Enhancing effect of negative polypropylene electret on in vitro transdermal delivery of cyclosporine A solution and its synergistic effect with ethyl oleate

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Abstract. In this study, the corona charged electrets at voltages of -500 V, -1000 V and -2000 V were made from polypropylene (PP) film. The cyclosporine A (CsA) and 10% ethyl oleate were chosen as the model drug and chemical enhancer, respectively. The charge storage stability of the electrets and the in vitro transdermal behaviour of the model drug in solution under different conditions were studied. The results indicate that the external electrostatic field of the negative PP electrets could penetrate through the rat skin and enhance the transdermal delivery of cyclosporine A. A synergistic effect on enhancing the transdermal delivery of cyclosporine A was observed by combining different surface potential negative PP electrets with 10% ethyl oleate, and the amount of transdermal delivery of CsA was greatly increased comparing with only application of electrets. Therefore, the combination application of electret and chemical enhancer could be a feasible strategy in enhancing transdermal delivery of small peptide drugs or some large molecular drugs.

1. Introduction
Skin is considered as a promising route for delivery of therapeutic molecules into the body due to its several advantages such as avoiding of the potential hepatic first pass effect, constant drug delivery, self-administration, non-invasive and improving patient compliance, and so on [1]. However, the stratum corneum (SC), the outmost layer of the skin, serves as a main barrier for transdermal delivery due to its rigid lamellar structure. Therefore, only small, potent and moderately lipophilic molecules could be effectively delivered via this route. However, the peptides, proteins and drugs which are often charged and highly hydrophilic in nature are limited to achieve the transdermal systemic delivery [2].

To overcome the barrier function of the SC and to increase the flux through the skin, several enhancing approaches have been investigated, such as chemical enhancers, iontophoresis, electroporation, electret, thermal energy, skin metabolism inhibitors, microneedles, etc [3, 4]. Among these approaches, chemical enhancers promote drug permeation through skin by increasing drug partitioning into the SC domain, the drug diffusivity in the SC domain or the combination of both [5]. Iontophoresis uses continuous current to drive peptide, proteins or charged drug molecules to

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cross the skin [5]. However, it has to use external power and electrodes, and erythema may present under the electrode application site [6]. Electret is a dielectric material whose external electrostatic field could penetrate through the skin and facilitate the drug to permeate through the skin by alteration of the skin structure [7, 8]. Besides, it has been proved that electret could increase the flux for both of the small molecules and some macromolecules with the molecular weight lower than 1000 Da [9].

Cyclosporine A (CsA) is a 3rd generation immunosuppressive agent used in organ transplantation. It is a cyclic nonpolar oligopeptide composed of 11 amino acid residues [10]. Its systemic administration might associate with narrow therapeutic index and side effects [11]. So topical delivery is desirable for treating skin disorders. However, this approach has been reported as ineffective in most case due to its large molecular weight (1202 Da). Therefore, we used the electrostatic field produced by PP electret to function as physical enhancer and 10% ethyl oleate as chemical enhancer, and the enhancing effects of single use of the electret or 10% ethyl oleate and the combinational use of both the strategies on transdermal delivery of CsA were studied.

2. Materials and Methods

2.1. Materials
The PP film with a thickness of 13 μm was obtained from Toray (Toray Industry Co. Japan). The cyclosporine A was purchased from Yuanye Biotechnology Co. Ltd. (Shanghai, China). The ethyl oleate was purchased from Feixiang Chemical Plant (Shanghai, China).

2.2. Preparation of electret
The double bare surface PP film was corona charged at constant voltage for 5 min using a corona charging system (Dalian University of Technology, China) with the point voltages of -10 kV and grid voltages of -500 V, -1000 V and -2000 V, respectively. The effective surface potentials of the electrets were measured by compensation method using a surface potentiometer (ESR102A, Beijing Huajinghui Technology Co. Ltd., China).

