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ScienceDirect
European Journal of Pharmaceutics and Biopharmaceutics 69 (2008) 1040–1045
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

Influence of the delivery systems using a microneedle array on the permeation of a hydrophilic molecule, calcein

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Received 6 December 2007; accepted in revised form 11 February 2008
Available online 19 February 2008

Abstract

Despite the advantages of drug delivery through the skin, such as easy accessibility, convenience, prolonged therapy, avoidance of the liver first-pass metabolism and a large surface area, transdermal drug delivery is only used with a small subset of drugs because most compounds cannot cross the skin at therapeutically useful rates. Recently, a new concept was introduced known as microneedles and these could be pierced to effectively deliver drugs using micron-sized needles in a minimally invasive and painless manner. In this study, biocompatible polycarbonate (PC) microneedle arrays with various depths (200 and 500 μm) and densities (45, 99 and 154 ea/cm^2) were fabricated using a micro-mechanical process. The skin permeability of a hydrophilic molecule, calcein (622.5D), was examined according to the delivery systems of microneedle, drug loading, depth of the PC microneedle, and density of the PC microneedle. The skin permeability of calcein was the highest when the calcein gel was applied to the skin with the 500 μm-depth PC microneedle, simultaneously. In addition, the skin permeability of calcein was the highest when 0.1 g of calcein gel was coupled to the 500 μm-depth PC microneedle (154 ea/cm^2) as well as longer microneedles and larger density of microneedles. Taken together, this study suggests that a biocompatible PC microneedle might be a suitable tool for transdermal drug delivery system of hydrophilic molecules with the possible applications to macromolecules such as proteins and peptides.

Keywords: Microneedle; Calcein; Permeation; Gel; Delivery systems

1. Introduction

Transdermal drug delivery offers many important advantages. For example, it is easy and painless, it protects the active compound from gastric enzymes, and avoids the hepatic first-pass effect. However, the skin is a natural barrier, and only a few drugs can penetrate the skin easily with the aid of penetration enhancers such as soybean phospholipids [1], long-chain fatty alcohols, cyclic monoterpenes [2,3] and non-ionic surfactants [4]. However, the stratum corneum limits these methods to drugs that are hydrophobic, low molecular weight and potent.

Biopharmaceuticals, such as peptides, proteins and the future use of DNA and RNA, are a rapidly growing segment of pharmaceutical therapies [5,6]. These biotechnology drugs are currently delivered almost exclusively through the parenteral route, which creates some problems such as expertise for delivery, accidental needle sticks or pain resulting in a reduced patient compliance.

Therefore, a new concept, a microneedle array, was introduced to merge the advantages of transdermal

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doi:10.1016/j.ejpb.2008.02.009

delivery and parenteral delivery [7–15]. Some studies on the efficacy of microneedles have been reported using peptides such as insulin [16] and desmopressin [17], genetic material including plasmid DNA and oligonucleotides [18,19] and vaccines directed against hepatitis B [20], anthrax [21] and Japanese encephalitis [22]. Briefly summarized, Henry et al. [7] used the silicon microneedle for transdermal delivery of calcein. Based on the previous publications, we developed new microneedle systems using biocompatible polycarbonate. Cormier et al. [17] fabricated the 225–600 μm of microneedles using titanium and applied the microneedles for peptide and protein, whereas, Park et al. [13] manufactured the microneedle array using PLGA (poly-lactide-co-glycolide) to encapsulate model drugs such as calcein and BSA.

However, there are few reports of the delivery systems of microneedle-based transdermal drug delivery. In order to develop microneedle-based drug products, the systems used for the components of microneedle array and drug need to confirm whether the synchronized systems or separated systems between microneedle array and drug are optimized. Therefore, this study examined the skin permeability to determine the optimal system between our own microneedle array and drug according to the method of application using a hydrophilic molecule, calcein, which was used as a low molecular weight tracer because it was a fluorescent dye and could be easily analyzed by a fluorescence spectrophotometer.

2. Materials and methods

2.1. Materials and apparatus

Calcein (Bis [*N*,*N*-bis (carboxymethyl) aminomethyl] fluorescein; $C_{30}H_{26}N_2O_{13}$, MW, 622.55) was purchased from Sigma (Steinheim, Germany). Bromocresol green was purchased from Samchun Co. (Pyongtack, Korea). The microneedle was supplied by KAIST (Daejeon, Korea). Carbomer 940 was used as received without further purification. All other chemicals and solvents were of analytical reagent grade and used without further purification.

