permeable and thus leads to a decrease in the MVTR²¹.

Moisture absorption study

A moisture absorption study was performed to investigate the affinity of the dressings to environmental moisture. As shown in Figure 4, it was observed that of the hydrogel-gauze dressings with 4% NaCMC, the amount of moisture gained was higher if the formulation had a higher amount of PG (F6) compared to formulations with a lower amount of PG (F3, F4 and F5). PG is a hydrophilic molecule with moisture absorption properties due to the presence of polar groups in its structure. PG will absorb moisture from the atmosphere into the dressings. In addition, PG is a humectants that is able to absorb moisture from the environment³¹. These moisture absorption properties help to keep the dressing moist and thus overcome the disadvantage of the current wet-wrap treatment, in which the bandage will dry up easily. Furthermore, it was observed that the moisture absorption of dressings increased with an increase in polymer amount due to the hydrophilic nature of NaCMC ($F6 > F2$ and $F5 > F1$). Similar findings were previously reported³², in which the moisture content increased with increasing concentration of a hydrophilic polymer.

After 12 weeks, the moisture absorption capacity of the formulations decreased compared to week 1, except for that of F1, which was higher at week 12. This result may be due to the degradation and evaporation of the plasticizer after a prolonged period of storage²¹. For F3, F5 and F6, there was a significant decrease in the moisture absorption capacity after week 12 ($p < 0.05$). For formulations F1, F2 and F4, there were no significant differences in the moisture absorption capacity between week 1 and week 12.

Mechanical properties

An ideal dressing should be durable, resistant to external stress, soft, flexible and elastic enough to cope with the stress caused by

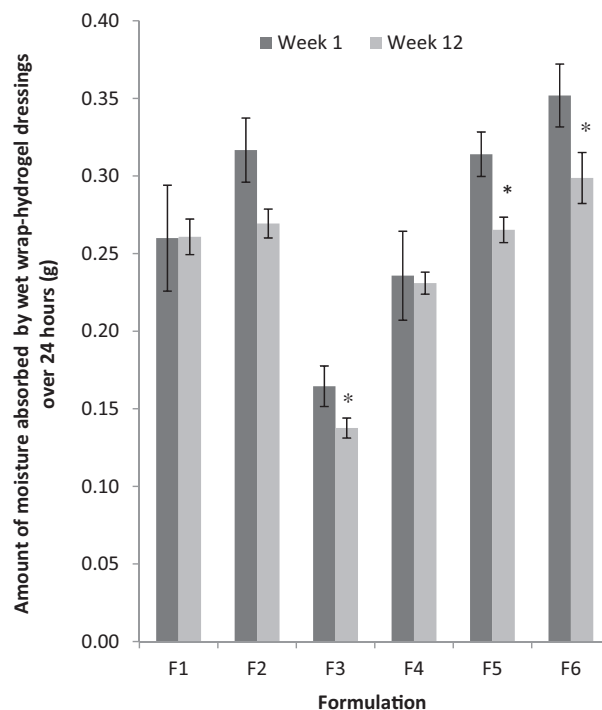


Figure 4. Moisture absorption of the hydrogel-gauze dressings over a period of 24 h for week 1 and week 12, $n=3$. *Represents significant difference in moisture absorption compared to week 1 ($p < 0.05$).

Table 2. Mechanical properties of hydrogel-gauze dressing dressings, $n=3$.

Formulation	Mechanical properties	
	Percentage elongation (% $\pm$ SD)	Tensile strength ($\text{N/mm}^2 \pm$ SD)
F1	90.74 $\pm$ 4.08	0.81 $\pm$ 0.04
F2	96.33 $\pm$ 4.93	0.59 $\pm$ 0.04
F3	84.09 $\pm$ 2.18	3.94 $\pm$ 0.12
F4	92.05 $\pm$ 2.89	1.88 $\pm$ 0.05
F5	89.80 $\pm$ 5.75	0.89 $\pm$ 0.05
F6	80.09 $\pm$ 5.58	0.61 $\pm$ 0.04

the movement of different body parts. This is important when dressings are applied on flexible surfaces, such as the elbow and knee. Thus, the mechanical properties are one of the important characteristics for eczema dressings. Tensile strength is the maximum force per unit area that can be applied before the dressings break and indicates the flexibility of the dressings. Meanwhile, the percentage elongation at break measures the percentage elongation of a dressing at its break point, which indicates the ductility and brittleness of the dressings¹⁹.

