Microneedles-Mediated Transdermal Delivery

M. Petchsangsai, N. Wonglertnirant, T. Rojanarata, P. Opanasopit, T. Ngawhirunpat

Abstract—The objective of the present study was to evaluate the potential of hollow microneedles for enhancing the transdermal delivery of Bovine Serum Albumin (MW~66,000 Da)-Fluorescein Isothiocyanate (BSA-FITC) conjugate, a hydrophilic large molecular compound. Moreover, the effect of different formulations was evaluated. The series of binary mixtures composed of propylene glycol (PG) and pH 7.4 phosphate buffer solution (PBS) was prepared and used as a medium for BSA-FITC. The results showed that there was no permeation of BSA-FITC solution across the neonatal porcine skin without using hollow microneedles, whereas the cumulative amount of BSA-FITC released at 8 h through the neonatal porcine skin was about 60-70% when using hollow microneedles. Furthermore, the results demonstrated that the higher volume of PG in binary mixtures injected, the lower cumulative amount of BSA-FITC released and release rate of BSA-FITC from skin. These release profiles of BSA-FITC in binary mixtures were expressed by Fick's law of diffusion. These results suggest the utilization of hollow microneedle to enhance transdermal delivery of protein and provide useful information for designing an effective hollow microneedle system.

Keywords—Hydrophilic macromolecules, Microneedles, Propylene glycol, Transdermal drug delivery

I. INTRODUCTION

SKIN is the largest organ of the body which covers an area of approximately 2 m^2 [1]. It acts as a protective barrier against the entry of foreign substances and possible invasion of pathogens or infectious agents. Skin consists of two distinct layers, the outer epidermis and the inner dermis. The dermis which forms the bulk of the skin is made up of connective tissue elements. The thickness of this layer is approximately 1-2 mm. The stratified epidermis (approximately 100-150 µm thick) comprises four distinct layers, from innermost to outermost of the body, i.e. stratum basale, stratum spinosum, stratum granulosum and stratum corneum. The stratum corneum, which is only 15-20 µm, can effectively provide the main barrier for permeation of permeant. It is known to be selectively permeable and allows relatively lipophilic molecules to diffuse to the skin layers [2], [3].

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In general, the permeant with a favorable physicochemical characteristics such as molecular weight less than 500 Da, logP around 2 and low melting point can penetrate across such barrier [4].

As a result, a hydrophilic macromolecular compound, e.g. DNA, siRNA, antisense oligonucleotides, and protein including insulin, cannot be easily penetrated across the skin. In order to enhance the transdermal transport of such molecules, there are two main types of enhancement methods, i.e. chemical and physical method to carry out these problems [5].

Microneedles (MNs) is one of the most interesting physical enhancement method. This method can disrupt stratum corneum barrier by creating large aqueous microchannels enough for molecules to pass through without skin damage. Nonetheless, the length of aqueous microchannel does not reach the dermis layer, which is filled with nerves and blood vessels. Consequently, the patient administered by MNs has no pain and bleeding [6], [7]. Normally, MNs can be divided into two types, i.e. solid and hollow MNs. Solid MNs were generally made of silicon, glass or metal. The transport of the drug from solid MNs occurred by diffusion. On the other hand, hollow MNs can facilitate active fluid flow through the needle bore and into the skin, which can lead to much faster rates of delivery. However, the hollow MNs are practically difficult to make and use [7].

Propylene glycol (PG) is well known to be chemical penetration enhancer. It acts by solvating the α-keratin structures of the cells and disordering lamellar lipid structures [8]. Moreover, PG can improve drug partitioning into the skin or solubility of drug in the formulation [9].

Up to now, there is a few research works relevant to the application of the combination strategies between chemical, i.e. PG, and physical, i.e. MNs for improving skin penetration of hydrophilic macromolecular compound. For this reason, the objective of this study was to investigate the effect of different volume of PG in binary mixtures comprised PG and PBS on the release rate and extent of hydrophilic macromolecule delivery, BSA-FITC, through microchannel created by single hollow microneedle.