2.2. Fabrication of microneedle

The biocompatible microneedle was fabricated by Han et al. [23]. First, the in-plane microneedles were fabricated using inclined UV lithography and electroforming with a sharp tip for a low insertion force and were made long enough to ensure sufficient penetration depth. In this step, it was easy to control the length, side shape, and tip sharpness. The in-plane microneedles were then converted into an out-of-plane microneedle array in order to increase the needle density. For mass production, a negative mold was fabricated by replicating the out-of-plane microneedle array. Finally, the out-of-plane microneedle sheets were produced using polycarbonate for biocompatibility using the negative mold in a hot-embossing machine. The heights of the fabricated PC microneedles were 200 and 500 μm, and the densities were 45, 99 and 154 ea/cm^2.

2.3. Calcein gel formulation

Calcein gel was prepared using carbopol 940 (1%). After the complete hydration of carbopol 940, calcein was added to the carbopol 940 solutions at a final concentration of 1 mM. The mixtures were stirred using a magnetic stirrer, and the calcein gel was formed with triethanolamine (0.1%). In addition, 1 mM of a calcein solution was prepared in order to compare its permeability into the skin with the calcein gel. A fluorescence spectrophotometer (Perkin-Elmer, UK, LS55) was used for analysis. When the calcein gel was applied to the skin with the 500 μm-depth PC microneedle, an indicated amount of calcein gel was loaded into the top of the PC microneedle array and the calcein gel was touched in the tip and the base of the PC microneedle.

2.4. Permeation study of calcein into the rat skin

The skin was obtained from male Sprague–Dawley rats weighing 250 ± 20 g. After carefully removing the hair with electric clippers (Thrive, Japan, Model 8000AD), a 5×5 cm patch of skin was excised from the dorsal region from each sacrificed rat. The subcutaneous tissue was trimmed and the excised rat skin was stored at -20 °C and used within one week of harvest. The extent and rate of skin permeation of calcein from the applied formulations were determined using Franz diffusion cells fitted with the excised rat skin. The effective diffusional area was 1.81 cm^2. The receptor compartment was filled with 11.8 ml of pH 7.4 phosphate-buffered saline. The temperature was maintained at 37 ± 0.5 °C using a thermostatic water pump (Labfine Scientific Instrument, Korea) and stirred at 600 rpm throughout the experiment. After 0.1 or 0.05 g of the calcein gel or 0.1 ml of calcein solution had been applied to the epidermal surface of the skin, 1.0 ml of the receptor medium was withdrawn every hour up to 12 h after application, and replaced immediately with an equal volume of fresh PBS equilibrated at 37 ± 0.5°C. The samples were analyzed by fluorescence spectroscopy (E_x 490 nm, E_m 510 nm). The cumulative amount of calcein permeating through the excised rat skins was plotted as a function of time. The slope and intercept of the linear portion were derived by linear regression analysis. The permeation rate at the steady state (Js, μg/cm^2/h) was calculated as the slope divided by the skin surface area. The cumulative amount of calcein permeated (Q_s, μg/cm^2) for 12 h was also calculated. The skin was removed temporarily from the diffusion chamber and placed on a supported surface, subsequently the PC microneedle was inserted into the equilibrated skin with clip for 30 min and then removed to identify the permeation behavior of calcein from calcein gel according to

the delivery systems of PC microneedle. The amount of calcein remaining in the skin after the permeation study was measured by isolating the skin from the Franz cell. The skin was cut into small pieces with scissors and forceps, and homogenized with 2 ml of PBS to extract the calcein. After centrifugation at 15,000 rpm for 10 min, the supernatant was taken for analysis. The specification of PC microneedles used for this permeation study was 500 μm-depth and 154 ea/cm^2 unless mentioned specially.