2.3. Preparation of skin
Male Sprague-Dawley (SD) rats with weight of 200±10 g were selected for transdermal permeation study. The study was conducted with the approval of Institutional Animal Ethical Committee of Second Military Medical University. The rat was anesthetized by intraperitoneal injection of 20% urethane (0.5 ml/kg body weight). The hair from the abdominal region was carefully shaved using an animal hair clipper before the day of the experiment. After a period of 24 h, the rat was sacrificed and the shaved region was incised and adherent fat and other visceral tissue were removed from the undersurface. Then the skin was washed with phosphate buffer solution (PBS), wrapped in aluminium foil and stored in deep freezer for use.

2.4. In vitro permeation experiment
A vertical Franz diffusion cells (receptor volume=6.75 ml, effective permeation area=2.8 cm²) were used to perform the in vitro permeation experiment. A piece of the excised skin was sandwiched between the donor cell and receptor cell with the SC facing the donor chamber. The donor cell was filled with saturated CsA solution, saturated CsA solution and 10% ethyl oleate in combination, saturated CsA solution covered by differently corona charged PP electret which was 1 mm away from the surface of the solution, or a combination of saturated CsA solution and 10% ethyl oleate with electret 1 mm away from the surface of the solution. Phosphate buffer solution (pH 7.4) was used in the receptor and stirred with magnetic bar. The cells were maintained at 32±0.5°C. Samples (0.3 ml) were withdrawn at regular time interval up to 24 h and were replaced with fresh PBS.
2.5. Data analysis

The cumulative amount \((Q, \mu g \text{ cm}^{-2})\) of CsA that permeated across the skin was calculated based on the equation:

\[
Q = (c_n + \frac{V}{V_0} \sum_{i=n}^{\infty} c_i) \times \frac{V}{A}
\]  

(1)

Where \(V\) is the volume of the receptor cell; \(A\) is the effective permeation area; \(V_0\) is the sampling volume; \(c_n\) and \(c_i\) are the concentrations of CsA in receptor cell at \(n\) h and \(i\) h, respectively.

The concentration of CsA was analysed by high performance liquid chromatograph (HPLC, Agilent Tech, USA) with a 250 mm x 4.6 mm DiamonsilTM ODS column (Dikma Tech Inc, China) and UV detection at 214 nm. The mobile phase consisted of acetonitrile, methanol and water in the ratio of 340:100:60 and delivered at a flow rate of 1.0 ml/min. The inter-day and intra-day S.D. of the HPLC analysis were below 5%.

2.6. Statistical analysis

Data were treated for statistical analysis using SPSS13.0 analytical software for single factor analysis of variance (ANOVA) and \(t\)-tests. \(P\) below 0.05 was considered to be significant.

3. Results and discussion

3.1. Stability of the electrostatic field penetrated through the rat skin

Figure 1 is the time courses of the effective surface potentials of negative PP electrets penetrated through the rat skin. It indicates that all the external electrostatic field of the electrets used in this study could effectively penetrate through the rat skin. A positive correlation between the penetrated effective surface potential and the initial surface potential of the electret was presented. The higher the initial surface potential of the electret, the larger the penetrated effective surface potential was. Although a quicker decay of the penetrated effective surface potential was observed when the electret had a higher initial surface potential, its absolute value of the remaining effective surface potential was still higher than those electrets with lower initial surface potentials. The penetrated electrostatic field of all the negative electrets could keep strong enough and relatively stable. Therefore, the PP electrets could be used in drug transdermal enhancement experiment.

3.2. Enhancing effect of electret for transdermal delivery of cyclosporine A

Figure 2 shows the percutaneous cumulative amounts of CsA from the saturated solution (control) and the saturated solution plus -500 V, -1000 V or -2000 V PP electrets. An 8 h lag time was observed for all the samples, which could be attributed to the higher molecular weight of the CsA. The natural barrier function of the SC to CsA was significantly increased, and the time for the drug to cross the SC and diffuse, the transfer as well the as metabolism in various layers of the skin was prolonged. Therefore, a delaying effect up to 8 h was presented and even could not be shortened after the application of negative PP electrets. Therefore, electret with the surface potentials in the range of -500 V to -2000 V could not effectively reduce the lag time of the CsA through the rat skin.

