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Research Paper

Enhanced skin permeation and hydration by magnetic field array: preliminary in-vitro and in-vivo assessment

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Abstract

Objectives The aim of the study was determine the effect of magnetic film array technology on the skin permeation of urea.

Methods A 5% urea gel was applied to human epidermal membrane *in vitro* and human skin *in vivo*. Application of gel with magnetic film array and plastic occlusive film was compared with application of gel with a plastic occlusive film and non-magnetic film. In-vitro epidermal penetration was determined using a Franz-type diffusion system. In-vivo permeation and changes in epidermal properties were visualised by optical coherence tomography.

Key findings The mean cumulative permeation of urea over 2 h for magnetic film array application was 89.54 ± 7.34 $\mu g/cm^2$ as compared with 20.83 ± 2.02 $\mu g/cm^2$ for passive occluded application (mean ± SEM, $n = 9/8$), representing greater than 4-fold increase over the 2-h application time period. Administration of urea with the magnetic film array resulted in the lag time being reduced from 40.58 ± 3.98 to 21.13 ± 6.27 min ($P < 0.02$), while steady state flux increased from 0.24 ± 0.03 to 0.75 ± 0.06 $\mu g/cm^2$ per min ($P < 0.0001$). Under active occlusion, the relative change in epidermal thickness as determined by optical coherence tomography increased by 16 and 11% at 30 and 60 min, respectively.

Conclusions Administration with a novel magnetic film array technology provided enhanced skin penetration of urea and increased epidermal hydration when compared with administration under an occlusive film only.

Keywords dry skin; magnetic field array; moisturising agents; optical coherence tomography; skin permeation

Introduction

Dry skin is the most common dermatological problem and is widely treated by topical application of moisturisers of many different compositions. Rawlings and Matts have provided excellent reviews on the role of water in the skin, dry skin and moisturisation.[1–3] Water content in and water loss from the stratum corneum play important roles in the hydration of the outer stratum corneum layers to maintain skin flexibility and in providing sufficient water to facilitate enzyme reactions involved in stratum corneum maturation, corneodesmolysis and desquamation.[4] A complex mixture of low molecular weight, water-soluble compounds known as natural moisturising factors is present in the stratum corneum to assist in moisture retention.[5]

Dry skin can be induced by a number of factors, including: low environmental temperature and humidity; abrupt changes in conditions associated with modern indoor climate-controlled environments; soap washing causing loss of lipid and natural moisturising factors from the stratum corneum; ageing and genetics.[6] Blank demonstrated that stratum corneum containing less than 10% water content is brittle.[7] If untreated, a 'dry skin cycle' is established, leading to scaly skin with increased hardness and brittleness.[3] This occurs because the superficial dehydration of the stratum corneum induces release of inflammatory mediators, hyperproliferation and disruption of epidermal differentiation. However, the dry skin cycle can be reversed by intervention with suitable moisturising agents.

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Humectants, occlusives and emollients are the most commonly used moisturiser components for the management of dry skin.[8] Other moisturising agents include bilayer-forming lipids including ceramides and phospholipids, hydroxyacids and agents that induce epidermal differentiation and lipogenesis, such as ligands for the peroxisomal proliferator-activated receptor (e.g. linoleic and other long-chain fatty acids), niacinamide and vitamin C. Rawlings and Matts recently reviewed the range of moisturisers, their properties and mechanisms of action.[6]

Urea has been used as a humectant in moisturising creams since 1943[9] and is a natural component of stratum corneum natural moisturising factors. Moisturisers containing urea have been reported to improve stratum corneum barrier function, reduce transepidermal water loss, increase skin capacitance and reduce irritation.[10,11] Occlusive formulations such as petroleum jelly, oils and occlusive dressings act as an occlusive film on the skin surface to reduce transepidermal water loss and thereby hydrate the skin. Many moisturisers contain combinations of agents to optimise the hydrating effect of the product on the skin.

In the current study, the influence of an un-powered magnetic film array on the permeation of urea into and through the epidermis was evaluated *in vitro* and the consequent hydration effect determined *in vivo*. In-vitro permeation of urea through human epidermis was evaluated in a Franz cell diffusion system using standard protocols. In-vivo determination of the hydration effects of the administered urea was carried out by optical coherence tomography (OCT) in human volunteers. Urea was administered as a simple measured dose of gel with occlusion film (control) or with occlusion film with the additional magnetic film array placed externally to the occlusive (active).