II. MATERIALS AND METHODS

A. Materials

Bovine Serum Albumin (MW~66,000 Da)-Fluorescein Isothiocyanate (BSA-FITC) conjugate was purchased from Sigma Aldrich (St. Louis, MO, U.S.A.). All other chemicals used in this study were analytical grade.

B. Preparation of Hollow Microneedles

Hollow MNs, Nanopass^{TM} (33-gauge hypodermic needle, i.d., 0.20 mm), were kindly provided by Terumo Co. (Tokyo, Japan). A hollow microneedle system used in this study was manufactured from a microneedle connected to a 27-gauge hypodermic needle (i.d., 0.22 mm; o.d., 0.40 mm; Terumo Co.). The microneedle was fixed with a triangular silicone sheet to maintain an angle of insertion (θ) of 40° and constant insertion depth in the skin barrier [10], [11]. Fig. 1 depicts schematic diagram of single hollow MNs connected to a 27 G hypodermic needle.
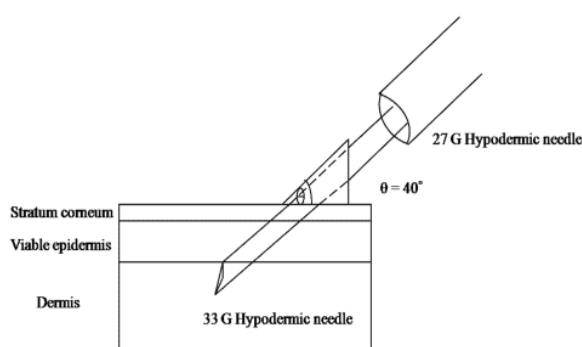

Fig. 1 Schematic diagram of hollow microneedle with 27 gauge hypodermic needle

C. Experimental Animals

Neonatal porcine skin was used as an animal skin model in this study because it shows a good model for human skin in terms of hair sparseness, and physical properties [12]. The neonatal porcine skin was obtained from stillborn piglets. The dorsal skin of stillborn piglets was excised and trimmed to a thickness of 1.2-1.5 mm using a dermatome. The skin was then stored in aluminium foil at -20 °C until used.

D. In Vitro Release Studies

In vitro release studies of BSA-FITC in binary mixtures comprised PG and PBS were evaluated using Franz diffusion cell apparatus, which have 2.022 cm^2 of diffusion area. The receptor compartments were filled with approximately 6 ml of PBS pH 7.4 and maintained the temperature at 32 °C using circulating water bath.

Before starting the experiment, skin samples were allowed to thaw for about 30 min in circulating batch which is maintained the temperature at 32 °C. Skin was then stretched on a board in order to provide mechanical support during MN insertion. BSA-FITC solution was manually delivered by applying gentle finger pressure. Each of the BSA-FITC formulations was emptied into the skin and MN was instantaneously removed. The volume of solution applied into the skin was kept constant at 10 µl for all formulations.

The skin was placed on the receptor compartments by giving the stratum corneum contact to the donor compartment. The donor and receptor compartment were then fixed by clamper and the top of the donor compartment was covered with parafilm® in order to prevent water loss from the skin during 8 h of experiment. The cumulative release profile of BSA-FITC was determined by sampling 500 µl of solution from the receptor compartment at specified time, i.e. 5, 15, 30 min, 1, 2, 4, 6, 8 h, and immediately replacing the same volume of PBS. The samples were then analyzed after finishing the experiments. All the tests were carried out in four experiments.

E. Preparation of Drug Solution

The different ratios of binary mixtures of PG and PBS pH 7.4 were prepared by mixing 12.5, 25.0, 50.0 and 75.0 % v/v PG in PBS pH 7.4. To each of these binary mixtures, BSA-FITC was added in order to obtain 40 µg/µl of solution. After that, the solutions were then vortexed to ensure that the drug was completely dissolved.

F. Viscosity Measurement

Brookfield DV-III LV programmable cone/plate rheometer with a CPE-40 spindle was used for measuring viscosity of the binary mixtures comprised PG and PBS. The sample volume was 2 ml. All formulations were carried out in triplicate.

