Influence of porous PTFE/LDPE/PP composite electret in skin ultrastructure

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Abstract Corona charging and heat melting method were used to prepare porous PTFE electret and porous PTFE/LDPE/PP composite electret, respectively. After 0.5, 1, 1.5, 3 and 4 hour’s action of fluorescein sodium (FINa) and -300V porous PTFE/LDPE/PP composite electret on the excised abdominal skin of rat, the skin structure was studied by means of scanning electron microscopy, transmission electron microscopy and confocal laser scanning microscopy, respectively, to probe the mechanism of electret on transdermal drug delivery. The results indicated that negative electret could increase the transdermal delivery of FINa due to its effect on changing the organized structure of stratum corneum, enlarging the hair follicles, which may be the mechanism of electret in enhancing transdermal drug delivery.

1. Introduction

Transdermal drug delivery (TDD) is the administration of therapeutic agents through intact skin for systemic effect. TDD offers several advantages over the conventional dosage forms such as tablets, capsules and injections. Skin consists of epidermis, dermis and hypodermis. The outmost layer of epidermis, the stratum corneum (SC), is considered the main barrier for transdermal drug delivery [1]. It is approximately 10-20 um thickness and acts as a protective membrane preventing water loss from the skin and limiting the ingress of chemicals from the environment. The stratum corneum has only 20% water and forms a highly lipophilic membrane. Because of the organized structure of SC, only limited drugs can be administrated transdermally. Usually, there are three pathways for drug to traverse the SC, namely intercellular, intracellular and appendage pathways. Barry’s lipid-protein theory indicated that drug permeate the skin via intercellular route in most case. The water soluble and ionic compounds are known to permeate the skin via appendages, that is hair follicles and sweat ducts. SC permeation is a rate-limiting process [2].

However, the permeation of drugs through skin can be enhanced by physical methods such as iontophoresis [3], electroporation [4], electret [5, 6] and by chemical penetration enhancers (CPE).
Electret is a kind of dielectric material which can keep the space and dipole charge for long time (e.g., negative electret of porous PTFE can keep the charge for 10^5 year [7]). As a new physical factor, its electrostatic potential and microcurrent can be used to regulate both the electret and electric state of skin, and to enhance transdermal drug delivery. Our previous studies indicated that -1000V and +1000V electret could increase methyl salicylate and lidocaine transdermal delivery, respectively. The increasing fold reached to 1.55 and 4.84 as compared with control group [5, 6].

Although it has been found that electret could be a new promising technique in drug transdermal enhancement, there is limited knowledge of how electret enhancing the drug delivery. Electron microscopy and confocal laser scanning microscopy (CLSM) have been important tools for visualize the ultrastructural details of skin [4]. Besides, CLSM has been used to study the improved drug transdermal delivery by chemical enhancement, iontophoresis, electroporation, sonophoresis. In this study, scanning electron microscope (SEM), transmission electron microscope (TEM) and CLSM were used to study the effect of negative porous PTFE/PE/PP composite film electrets on fluorescein sodium transdermal delivery and the mechanism of electret on transdermal enhancement.

2. Materials and methods

2.1. Reagents

Male Sprague-Dawley (SD) rats (220±10g) were obtained from the Animal Centre of Second Military Medical University. Fluorescein sodium (FINa), paraformaldehyde and osmium tetroxide were purchased from Sigma-Aldrich Chemicals, USA. Other reagents used were uranyl acetate, Epon 812, acetate, ethanol, urethane, PBS solution. All chemicals were used as supplied.

2.2. Preparation of electret

Porous PTFE films, 50 μm thickness with porosities of 70% prepared by means of unidirectional stretching at a suitably high temperature, were purchased from Shanghai Plastic Institute. PP with the thickness of 13μm (Toray Fan Co. Japan) constituted the upper layer of the composite membrane as a water resistant layer. The middle layer was LDPE products. To ensure the structure similarity of the sample, all the films were from the same roll through out the experiment.

Porous PTFE films were corona charged (CORONATROL, Model 152A, Monroe Electronics Co., USA) at room temperature for 5 min with the point voltages of -8kV and grid voltages of -300V. Subsequently, the LDPE film was sandwiched between porous PTFE electret and PP and heat melted for 30min at 105°C. Afterwards, it was cooled to form porous PTFE/LDPE/PP composite electret [8]. The surface potentials of the charged samples were measured by potentiometer (Monroe electronics, Isoprobe, Model 244, USA).

2.3. Experiments

2.3.1. Electret group and control group. On each experiment, 6 of 10 SD rats were selected randomly. After rat being anaesthetized by 20% urethane, the abdominal region of 8cm×6cm area was shaved and divided into left and right parts, respectively. The left part with porous PTFE/LDPE/PP attached was taken as electret group, while the right part was the control group. At the time interval of 0.5, 1, 1.5, 3 and 4h, rat was killed and three pieces of skin were cut from each part. After the adhering fat and other visceral tissue were removed, the skin was fixed in 4% paraformaldehyde solution for more than 24h (prefixed sample) to prepare the SEM, TEM and CLSM samples, respectively.

2.3.2. Experimental group. 3 of 15 SD rats were selected randomly. The abdominal hair was shaved by method described above. The shaved area was divided into 4 regions with bilateral symmetry. Then the regions were wiped with 3.0ml 0.125% FINa solution. After the skin was dried, porous PTFE/LDPE/PP electret was attached to the left region and taken as fluorescent electret group, while the porous PTFE was attached to the right region as fluorescence group. At the same time interval as
above, rat was killed and the corresponding skins were cut. The skins were treated by the same method as described in 2.3.1 for SEM, TEM and CLSM sample preparation.