Materials and Methods

Materials

All the chemicals and reagents listed below were used as supplied: urea gel was supplied as a 5% w/w urea in VersaBase Gel (PCCA, Huston, TX, USA) as supplied from Compounding on Oxford (Perth, WA, Australia); phosphate-buffered saline solution pH 7.4 (PBS) was prepared according to the United States Pharmacopeia. *p*-Dimethylaminobenzaldehyde (DMAB) was purchased from BDH Laboratory Chemicals Group (Poole, England). Ethanol was obtained from CSR Distilleries Group (Sydney, Australia) and concentrated sulfuric acid was from Lab Scan Asia Co Ltd (Bangkok, Thailand). Passive occlusive material used for in-vitro and in-vivo studies consisted of a polymer film of similar thickness and cut to the same dimensions as the active magnetic polymer material. In addition, all in-vitro cells and in-vivo sites were further occluded with Parafilm M polymer film.

Magnetic film array material consisted of 35 mm × 35 mm sections of unpowered flexible array matrix (ETP Type 008), a proprietary enhanced transdermal delivery array film developed by OBJ Ltd (Perth, WA, Australia). The magnetic film array (ETP Type 008) is a thin flexible polymer matrix containing multiple magnetic elements arranged to produce complex 3-dimensional magnetic gradients. The material has a peak magnetic field strength of 40 mT. However, the arrangement and distribution of alternating poles across the surface of the material results in a total magnetic gradient of 2 T/m^2.

Spectroscopic analysis

Urea quantification was based on the analytical method of Knorst *et al.*,[12] a modified derivatisation by DMAB into a coloured compound. Derivatisation of urea in skin diffusion samples employed equal volumes of sample solution and the DMAB reagent (4% w/v) prepared with concentrated sulfuric acid (4% v/v) in alcohol (95%). In this case, 200 μl of the urea sample was mixed with 200 μl of the DMAB reagent. After 10 min the absorbance of the coloured derivatised solution was measured at 420 nm using a UVmini-1240 UV-vis spectrophotometer (Shimadzu Scientific Instruments, Sydney, NSW, Australia) against an appropriate reagent blank (receptor solution processed as for skin permeation receptor solution samples).

In-vitro skin diffusion studies

Preparation of epidermal membrane

Epidermal membranes were obtained from human skin sourced from Perth hospitals (abdominal region following abdominoplasty surgery; three female donors aged 50, 39 and 44 years, respectively) with ethical approval from the Human Research Ethics Committee of Curtin University. Epidermal membranes were obtained by the heat separation method.[13] Briefly, the subcutaneous tissue was removed by dissection and then full-thickness human skin was immersed in water at 60°C for 1 min. The epidermal membrane was teased off the dermis, placed onto aluminium foil with the stratum corneum facing upward, air dried for 15 min and then stored at −20°C (for not more than 2 months) until required.

In-vitro diffusion studies

In-vitro diffusion studies across human epidermis were performed using Pyrex glass Franz-type diffusion cells (enabling permeation across epidermal membranes of cross-sectional area 1.18 cm^2); receptor volume was approximately 3.5 ml. The membrane was placed between the donor and receptor compartment of the cell and allowed to equilibrate for 1 h with PBS in the receptor compartment, which was stirred continuously with a magnetic stirrer. PBS (1 ml) was placed in the donor and receptor compartments of the cell, which was placed in a water bath maintained at 37 ± 0.5°C. Epidermal membrane integrity was then determined by visual inspection over a bright light and electrical resistance (kΩ), capacitance (nF) and impedance (kΩ) measurements using a digital portable LCR meter (TH2821/A/B, Tonghui (Changzhou) Electronic Co., Ltd, Changzhou, China). The measurements were taken by immersing the stainless steel probe lead tips, one each in the donor and receptor compartments.[14] Membranes exhibiting an electrical resistance of less than 20 kΩ were rejected from the study. The diffusion cells were emptied, and receptor compartments refilled with fresh preheated PBS at 37 ± 0.5°C. Urea gel (0.5 g) was placed in the donor compartment. Sections of magnetic film array were cut to a