Table 2 shows that F3 has the highest tensile strength ($3.94 \pm 0.12 \text{ N/mm}^2$), while F2 has the lowest tensile strength ($0.59 \pm 0.04 \text{ N/mm}^2$). The results showed that formulations with higher amounts of PG had the lowest tensile strength. Both F1 and F5 contained the same amount of plasticizer but different amounts of NaCMC polymer. The results showed that the tensile strength of F1 was less than that of F5. As the plasticizer content increased (F3 to F6), the tensile strength decreased, and this decrease was statistically significant. From this study, it can be inferred that the plasticizer greatly influences the tensile properties of the dressings. PG has a small molecular weight and is therefore able to occupy the space between polymer chains. This increases the free volume between the polymer chains by reducing the interactions between the chains. The polymer chains easily slide

past one another, resulting in lower values of tensile strength and reduced brittleness²². This phenomenon made the dressings more flexible as the mobility of the polymer increased in the presence of the plasticizer compared to an unplasticized state. However, it was observed that all dressings have high values (80%–96%) of percentage elongation at break. The difference between percentage elongation for dressings F3, F4, F5 and F6 were not statistically significant, although PG content increased from F3 to F6. Thus, it was shown that the plasticizer has a significant effect on tensile strength but not on percentage elongation. The presence of a plasticizer in the polymer structure lowers the glass transition temperature of the dressings, which makes the dressings change from a rigid glassy polymer to a rubbery state, resulting in the formation of less stiff and more elastic hydrogel dressings²². A drawback to the mechanical study is that, unlike the MVTR and TEWL, measurements were not carried out at week 12. Further investigation is required to determine the hydrogel-gauze mechanical properties after 12 weeks of storage.

Animal study

Based on the study done by Shiohara et al.³³, NC/Nga mice are the most extensively studied animal model of AD since the inbred strain was established in 1957. These mice spontaneously develop AD-like eczematous skin lesions when kept in air-uncontrolled conventional housing but not when maintained under specific pathogen-free (SPF) conditions. However, when these mice are maintained under conventional conditions, the low incidence of AD-like lesions is a drawback. In contrast, the application of a chemical hapten, 2,4-dinitrofluorobenzene (DNFB) or 2,4-dinitrochlorobenzene (DNCB) produces 100% reproducible AD-like lesions in these mice³⁴. Because the clinical symptoms displayed in the NC/Nga mice treated with chemicals are similar to humans with AD, these mice are considered to be an AD animal model suitable for understanding and evaluating effective therapeutic strategies for AD.

Transepidermal water loss

Transepidermal water loss (TEWL) measures the rate at which water vapor is lost from the body through the skin³⁵ and is an important measurement in evaluating barrier functionality³⁶. The results of the TEWL experiments are presented in Figure 5. The TEWL reading increased after sensitization of mice by DNFB in this study, suggesting that abnormal epidermal barrier function had developed¹³. Damage to the stratum corneum and superficial skin layers not only results in physical vulnerability but also results in an excess rate of water loss. The TEWL reading decreased after treatment with either saline, hydrogel-gauze, Cetaphil Cream, Sebamed Lotion, Physiogel Cream or Aqueous Cream (Figure 5). There was a significant difference between the TEWL before and after treatment ($p < 0.05$) for all topical formulations. The saline-control group showed a decrease in TEWL from 44.4 g/m² to 43 g/m², a 2.98% reduction. Among all the topical formulations compared in this study, the hydrogel-gauze was the most effective, decreasing TEWL from 45.3 g/m² to 7.4 g/m² after 8 days of treatment, an 83.67% reduction. The next most effective treatment was Cetaphil Cream, which decreased TEWL by 77.01%, followed by Aqueous Cream (73.87%), Sebamed Lotion (70.64%) and, lastly, Physiogel Cream (50.18%).