2.5. Mathematical modeling regarding release profile

In our study, the drug release rates were evaluated according to the simplified Higuchi diffusion Eq. (1), depicting the drug release from one side of a semisolid layer in which the drug is dissolved.

$$q = 2C_0(D_t/\pi)^{1/2} \quad (1)$$

where q is the amount of drug released into the receptor medium per unit area of exposure, C_0 is the initial drug concentration in vehicle, D is the apparent diffusion coefficient of drug and t is the time elapsed since the start of drug release. In the case of passive diffusion, the steady-state flux through the unit area of a membrane is given by Fick's law,

$$J = P \cdot (C_d - C_r) \quad (2)$$

where J is the flux per unit area, P represents the permeability coefficient and C_d, C_r are the concentrations in the donor and receptor solutions, respectively. In case sink conditions are maintained on the receptor side, $(C_d - C_r)$ is replaced by C_d.

$$J = P \cdot (C_d) \quad (3)$$

The permeability coefficient, P, is the constant for a given drug under the same experimental conditions. There should be a linear relationship between the flux and the donor concentration.

2.6. Optical images of skin in the presence of a microneedle

The ability of the microneedles to create transport pathways across the stratum corneum was assessed by applying microneedle arrays with various densities (154, 99 and 45 ea/cm^2) onto the shaved rat skin for 30 min and examining the images by a digital camera, COOLPIX 4500 (Nikon Co. Ltd., Tokyo, Japan). Optical observations were carried out to correlate the permeation study of calcein according to the method of application between an array of 500 μm-depth-microneedles with a density of 154 ea/cm^2 and the calcein gel with the ability of the microneedle to make a transport pathway. The calcein gel was mixed with bromocresol green and administrated to the rat skin for 30 min by gripping the microneedles with two clips using the following three methods: calcein gel coupled to the microneedle, loading the calcein gel onto the region applied by the microneedle and microneedle application after loading the calcein gel.

2.7. Statistical analysis

A student t test was used to compare two different groups of samples. A p value < 0.05 was considered significant.

3. Results and discussion

3.1. Transdermal transport facilitated by solid microneedles

Most of the geometrical characteristics of out-of-plane microneedles can be controlled for real life applications in drug delivery, cosmetic delivery and mesotherapy. Since the PC microneedles fabricated for this study could be mass-produced and consisted of a biocompatible polymer, these have a sufficient potential for industrial applications. The insertion effect into the skin by the PC microneedle having the various array densities (154, 99 and 45 ea/cm^2) was observed under the same pressure using a clip for 30 min to identify the integrity of the tip of the PC microneedle and to assess the ability of the PC microneedle to create the transport pathways across the stratum corneum. Fig. 1 shows the piercing effect into the stratum corneum according to the density of the PC microneedle arrays at the dermal side of the skin. The results showed that there were an increasing number of holes with an increasing density of the PC microneedle arrays. The green spots in the shape of the microneedle array indicate that the dye rapidly permeates across the epidermis through the holes created by the microneedles.

3.2. Effect of application modes of microneedle on the permeation of calcein

First of all, skin permeation study was carried out to identify the effect of the dosage forms of calcein, such as the solution and gel, for applying the calcein into the microneedle. Fig. 2(A) shows a similar permeation profile of the calcein from the calcein solution and calcein gel into the rat skin. Therefore, the skin permeability study of calcein using the calcein gel was carried out to identify the effect of the delivery systems of the PC microneedles on the permeation of calcein. The aim of this work was to figure out the delivery method of hydrophilic molecule, calcein using a microneedle array. So calcein is needed to be coated into the tested microneedles. Naturally, the formulation of calcein was needed to prevent it from spilling out of the tested microneedles. Therefore, calcein gel formulation showing quite similar permeation profiles with calcein solution should be selected. The different delivery systems are as follows: the PC microneedle was inserted into the skin for 30 min ahead of applying the calcein gel into the skin; the calcein gel was loaded onto the rat skin directly and then the PC microneedle was inserted into the same site for 30 min; and the calcein gel was applied into the skin with the PC microneedle, simultaneously. This experiment was carried out to determine if