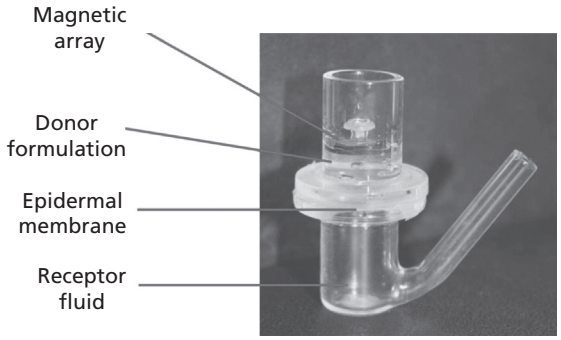

Figure 1 In-vitro setup showing placement of ETP film

size suitable for insertion into the donor compartment of the Franz-type cell and suspended above the gel (Figure 1), while passive cells had a non-magnetic polymer film of similar dimensions placed above the gel. All cells were also occluded by sealing the top of the donor compartment of the cell with Parafilm. Aliquots from the receptor phase were withdrawn from the sampling arm and replaced with fresh pre-heated (37°C) PBS over a 2-h period. The total urea content permeating the epidermal membrane to the receptor solution samples obtained from individual experiments was determined by spectroscopic analysis. After 2 h the donor and receptor fluids were recovered, the cell disassembled and the skin epidermal membrane examined for obvious tears (any cells with torn membranes were rejected). Experiments were repeated nine times for magnetic film array enhanced (active) and eight times for passive (control) diffusion experiments. The cumulative amount of urea permeating through the epidermis to the receptor compartment (μg/cm^2) was plotted as a function of time (h) and the steady state flux (μg/cm^2 per h), permeability coefficient (cm/min) and lag time (min) were determined.

Statistical analysis

Magnetic film application and passive application were analysed using a linear mixed effects model. Data were transformed using the square-root of the measures to stabilise the variance. The within-cell correlation over time was accommodated using a first-order autoregressive correlation structure. Post-hoc comparisons between conditions at each time point were evaluated using the Mann–Whitney *U*-test. Permeation parameters were calculated from the data: steady state flux, permeability coefficient, lag time and cumulative amount of urea permeated at 2 h. Comparison of the parameters obtained for active and control administration were compared by unpaired *t*-tests.

In-vivo hydration: OCT assessments

Direct measurement of penetration of urea into the skin *in vivo* is difficult and so OCT was used to measure the physical changes in skin properties. Over the past decade OCT has emerged as a high-resolution optical diagnostic imaging modality widely used in ophthalmology[15,16] and dermatology.[17–19] Although the spatial resolution (typically 5–20 microns) of OCT is not as good as that of histology, it enables non-invasive, in-vivo imaging of the internal tissue microstructures. This resolution is much higher than most of the current clinical diagnostic technologies such as X-ray, computed tomography or magnetic resonance imaging. In addition, unlike X-ray and computed tomography, OCT does not use ionising radiation, instead using light waves in the near infrared. While this significantly reduces the penetration depth to a few millimetres (approx. 3 mm) due to the optical scattering of tissue, it is sufficient to reveal images of the stratum corneum, epidermis, upper dermis and hair follicles as well as sweat glands, where most skin pathologies and conditions occur, including skin cancers.[17,19–21] Crowther *et al*.[22] recently demonstrated a strong, positive correlation in measurement of stratum corneum thickness between OCT and confocal Raman spectroscopy, with the latter technology offering higher resolution for thinner stratum corneum sites ($\approx$<15 μm).

The operation of OCT is based on interference of light from a low coherence broadband light source. The working principle is analogous to ultrasound imaging,[19,23] being based on the reflection of a signal from the tissue but utilising light rather than acoustic waves. Unlike ultrasound, no direct tissue coupling is required for OCT imaging. As the tissue sample consists of different types of cells, organelles and microstructures, each with different reflective index, the incoming light beams are reflected and backscattered from different boundaries within the tissue. Thus, OCT is an important tool in monitoring transdermal delivery of drugs that alter the skin structure.