The reduction of TEWL in treated mice implies that the damaged epidermal barrier was recovering¹³. This study shows that the hydrogel-gauze-treated group had the highest percentage of TEWL reduction, demonstrating that the hydrogel-gauze dressing may have a superior therapeutic performance in lowering TEWL compared to other commercial formulations and may accelerate the recovery of the damaged epidermal barrier in

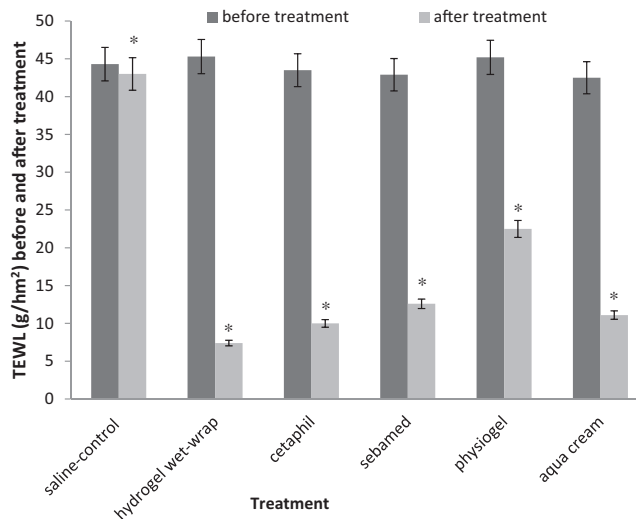


Figure 5. Transepidermal water loss (TEWL) of NC/Nga mice before and after treatment for eight days, $n = 5$. *Represents significant difference in TEWL compared to before treatment ($p < 0.05$).

NC/Nga mice. The suggested mechanism for this hydrogel-gauze dressing is that it acts as an occlusive dressing that rehydrates dry and damaged stratum corneum by promoting a moist healing environment. In addition, it reduces dry skin pain by cooling the dry skin surface, and it has a gauze layer to protect dry skin from secondary damage and to act as a mechanical barrier.

Histological examination

Histological examination of mice tissue was conducted by staining with H&E. Hematoxylin colors the nuclei of cells blue. The nuclear staining was followed by counterstaining with eosin, which colors eosinophilic structures in various shades of red, pink and orange³⁷. The parameters used in the histology examination to compare the performances of the hydrogel-gauze dressing and other commercial formulations were the presence or absence of leukocyte infiltration, keratin deposition and the ability to improve the integrity of the stratum corneum. These parameters are among the important pathological hallmarks of moderate-to-severe AD.

The staining of the tissue from the normal group showed an absence of keratin deposition, an absence of leukocyte infiltration and complete integrity of the stratum corneum (Figure 6a). There are five major layers of keratinocytes in the epidermis. The bottom layer of epidermis, the stratum basale, has column-like cells. The cells divide and push previously formed cells into higher level towards the stratum corneum. As cells move into the higher layers, they flatten and eventually die and slough off³⁸. The normal cycle of maturation of keratinocytes is 28–30 days. In dry skin, this cycle is accelerated to 3–4 days. In other words, the replacement of cells is accelerated by injury. This phenomenon can be seen in the DNFB-control group shown in Figure 6(b(i)), in which keratin deposition occurred and the cells could not reach the outer-most layer of the epidermis. The other treatment groups did not show keratin deposition. The stratum corneum was severely disrupted in DNFB-control mice (Figure 6b(ii)). Saline treatment showed negative effects in regenerating stratum corneum after 8 days as the histology examination revealed that the stratum corneum remained disrupted (Figure 6c). The hydrogel-gauze dressing and other commercial formulations were able to preserve the integrity of the stratum corneum as the five layers of the stratum corneum remained intact.

Infiltration is the diffusion or accumulation of substances that are either not normally present or are present in excess amounts³⁹.