However, an enhancing effect of the electret on CsA cross the rat skin was observed within the time interval of 8 h to 24 h. The cumulative amounts of the drug at 24 h after application of -500 V, -1000 V and -2000 V PP electrets were 7.52±0.70 \(\mu g \text{ cm}^{-2}\), 9.28±1.87 \(\mu g \text{ cm}^{-2}\) and 8.86±4.57 \(\mu g \text{ cm}^{-2}\) respectively, which were 1.2-fold, 1.48-fold and 1.42-fold with respect to the control. The highest enhancing effect on permeation was obtained when the PP electret surface potential was -1000 V, suggesting a “potential window” was exist for electret transdermal enhancing effect. And this result has been proved by our previous studies [12]. Our scanning electron microscopy (SEM) study indicates that among the three negative electrets, -1000 V PP electret had the most significant effect on loosening the SC and disengaging the compact SC lipid structure, which were in favour of drug transdermal delivery.
Figure 1. Skin penetrated surface potential decay of negative PP electrets.

Figure 2. Time course of cumulative amount of CsA through rat skin.

3.3. Synergistic effect of electret and ethyl oleate for transdermal delivery of CsA

Figure 3 shows the cumulative amounts of CsA from the saturated solution, the saturated solution plus 10% ethyl oleate, and the saturated solution plus 10% ethyl oleate and negative PP electret. It indicates that ethyl oleate could significantly increase the cumulative amount within 24 h as compared with the control. Besides, the lag time for CsA penetrating through the rat skin was shortened. The cumulative amounts of the drug at 8 h and 24 h were 18.94±21.72 μg cm⁻² and 89.69 μg cm⁻², respectively, which were 18.94-fold and 14.30-fold with respect to those of the control. Ethyl oleate, a kind of fatty acids, is proved to be an effective enhancer for transdermal drug delivery [13]. The action mechanism was that Ethyl oleate as a separate phase within the bilayer lipids can disturb the intercellular lipid packing and thus facilitating the permeation of the drug penetrating through the skin.

Figure 3 also indicates that when -500 V, -1000 V or -2000 V PP electret was used in combination with 10% ethyl oleate, the cumulative amounts of CsA at 24 h were respectively 109.86±45.12 μg cm⁻², 127.15±38.63 μg cm⁻² and 142.51±42.71 μg cm⁻², which were 17.50-fold, 20.25-fold and 22.69-fold increases with respect to the control, and 1.1-fold, 1.28-fold and 1.43-fold with respect to single use of 10% ethyl oleate. Therefore, the combinational use of both the electret and chemical enhancer had the synergistic effect on CsA transdermal delivery. The higher the corona voltage of the PP electret, the more the synergistic effect was. The mechanism could be explained as follows: electret could not only regulate the electric polarized state of the skin, but also change the structure of the SC to form many...
reversible ducts. The ethyl oleate diffused to SC and various layers of the skin via these ducts, further disturbed the arrangement of the lipid bilayers of the SC and increased the fluidity of the lipids. Therefore, more drugs could dissolve and trap in the skin, and then penetrate through various layers of the skin into subcutaneous tissue and further into blood circulation under the action of natural diffusion and electrostatic field of the PP electret.

4. Conclusions
Negative electret alone or in combination with ethyl oleate could increase the in vitro transdermal delivery of CsA. -1000 V electret had the best enhancing effect when the electret was used alone. However, -2000 V electret exhibited the best synergistic enhancement when it was in combination with the ethyl oleate. These findings suggest that the electrostatic field produced by the PP electret could be a potential physical factor to enhance the transdermal delivery of macromolecule, especially used in combination with chemical enhancers.

Acknowledgments
The authors thank the National Nature Science Foundation of China (Grant No. 50977089) and Natural Science Foundation of Shanghai (Grant No. 10ZR1437600) for financial support provided.

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