OCT measurements were carried out using a commercial swept-source FD-OCT system (Thorlabs, Newton, NJ, USA). This system comprised a broadband, high-speed frequency swept laser and a fibre-based Michelson interferometer with a balanced detection scheme to perform depth profiling and 3-dimensional image reconstruction at video rates. The system had a handheld probe to enable in-vivo scanning of the skin. The light source had a centre wavelength of 1325 nm and a full-width at half-maximum bandwidth of 100 nm. The average output power to the skin was 10 mW, with a maximum axial scan rate of 16 kHz. Axial resolution was 12 μm and transverse resolution was 15 μm. Maximum imaging depth was 3 mm, with a maximum imaging width of 10 mm. The scanning process involved axial and transverse scanning of the tissue sample. An axial or depth scan (A-scan) was obtained by translating the reference arm length, resulting in localised interference fringes with amplitudes related to sample reflectivity.[19] Adjacent A-scans were combined to produce B-scans (i.e. 2-dimensional images), which depict the tissue cross-sectional subsurface structures of the tissue.

The study was conducted on a human volunteer (male, 55 years old) with a healthy skin type with ethical approval from the University of Western Australia. The OCT measurements were carried out on two regions of skin. The first region on the inside right forearm had 5% urea gel topically applied in conjunction with occlusive Parafilm with the addition of an external ETP Type 008 (active). The second region, in a similar position on the left arm, had the same amount of 5% urea gel applied and then occluded using Parafilm and a passive polymer sheet of the same dimensions as the ETP Type 008. OCT images were acquired at 0, 30 and 60 min in triplicate. Four sites were measured from each

region, resulting in 72 images that were used to determine changes in epidermal thickness in response to penetration of the active ingredient, urea. The epidermal thickness was calculated from OTC images by boundary differentiated pixel counts of the region encompassed by the stratum corneum and dermal/epidermal boundary from each image. This was then coloured using a primary fill and the thickness averaged over 5-mm widths across the image (Figure 2). The average thickness was calculated for each site at each time point and the percentage increase in thickness calculated for times 30 and 60 min relative to time 0. The error of the thickness measurement was 10–15 μm.

Results

Urea analysis

Spectrophotometric analysis was carried out for quantitative analysis of urea that permeated the skin. Upon derivatisation with 4% DMAB reagent, a coloured derivatised compound was formed in the presence of urea. All calibration curves of urea standards showed good linearity over the concentration range of 7.8–125 μg/ml ($r^2 = 0.99$; $n = 5$). The limit of detection (LOD) and limit of quantitation (LOQ) of the assay were 0.83 and 2.5 μg, respectively.

In-vitro skin diffusion study

The in-vitro permeation profiles of urea across human epidermis are presented in Figure 3 and permeation parameters are given in Table 1. The results were compiled from nine active cells (occlusion plus magnetic film) and eight control cells (occlusion plus control film). A comparison of the cumulative amount of urea penetrating the epidermis to the receptor solution versus time was plotted for passive and magnetic array enhanced applications (Figure 3). A linear mixed effects model analysis showed a significant difference between groups ($F_{1,15} = 19.92$; $P = 0.0005$), a significant difference over time ($F_{1,134} = 156.45$; $P < 0.0001$) and a significant group–time interaction ($F_{1,134} = 23.02$; $P < 0.0001$). There was a significant difference between groups at all time points ($P < 0.02$). Mann–Whitney U values for each comparison are included in Table 2.

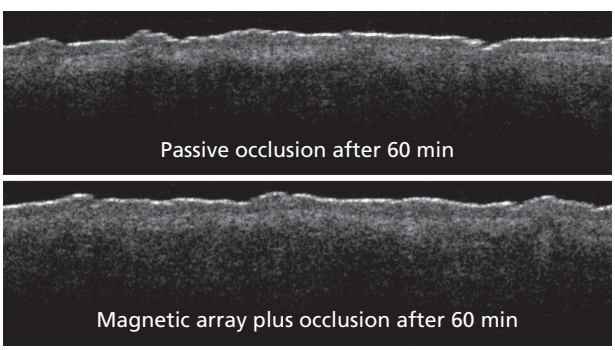

Figure 2 Optical coherence tomography images. Examples of optical coherence tomography images of human skin at 60 min after application of urea gel with passive occlusion and ETP magnetic array with occlusion