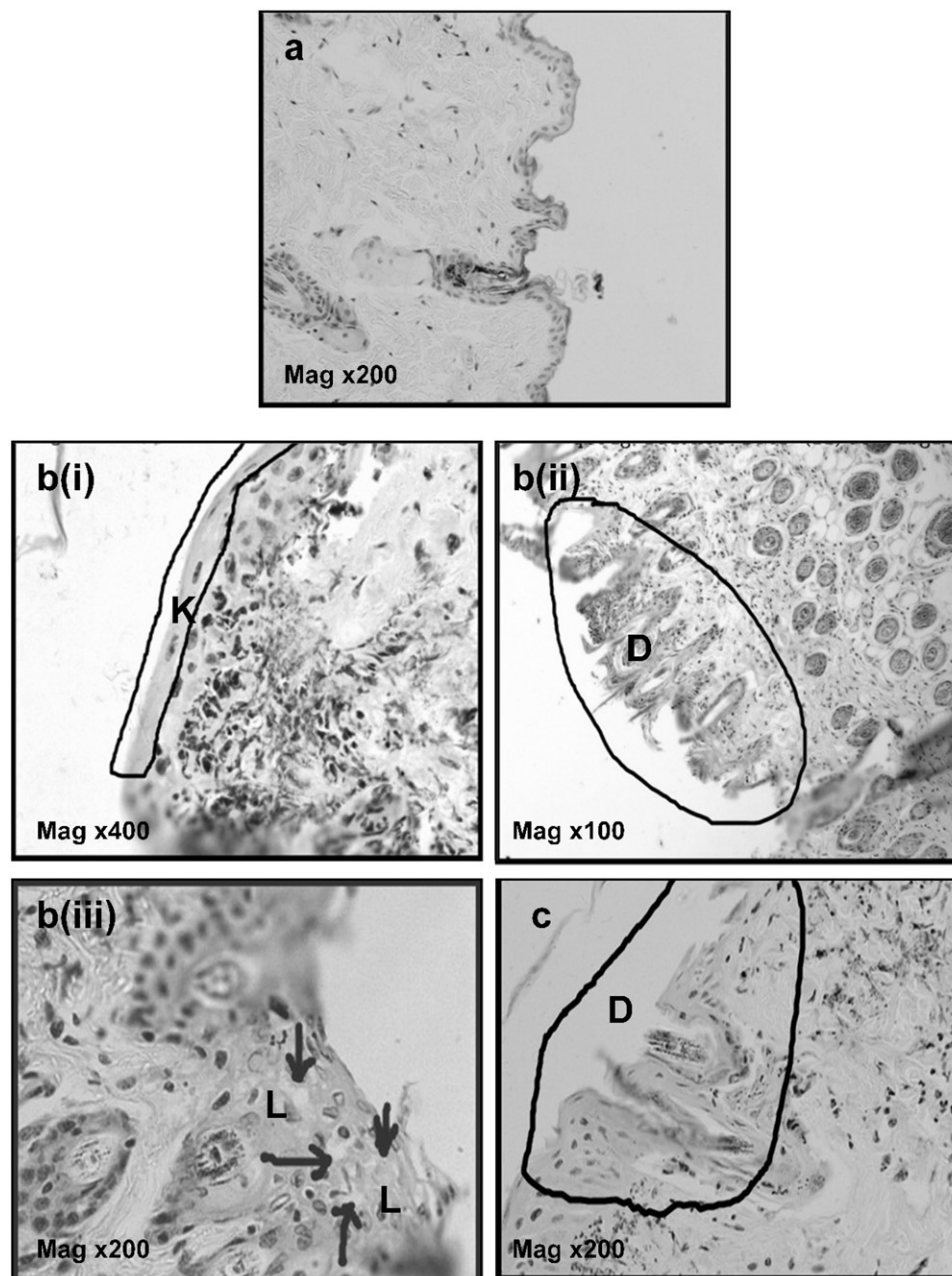


Figure 6. Histological examination of NC/Nga mice after 8 days of (a) normal group, (b)–(d) DNFB-control group and (e) saline-control group. K indicates keratin deposition in stratum corneum, D indicates stratum corneum disruption, L and black arrows indicate leukocyte infiltration.

In the DNFB-control group, the epidermis and dermis became thick because of leukocyte infiltration (Figure 6b(iii)), induced by the infiltration of immunocytes on the skin such as T cell, mast cell and eosinophils, which are the main features of AD⁴. Leukocyte infiltration was also observed in the Cetaphil-treated group (Figure 7b) and the Sebamed-treated group (Figure 7c), demonstrating that the skin did not fully recover after 8 days. In contrast, no leukocytes were found in the hydrogel-gauze group on the 8th day of the treatment (Figure 7a). The whole epidermis layer of the Aqueous Cream-treated group looked thinner (Figure 7e). This result may be due to the skin-thinning effect of Aqueous Cream because a previous study found that prolonged application of Aqueous Cream can cause thinning of the skin's outermost layer⁴⁰.