G. Quantitative Analysis

The amount of BSA-FITC released through neonatal porcine skins was quantized by fluorescence spectrophotometer with an excitation wavelength of 400 nm and emission wavelength of 535 nm (Microplate reader, Packard Bioscience; A153601).

III. RESULTS AND DISCUSSION

A. MNs-mediated transdermal delivery

It is well known that stratum corneum is the important barrier to permeation of permeant including therapeutic agents, e.g. protein and DNA. The use of MNs is a potential physical method to enhance the permeation of such molecules [13]-[15]. This useful enhancement method bypasses the stratum corneum barrier and creates transient aqueous channel pathway along the stratum corneum through the deeper skin.

In order to investigate the potential of MNs for macromolecules delivery, BSA-FITC was used as a high molecular weight protein model in this study.

Fig. 2 shows the relationship of the cumulative amount of BSA-FITC in PBS released and time. The plotted data obtained from intact skin (400 µg/ml of BSA-FITC in PBS without MNs) and MNs-treated skin with 10 µl BSA-FITC in PBS (40 µg/µl). The results showed that MNs dramatically increased BSA-FITC permeability through neonatal porcine skin, whereas BSA-FITC could not easily penetrate skin by passive diffusion. This could be due to its physicochemical characteristics, i.e. hydrophilic molecule and high molecular weight compound.
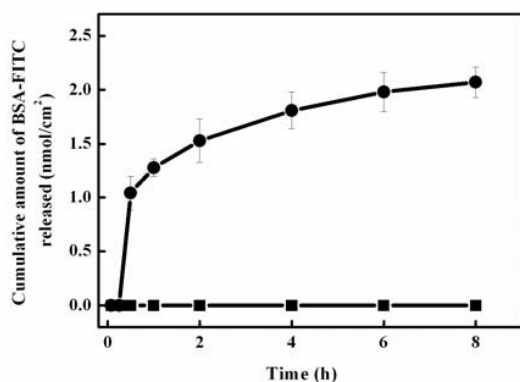
Fig. 2 *In vitro* release of BSA-FITC following treatment with MNs
(•) and without MNs (■). Each point represents the mean ± S.E. of
four experiments

*B. Effect of Volume of Propylene Glycol in the Formulation
on BSA-FITC Released*

Propylene Glycol (PG) is normally known to be a chemical penetration enhancer. For this reason, it should be anticipated that the combination of chemical, i.e. PG, and physical, i.e. MNs, could facilitate in skin penetration.

In this study, a series of four binary mixtures of PG and PBS pH 7.4 containing equal concentrations of BSA-FITC (40 µg/µl) were investigated.

Fig. 3 shows the cumulative amount of BSA-FITC released from different formulations versus time. The results demonstrated that the lower amount of BSA-FITC released were found when the higher volume of PG was incorporated in the formulation. Moreover, the cumulative amount and percentage of BSA-FITC released from all formulations through the skin over 8 h are shown in Table I.
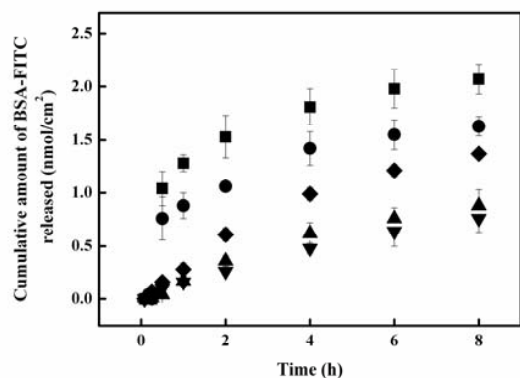
TABLE I
CUMULATIVE AMOUNT AND PERCENTAGE OF BSA-FITC RELEASED
FROM SKIN OVER 8 H AFTER ADMINISTRATION BY MNs