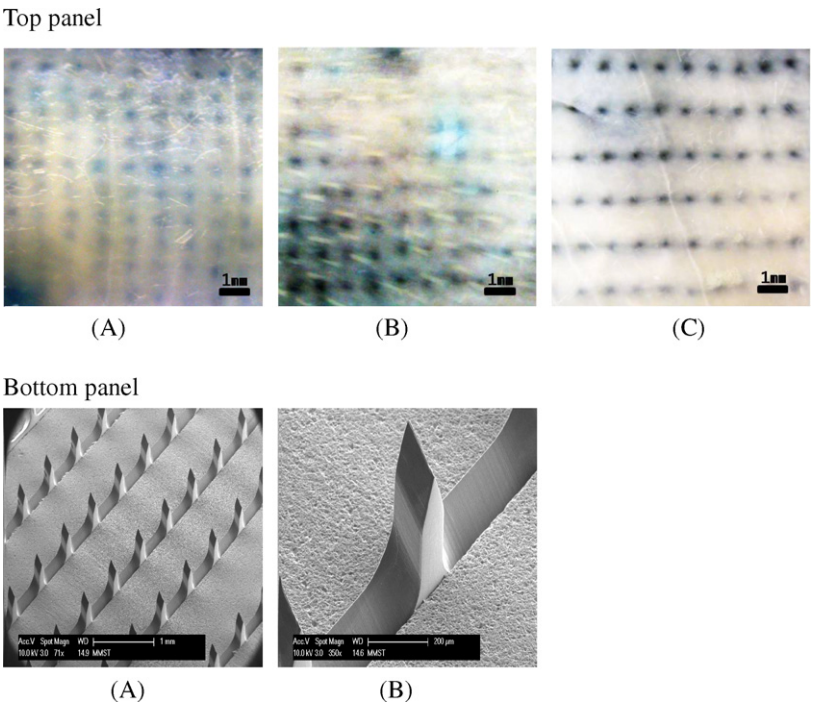

Fig. 1. Photographs showing the piercing effect of the PC microneedle and fabricated microneedles. Top panel, piercing effect according to the density of microneedles. The number of piercing holes increased with increasing microneedle density. (A) Microneedle array with a density of 154 ea/cm^2. (B) The microneedle array with a density of 99 ea/cm^2. (C) Microneedle array with a density of 45 ea/cm^2. A dye, bromocresol green, was added to the calcein gel; Bottom panel, (A) photomicrographs showing the overall structures of the microneedles fabricated with 500 μm-depth at a density of 154 ea/cm^2 (B) photomicrographs showing the enlarging structure of the microneedles fabricated.

synchronized or separate systems between the PC microneedle array and the calcein gel could be optimized for the development of a transdermal drug delivery system. The highest permeation profiles were obtained when the calcein gel was applied into the skin together with the PC microneedle (Fig. 2(B)). The effect of the amount of calcein loaded into the microneedle was examined after first confirming that the calcein gel coupled directly to the microneedle showed the highest level of permeation. The skin permeation study was performed after loading different amounts of calcein (0.05, 0.1 g) into the microneedle. The microneedle coupled with 0.1 or 0.05 g of the calcein gel showed an approximately 5- and 3.6-fold increase in calcein permeation into the rat skin, respectively, compared with the case of loading the calcein gel alone (Fig. 3). The effect of the depth (500, 200 μm) of the microneedles on the level of the calcein permeation was examined. The 500 μm-depth-microneedle showed a 5.46-fold increase in permeation compared with the control group, whereas the 200 μm-depth-microneedle showed a 3.5-fold increase compared with the control group (Fig. 4(A)). Fig. 4(B) shows that the amount of calcein permeation increased with increasing density of microneedles from 45 to 154 ea/cm^2. This suggests that the number of holes created by the microneedles reflect the permeation results of calcein into the rat skin. Tables 1–3 show the permeation parameters of calcein from the calcein coupled with the microneedle through the rat skin according to the amount of calcein, the depth of the microneedle and the density of the microneedle. The higher calcein flux from the calcein coupled with the microneedle was most likely due to the creation of holes induced by the microneedles, which act as a transdermal pathway to facilitate a transdermal drug delivery.

Our results showed a minimal increase of the permeation of calcein in the presence of a microneedle when compared to another publication [13]. Especially, Henry et al. [7] reported the calcein permeation increase by the order of 1000–10,000 into the skin under the microneedle application. Attention is to be paid to the difference of the material composed of microneedles of Henry et al. and those of ours as well as experimental condition. Henry et al. fabricated their microneedles with silicon, we fabricated microneedle arrays using biocompatible polycarbonate. Henry et al. used human epidermis which was isolated from dermis using a standard heat separation to evaluate the transdermal permeability of calcein; however, we used rat full skin which was used to perform the transdermal permeability of calcein for the clinical applications in future.

Therefore, this paper might suggest more invasive, comfortable and convenient transdermal drug delivery systems using the PC microneedle. However, the future work on the delivery systems of the PC microneedle with the study on the formulation of the drug to be loaded into the PC microneedle will be performed.