Thus, based on a histological examination, it can be concluded that both Physiogel Cream and the hydrogel-gauze dressing have superior therapeutic abilities in treating the AD condition

compared to the other treatments because both the hydrogel-gauze dressing and the Physiogel-treated groups exhibited an intact stratum corneum, the absence of leukocyte infiltration and the absence of keratin deposition (Figure 7a and d) after 8 days of treatment.

Conclusion

Hydrogel-gauze dressings were successfully developed using NaCMC and PG. Among the six formulations, F4 showed the most desirable physicochemical properties in terms of thickness, weight, mechanical strength, TEWL, and moisture absorption and was the most stable over 3 months. Using NC/Nga mice, the hydrogel-gauze dressing showed superior therapeutic performance as an occlusion dressing compared to commercial products. Hydrogel-gauze dressings may be a useful alternative to wet-wrap therapy for chronic AD in children as continuous remoistening is

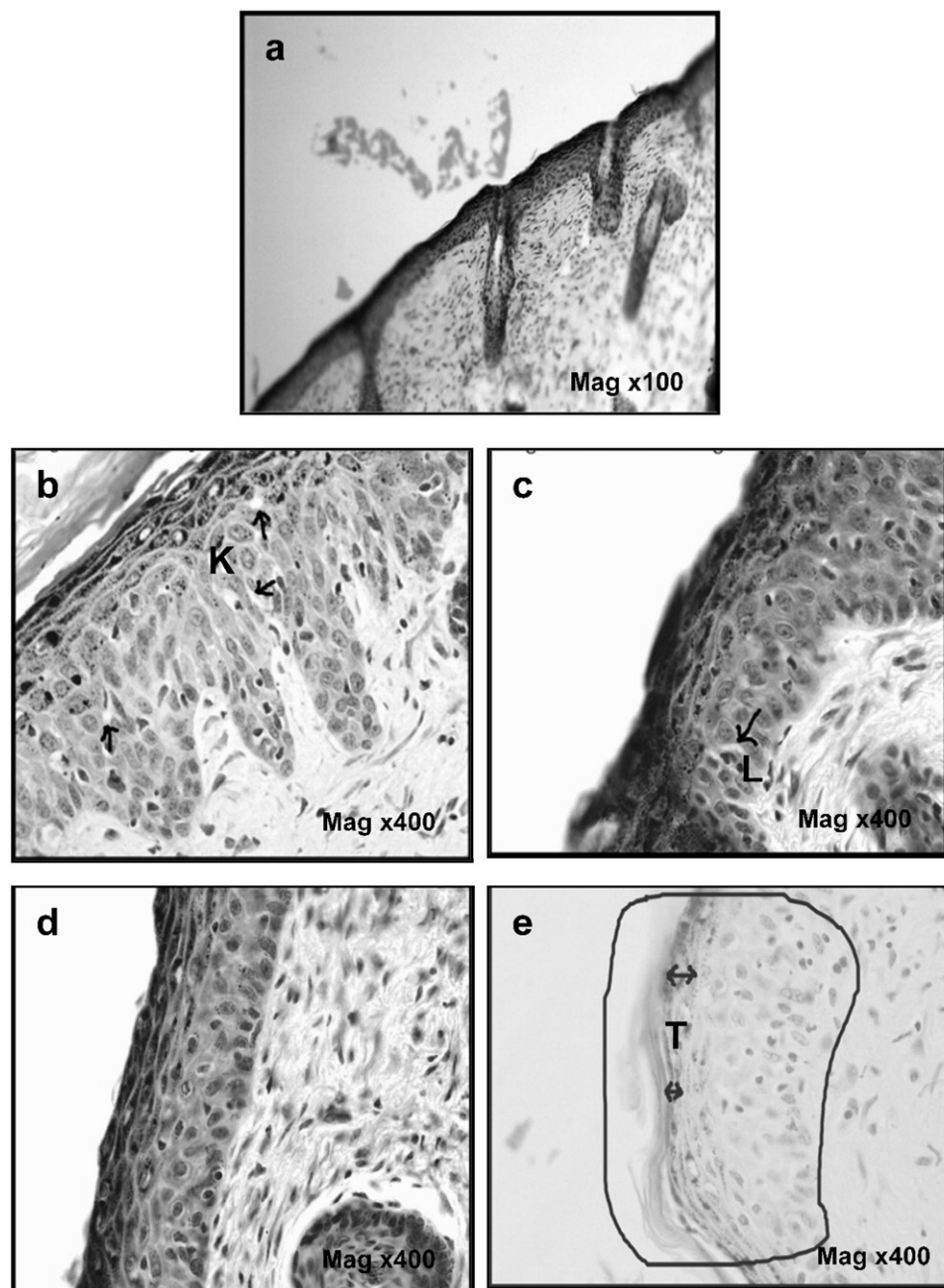


Figure 7. Histological examination of NC/Nga mice after 8 days with treatment of (a) hydrogel gauze wrap, (b) Cetaphil, (c) Sebamed, (d) Physiogel group and (e) Aqua cream. K indicates keratin deposition in stratum corneum, L indicates leukocyte infiltration and T shows the thinning of epidermis layer.