Volume of PG in the binary mixtures (% v/v)	Cumulative amount of BSA-FITC released (nmol/cm^2)	Percentage of BSA-FITC released
0.0	2.07 ± 0.14	69.04 ± 4.65
12.5	1.63 ± 0.09	58.82 ± 7.72
25.0	1.37 ± 0.04	45.58 ± 1.19
50.0	0.76 ± 0.13	24.69 ± 3.71
75.0	0.88 ± 0.15	22.07 ± 6.80

The results demonstrated that cumulative amount and percentage of BSA-FITC released decreased obviously with an increase in volume of PG in binary mixtures, except for 75 % v/v which was similar to that of 50 % v/v in both quantitative data.

The release rate (k) of BSA-FITC obtained from simplified Higuchi model (1) were plotted and presented in Fig. 4 and Table II.

$$Q = kt^{1/2} \qquad (1)$$

Fig. 4 demonstrated that the release rate gradually decreased in the formulation with the higher volume of PG. However, it was found that the relationship was a non-linear.

In order to describe the possible reason for explanation related to the reduction in cumulative amount and release rate of BSA-FITC, the viscosities of all binary mixtures were measured and presented in Table III. The results show that the viscosities of binary mixtures increased with an increase in the volume of PG.

Considering the cumulative amount of BSA-FITC released from all formulations (Table I) and the viscosities (Table III), the results could be implied that the reduction of cumulative amount of BSA-FITC released corresponded to the increase in the viscosities of binary mixtures.

Fig. 3 *In vitro* release profile of BSA-FITC following treatment with MNs; (■) No PG, (•) 12.5 %, (◆) 25.0 %, (▲) 50.0 %, and (▼) 75.0 % v/v of PG in binary mixtures. Each point represents the mean ± S.E. of four experiments
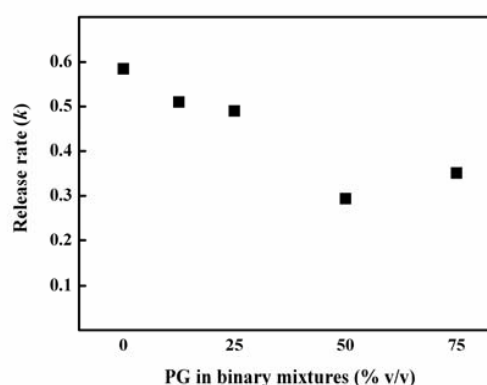

Fig. 4 Release rate of 40 µg/µl of BSA-FITC in binary mixtures from neonatal porcine skin

TABLE II
RELEASE KINETICS OF BSA-FITC FROM DIFFERENT FORMULATIONS BY
SIMPLIFIED HIGUCHI MODEL

Volume of PG in the binary mixtures (% v/v)	Kinetic constant (k)	R^2
0.0	0.585	0.986
12.5	0.510	0.989
25.0	0.490	0.999
50.0	0.294	0.980
75.0	0.352	0.996

TABLE III
THE VISCOSITIES OF BINARY MIXTURES COMPRISED PROPYLENE
GLYCOL AND PHOSPHATE BUFFER SOLUTION

Quantity of PG in the binary mixtures (% v/v)	Viscosities (cP)
0.0	1.223 ± 0.04
12.5	1.710 ± 0.035
25.0	2.597 ± 0.064
50.0	6.037 ± 0.029
75.0	14.877 ± 0.401

In the present study, the resistance of stratum corneum to drug penetration was eliminated by MNs. The resistance from the microchannel and the dermis below microchannel become important. It is also expected that the dermis layer would present a higher resistance compared to the microchannel [4].

This could be suggested that microchannel and dermis layer would be affected by the presence of PG in the formulations which is expected to increase the diffusional resistance of those layers [4].

IV. CONCLUSION

The use of MNs is the potential physical enhancement method for delivering hydrophilic macromolecular compound, i.e. BSA. The combination strategies of PG and MNs are not as good as expected. This could be due to the higher viscosity in those formulations given by the higher volume of PG resulting in the increase in the resistance in the microchannels and dermis layer.

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