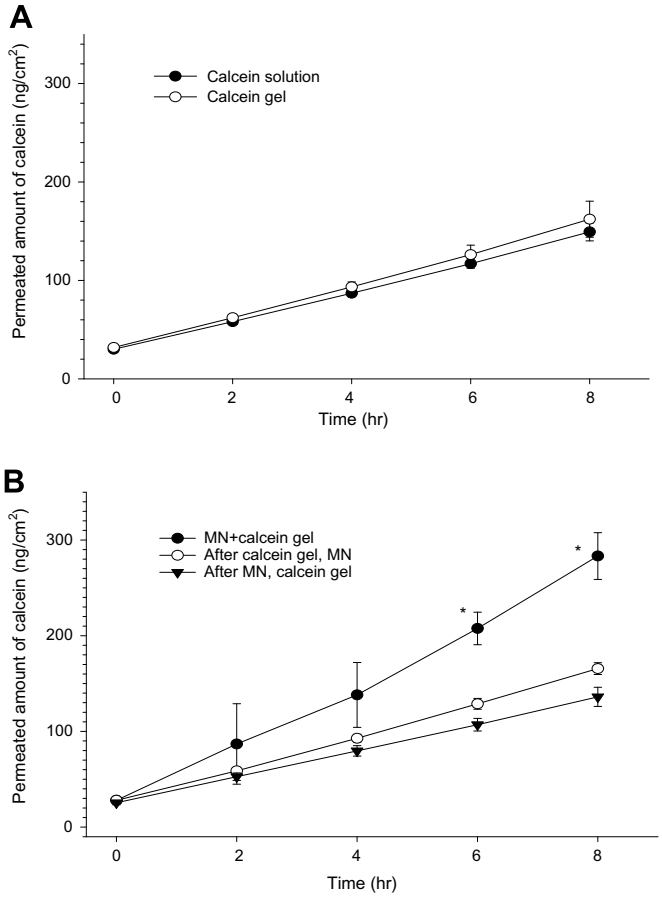

Fig. 2. The permeation profile of calcein through the rat skin. (A) The calcein solution and calcein gel were loaded into the diffusion cell fitted with the rat skin to compare the permeability of the two formulations. (B) The skin permeability test was classified into the following delivery systems of the PC microneedle: the PC microneedle was inserted into the skin for 30 min ahead of applying the calcein gel into the skin; the calcein gel was loaded onto the rat skin directly and then the PC microneedle was inserted into the same site for 30 min; and the calcein gel was applied into the skin with the PC microneedle, simultaneously.

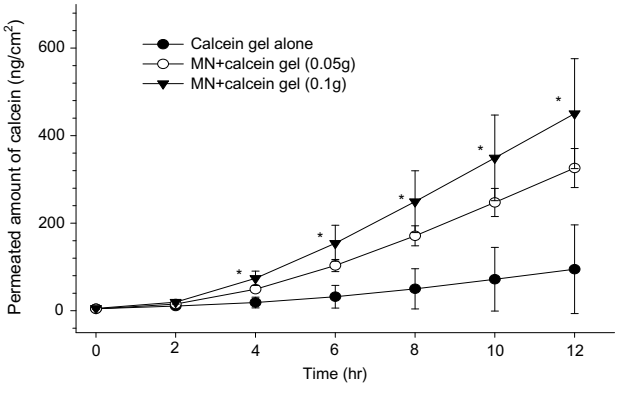

Fig. 3. The permeated calcein from the calcein gel coupled to the PC microneedle according to the amount of calcein gel.

4. Conclusion

The PC microneedle arrays with various depths (200 and 500 μm) and densities (45, 99 and 154 ea/cm^2) were fabricated using a micro-mechanical process, and were applied to improve the level of the skin permeation of a hydrophilic molecule, calcein. The highest permeation profiles were obtained with the calcein gel coupled directly with the microneedle loaded onto the rat skin. In addition, 0.1 g of calcein gel coupled directly with the 500 μm-depth-microneedle showed a 5.46-fold increase in permeation after 12 h permeation into the rat skin compared with the control group, calcein gel alone. Overall, this report