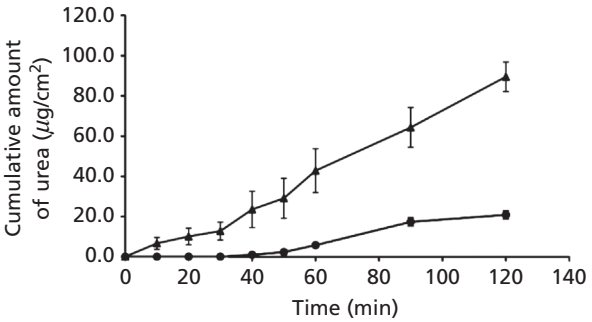

Figure 3 In-vitro permeation profile of urea across human epidermis. Cumulative amount of urea (μg/cm^2) permeating human epidermis to the receptor compartment during application of 5% urea gel with ETP 008 (▲) verses passive occluded (●) conditions (mean ± SEM of nine and eight cells, respectively)

There was a significant increase in the mean cumulative permeation of urea over 2 h for ETP application (89.54 ± 7.34 μg/cm^2) as compared with passive occluded application (20.83 ± 2.02 μg/cm^2; $P < 0.0001$, unpaired t-test). All permeation parameters were significantly enhanced by magnetic application (based on unpaired t-tests). The lag time was reduced from 40.58 ± 3.98 to 21.13 ± 6.27 min ($P < 0.02$), while steady state flux increased from 0.24 ± 0.03 to 0.75 ± 0.06 μg/cm^2 per min ($P < 0.0001$) by administration of urea with the magnetic film array (Table 1).

In-vivo hydration: OCT assessments

Examples of OCT images are provided in Figure 2. Over all measurements, OCT showed that 5% urea gel under passive occlusion increased the relative epidermal thickness by 3 and 6% at time points 30 and 60 min, respectively, compared with time 0. Under active occlusion, the relative change in epidermal thickness increased by 16 and 11% at 30 and 60 min, respectively. These results are displayed in Figure 4; the errors of the relative change were calculated from the error in measuring the epidermal thickness. At 30 min after topical application of the urea gel the OCT measurements revealed a greater than 5-fold increase in epidermal thickness using the active ETP compared with passive occlusion. The effect was less marked at 60 min but there was still an almost 2-fold difference in the relative epidermal thickness comparing the active and passive occlusion.

Discussion

A comparison of the cumulative amount of urea penetrating the epidermis to the receptor solution versus time was plotted for passive and magnetic film array applications. In all cases, cells were occluded and non-magnetic polymer film of similar dimensions was used as a control to ensure that any increase in urea penetration was due to the magnetic field energy in the array film and not simply the occlusive effect of magnetic film in proximity to the gel. Urea penetration was significantly increased by magnetic film array compared with passive application over the time period of the experiment ($P < 0.001$). All urea permeation parameters were significantly enhanced in the presence of the magnetic field.

Table 1 In-vitro skin permeation of urea across human epidermis

Parameters	Treatment	
	Passive	ETP
Mean cumulative permeation ($\mu g/cm^2$)	20.83 ± 2.02	89.54 ± 7.34
Steady state flux ($\mu g/cm^2$ per min)	0.24 ± 0.03	0.75 ± 0.06
Lag time (min)	40.58 ± 3.98	21.13 ± 6.27
Permeability coefficient (cm/h)	$2.98 \times 10^{-5} \pm 0.23 \times 10^{-5}$	$9.61 \times 10^{-6} \pm 1.02 \times 10^{-6}$

In-vitro skin permeation parameters (0–120 min) for urea following administration as 5% gel with ETP versus passive occluded application. Values are mean ± SEM. All measures were significantly different when compared for the two treatments ($P < 0.05$; unpaired *t*-test).

Table 2 Statistical difference in urea permeation for magnetic versus passive administration

	Time (min)							
	10	20	30	40	50	60	90	120
P value	0.021	0.021	0.006	0.006	0.002	0.001	0.000	0.000

Statistical analysis of the difference in in-vitro skin permeation of urea over time for magnetic versus passive administration (Mann–Whitney *U*-test).