not required. This research shows that hydrogel-gauze dressings are a potential treatment method for moderate-to-severe AD.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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References

- Chan SK, Burrows NP. Atopic dermatitis. *Medicine* 2009;37:242–5.
- Krakowski AC, Dohil MA. Topical therapy in pediatric atopic dermatitis. *Semin Cutan Med Surg* 2008;27:161–7.
- Shekariah T, Kalavala M, Alfaham M. Atopic dermatitis in children: a practical approach. *J Paediatr Child* 2011;21:112–18.
- Yun MY, Yang JH, Kim DK, et al. Therapeutic effects of Baicalein on atopic dermatitis-like skin lesions of NC/Nga mice induced by *Dermatophagoides pteronyssinus*. *Int Immunopharmacol* 2010;10:1142–8.
- Guttman-Yassky E, Nograles KE, Krueger JG. Contrasting pathogenesis of atopic dermatitis and psoriasis – Part I: clinical and pathologic concepts. *J Allergy Clin Immunol* 2011;127:1110–18.
- Leung DYM, Jain N, Leo HL. New concepts in the pathogenesis of atopic dermatitis. *Curr Opin Immunol* 2003;15:634–8.
- Camfferman D, Kennedy JD, Gold M, et al. Eczema and sleep and its relationship to daytime functioning in children. *Sleep Med Rev* 2010;14:359–69.
- Hong J, Buddenkotte J, Berger TG, et al. Management of itch in atopic dermatitis. *Semin Cutan Med Surg* 2011;30:71–86.
- Wingfield C. Skin care: managing dry skin conditions. *Wound Essentials* 2011;6:50–8.
- Goodyear HM, Spowart K, Harper JI. Wet-wrap dressings for the treatment of atopic eczema in children. *Br J Dermatol* 1991;126:604.