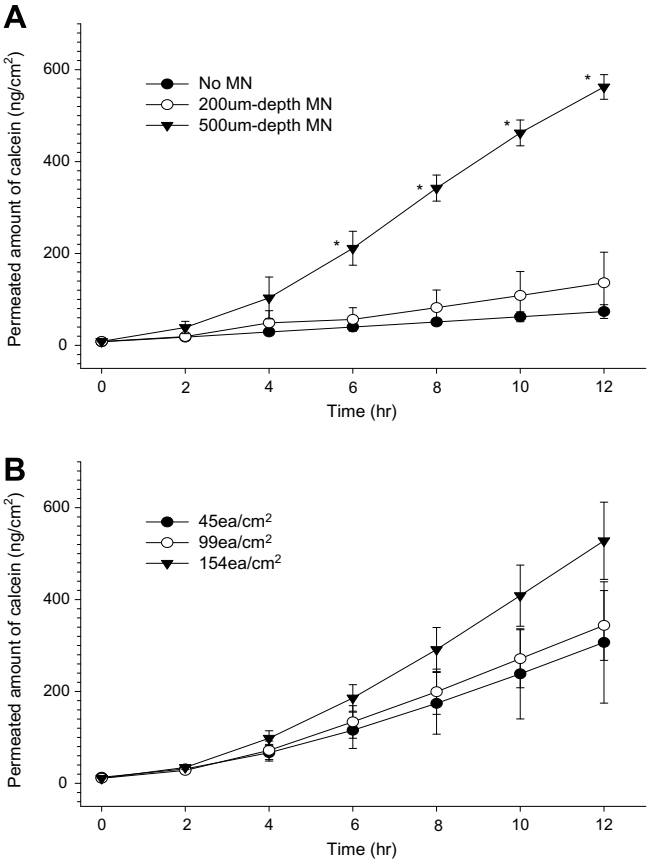

Fig. 4. Effects of the parameters of the PC microneedle on the permeation of calcein from the calcein gel coupled to the PC microneedle. (A) 500 μm-depth and 200 μm-depth PC microneedle were used for the permeation study of calcein. (B) PC microneedle with densities of 45, 99 and 154 ea/cm^2 were used for the permeability study.

Table 1
Permeation parameters of calcein from the calcein gel coupled with the microneedle through the rat skin

	Flux (ng/cm^2/h)	Q_s[a] (ng/cm^2)	Skin deposition after 24 h (μg/cm^2)
Calcein gel	9.612 ($r^2 = 0.989$)	94.8 ± 101.3	22.6 ± 5.01
MN + calcein gel (0.05 g)	34.901 ($r^2 = 0.995$)	325.99 ± 44.55	6.98 ± 1.24
MN + calcein gel (0.1 g)	47.363 ($r^2 = 0.998$)	450.22 ± 125.6	8.93 ± 1.11

[a] Cumulative amount of calcein permeated for 12 h.

Table 2
Permeation parameters of calcein from the calcein gel coupled with the microneedle according to the depth of the microneedle through the rat skin

	Flux ($ng/cm^2/h$)	Q_s[a] (ng/cm^2)	Skin deposition after 24 h ($\mu g/cm^2$)
No MN	5.545 ($r^2 = 0.999$)	73.50 ± 14.97	11.159 ± 3.56
200 μm-depth-MN	26.175 ($r^2 = 0.997$)	257.31 ± 214.991	6.33 ± 2.15
500 μm-depth-MN	39.151 ($r^2 = 0.999$)	401.69 ± 217.411	4.87 ± 2.08

[a] Cumulative amount of calcein permeated for 12 h.

Table 3
Permeation parameters of calcein from the calcein gel coupled with the microneedle according to the density of the microneedle through the rat skin

	Flux ($ng/cm^2/h$)	Q_s[a] (ng/cm^2)	Skin deposition after 24 h ($\mu g/cm^2$)
45 ea/cm^2	30.141 ($r^2 = 0.996$)	306.62 ± 131.96	7.87 ± 0.91
99 ea/cm^2	34.072 ($r^2 = 0.998$)	343.69 ± 75.92	7.69 ± 1.97
154 ea/cm^2	54.127 ($r^2 = 0.996$)	528.08 ± 84.03	6.79 ± 1.05

[a] Cumulative amount of calcein permeated for 12 h.

showed significant increases in the permeation of a hydrophilic molecule, calcein, which is generally considered to be impermeable through the skin.

Acknowledgement

This study was financially supported by the research fund of Chungnam National University in 2006.

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