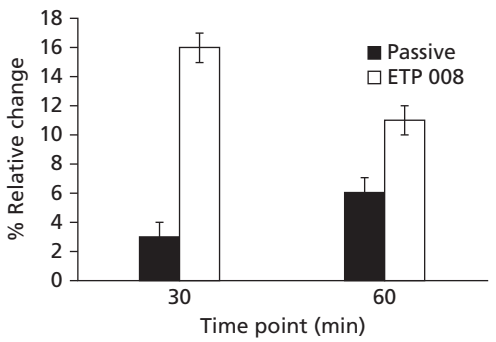

Figure 4 Relative change in epidermal thickness. Difference in epidermal thickness at time points 30 and 60 min relative to 0 min due to administration of 5% urea gel with passive and active (ETP 008) occlusion giving the percentage change (mean ± error calculated from the error in measuring the epidermal thickness; 72 OCT images)

As direct measurement of penetration of urea into the skin *in vivo* is difficult, OCT was used to visualise and measure the physical changes in skin properties due to hydration. As expected, the epidermal depth was increased by hydration due to occlusion and the presence of the topically administered hydrating agent, urea. OCT permitted monitoring of the time-dependent effects on skin properties in response to hydration. In this study, the application of 5% urea gel with occlusion increased epidermal thickness by 3 and 6% after 30 and 60 min, respectively. However, when urea gel was applied with the magnetic film (ETP 008), the epidermal thickness increased by 16% after 30 min and 11% at 60 min. This suggests that the magnetic field array material increases hydration by enhancing the rate of urea permeation into the skin, and confirms the in-vitro epidermal penetration data obtained in the study. It appears from the OCT measurements that the changes in the epidermal properties maximise after about 30 min and then stabilise or even decrease over longer periods of time. Given the difficulty in making repeat measurements in exactly the same place (positioning accuracy equals the transverse resolution of the OCT imaging system which is 15 μm), this may be due to changes in tissue morphology due to skin appendages such as hair follicles or sweat ducts. There is the added complication that the changes in the levels of hydration may also change the refractive index of the epidermis; here, for all thickness measurements we have assumed the refractive index to be 1.4. Further studies with more subjects and multiple time points are required to fully understand the in-vivo changes.

Magnetic fields have been shown to induce changes in a number of cell types including fibroblasts, endothelial cells and keratinocytes.[24–26] They have been reported to induce wound healing[27,28] and improve chronic skin ulcers,[29,30] stimulate collagen[31] and bone growth,[32] and enhance the photodynamic effect on cancer cells.[33] Murthy and Hiremath reported enhanced skin permeation of benzoic acid, salbutamol sulfate and terbutaline sulfate by magnetophoresis, which involved the use of stationary permanent magnets.[34–36] We have previously demonstrated enhanced skin penetration of 5-amino-levulinic acid, a dipeptide and naltrexone hydrochloride by a pulsed electromagnetic field.[37–39] In the latter study, a preliminary investigation to elucidate the mechanism of magnetic enhanced delivery was undertaken by applying 10-nm gold nanoparticles to human epidermis with and without electromagnetic fields. Multiphoton microscopy with fluorescent lifetime imaging microscopy showed skin permeation of the nanoparticles with the pulsed electromagnetic field with no evidence of passive permeation. This suggests that the magnetic energy may induce channels within the stratum corneum of at least 10 nm in diameter. It must be noted that these studies involved a range of different types of magnetic fields to influence skin permeation of various compounds. The precise mechanism of skin permeation enhancement may vary with the magnetic field characteristics.

Conclusions

Urea gel was applied as a moisturiser. Its penetration across human epidermis was determined *in vitro* and epidermal hydration effect visualised *in vivo* by OCT. Administration

with a novel magnetic film technology provided enhanced skin penetration of urea and increased epidermal hydration when compared with administration under an occlusive film. The practical benefits of an unpowered, thin and flexible magnetic film array that is suited to fabrication as a drug patch or cosmetic mask with enhanced skin delivery warrants further investigation.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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