11. Dabade TS, Davis DMR, Wetter DA, et al. Wet dressing therapy in conjunction with topical corticosteroids is effective for rapid control of severe pediatric atopic dermatitis: experience with 218 patients over 30 years at Mayo Clinic. *J Am Acad Derm* 2012;67:100–6.
12. Hon EKL, Wong KY, Cheung LK, et al. Efficacy and problems associated with using a wet-wrap garment for children with severe atopic dermatitis. *J Dermatol Treat* 2007;18:301–5.
13. Lee JH, Lee SJ, Kim DS, et al. The effect of wet-wrap dressing on epidermal barrier in patients with atopic dermatitis. *J Eur Acad Dermatol Venereol* 2007;21:1360–8.
14. Kulkarni RV, Sreedhar VS, Mutalik C, et al. Interpenetrating network hydrogel membranes of sodium alginate and poly(vinyl alcohol) for controlled release of prazosin hydrochloride through skin. *Int J Biol Macromol* 2010;47:520–7.
15. Ramli NA, Wong TW. Sodium carboxymethylcellulose scaffolds and their physicochemical effects on partial thickness wound healing. *Int J Pharm* 2011;403:73–82.
16. Kim JO, Park JK, Kim JH, et al. Development of polyvinyl alcohol–sodium alginate gel-matrix-based wound dressing system containing nitrofurazone. *Int J Pharm* 2008;359:79–86.
17. Karki R, Jung M-A, Kim K-J, et al. Inhibitory effect of *Nelumbo nucifera* (Gaertn.) on the development of atopic dermatitis-like skin lesions in NC/Nga mice. *Evid Based Complement Alternat Med* 2012;2012:153568.
18. York P. The design of dosage forms. In: Aulton ME, ed. *Pharmaceutics: the science of dosage form design*. 2nd ed. London: Churchill Livingstone; 2001:1–12.
19. Boateng JS, Matthews KH, Stevens HNE, et al. Wound healing dressings and drug delivery systems: a review. *J Pharm Sci* 2007;97:2892–923.
20. Asane GS, Nirmal SA, Rasal KB, et al. Polymers for mucoadhesive drug delivery system: a current status. *Drug Dev Ind Pharm* 2008;34:1246–66.
21. Heng PWS, Chan LW, Ong KT. Influence of storage conditions and type of plasticizers on ethylcellulose and acrylate films formed from aqueous dispersions. *J Pharm Sci* 2003;6:334–44.
22. Boateng SJ, Stevens HNE, Eccleston GM, et al. Development and mechanical characterisation of solvent-cast polymeric films as potential drug delivery systems to mucosal surfaces. *Drug Dev Ind Pharm* 2009;35:986–96.
23. Seaman S. Dressing selection in chronic wound management. *J Am Pediatr Medical Assoc* 2002;92:24–33.
24. Pal K, Banthia AK, Majumdar DK. Polymeric hydrogels: characterisation and biomedical applications – a mini review. *Des Monomers Polym* 2009;12:197–220.
25. Di Nardo A, Wertz P, Giannetti A, et al. Ceramide and cholesterol composition of the skin of patients with atopic dermatitis. *Acta Dermatol Venereol* 1998;78:27–30.
26. Pongjanyakul T, Puttipatkhachorn S. Alginate-magnesium aluminum silicate films: effect of plasticizers on film properties, drug permeation and drug release from coated tablets. *Int J Pharm* 2007;333:34–44.
27. Wittaya-areekul S, Prahsarn C. Development and in vitro evaluation of chitosan-polysaccharides composite wound dressings. *Int J Pharm* 2006;313:123–8.
28. Dey SMA. Preparation of carvedilol transdermal patch and the effect of propylene glycol on permeation. *Int J Pharm Pharm Sci* 2010;2:137–43.
29. Lim SJ, Lee J, Piao M, et al. Effect of sodium carboxymethylcellulose and fucidic acid on the gel characterization of polyvinylalcohol-based wound dressing. *Arc Pharm Res* 2010;33:1073–81.
30. Vieira MGA, Silva MA, Santos LO, et al. Natural-based plasticizers and biopolymer films: a review. *Eur Polym J* 2011;47:254–63.
31. Amnuait C, Ikeuchi I, Ogawara K, et al. Skin permeation of propranolol from polymeric film containing terpene enhancers for transdermal use. *Int J Pharm* 2005;289:167–78.
32. Chauhan I, Bajpai M. Formulation and evaluation of transdermal drug delivery of raloxifene hydrochloride. *Int J Pharm Sci Res* 2010;1:72–9.
33. Shiohara T, Hayakawa J, Mizukawa Y. Animal models for atopic dermatitis: are they relevant to human disease? *J Dermatol Sci* 2004;36:1–9.
34. Taek KK, Kim J-C. In vitro skin permeation and anti-atopic efficacy of lipid nanocarriers containing water soluble extracts of *Houttuynia cordata*. *Drug Dev Ind Pharm* 2013. [Epub ahead of print]. doi: 10.3109/03639045.2013.819883.
35. Francesco G, Leonardo C. The dynamics of TEWL from hydrated skin. *Skin Res Technol* 2002;8:178–86.
36. Laudanska H, Reduta T, Szmikowska D. Evaluation of skin barrier function in allergic contact dermatitis and atopic dermatitis using method of the continuous TEWL measurement. *J Dermatol Venereol* 2003;48:123–7.
37. Llewellyn BD. Nuclear staining with alum-hematoxylin. *J Biotech Histochem* 2009;84:159–77.
38. James W, Berger T, Elston D. Andrews' diseases of the skin. *J Clin Dermatol* 2005;10:5–6.
39. Kawakami Y, Uchiyama Y, Esteban CR, et al. Sall genes regulate region-specific morphogenesis in the mouse limb by modulating Hox activities. *Development* 2009;136:585–94.
40. Tsang M, Guy RH. Effect of aqueous cream BP on human stratum corneum in in vivo. *J Dermatol* 2010;163:954–8.