2.3.3. **Scanning electron microscopy.** Prefixed skin samples were washed in PBS solution three times, fixed by 1% osmium tetroxide for 2 h and subsequently dehydrated in graded series of ethanol. Then samples were air dried for 1 h, fixed to a bracket and filmed for 10 min. Samples were observed under a Hitachi S-520 SEM.

2.3.4. **Transmission electron microscopy.** The graded dehydrated samples as described in 2.3.3 were soaked in acetone for 20 min, immersed in Epon812/acetone mixture for 1 h and then overnight in Epon812. Subsequently, the samples were thin sectioned, stained with uranyl acetate and lead citrate and examined by Hitachi H-800 TEM.

2.3.5. **Confocal laser scanning microscopy.** Prefixed skin samples were dehydrated, embedded in paraffin and sectioned for CLSM analysis. The CLSM used was a Leica TSC SP2 (Leica, Germany) equipped with an Ar/ArKr ion laser, for excitation at 488 nm and transmission at 500–599 nm, and with an image resolution of 1024×1024 pixels, numeric aperture of 0.85. Every pixel represents a value of intensity, from 0 to 255, where 0 is no fluorescence detected and 255 is full saturation.

3. **Results and discussion**

3.1. **Charge storage stability of porous PTFE/LDPE/PP electret**

Fig. 1 is the isothermal surface potential decay profile of -300V porous PTFE/LDPE/PP composite electret at relative humidity (RH) of 98%. It showed that porous PTFE/LDPE/PP composite electret could hold 98% of its initial surface potential after being kept at high RH surrounding for 24 h, indicating better glutinosity between porous PTFE and PP [9]. PP effectively interdicted the influence of water at high RH in porous PTFE of its dielectric and charge storage property, improved the charge storage stability of porous PTFE/LDPE/PP. Similar result was observed after the composite electret being applied to rat skin for 4 h. It could be seen from Fig.2 that surface potential decayed only 10% of its initial value, indicating further that negative porous PTFE/LDPE/PP electret had excellent performance in resisting water influence in charge storage stability, though the weak acidic liquid on skin epidermis that caused by water evaporation and sebum secretion from the skin might easily cause the charge decay of electret. Negative porous PTFE/LDPE/PP electret not only exhibited better humidity resistance and excellent charge storage stability, but also could be used as a novel driving force in study of transdermal drug delivery due to its stable electrostatic field.

![Fig.1 Surface potential decay of negative porous PTFE/LDPE/PP electrets at RH=98%](image1)

![Fig.2 Surface potential decay of negative porous PTFE/LDPE/PP electret after skin contacting](image2)

3.2. **Influence of negative porous PTFE/LDPE/PP electret in skin structure**
With scanning electron microscopy, the horizontal plane of the epidermis was inspected. Fig. 3 is the microphotograph of normal epidermis. It showed closely united assembly of squamous cells, with a ridge and furrow pattern [10]. The cells showed irregular folds convolutions and occasionally a villous appearance. Cell margins were difficult to observe as adjacent cells appear to merge because of their close apposition. The central bulge or depression present on few cells might be attributed to the position of nucleus before cell death. These cells enclosed keratin filaments embedded in amorphous matrix of mainly lipid and non-fibrous protein. All the cells showed irregular folds and convolutions.

SEM of the skin samples after treated with electret showed interesting changes of the lipid arrangement of stratum corneum (Fig. 4). It could be seen that epidermal cells disengaged, compact structure loosed, epidermal cranny increased, cells turned to flatten and calibre of hair follicles enlarged. Therefore, the looseness and cell separation of stratum corneum, broaden of hair follicles and alteration of fluid properties of the stratum corneum lipid that resulted from electrostatic field the electret produced, might led to the decreased permeation resistance and enhanced transdermal drug delivery.

The morphological change of the skin after electret treatment was further confirmed by transmission electron microscopy [11]. Fig. 5 is the TEM microphotography after skin was applied with FINa for 3 h. It can be seen that stratum corneum was acidophilic and epidermis displayed an even zonal distribution. The stratum corneum and granulocyte layer engaged closely and epidermal cells showed integrity and continuity. There were no observable changes in connection of granulocyte and prickle cell, and either the dermis structure. However, considerable changes could be observed for the skin from fluorescent electret group after 3 hours treatment [Fig 6]. The stratum corneum separated from epidermis and desquamated, but only with a lamina joined to epidermis. Besides, epidermis seemed to have loosened. Therefore, by loosening of the SC arrangement and desquamating, electret could reduce the barrier function of skin and ensure the transdermal drug delivery, which might be the explanation of enhancing effect the electret produced in transdermal drug delivery.
3.3. Effect of porous PTFE/LDPE/PP electret on transdermal permeation of FINa
To better understand the pathways involved, the negative porous PTFE/LDPE/PP electret enhanced permeation of fluorescent molecule was investigated. CLSM with dual-channel imaging was used to visualize the distribution of FINa [12]. The fluorescent intensities in epidermis were $116.47 \pm 18.81$, $85.67 \pm 5.95$, $75.65 \pm 11.12$ and $74.61 \pm 4.23$, respectively, after 0.5, 1, 1.5 and 3 hour’s application of FINa on rat skin. However, the fluorescent intensities in epidermis were changed to $145.8147 \pm 18.91$, $132.54 \pm 8.29$, $170.96 \pm 14.39$ and $110.18 \pm 11.85$ respectively, when -300V porous PTFE/LDPE/PP electret in combination with FINa were applied to rat skin for the same duration mentioned above, indicating a fluorescent intensity increase of 1.25, 1.55, 2.26 and 1.48, respectively, as compared with fluorescent group, which had statistical significance ($P<0.001$).

Furthermore, a maximum fluorescent intensity was obtained at 1.5 hour’s electret experiment, then it weakened gradually as time passed. 4 hour’s application of electret resulted in a fluorescent intensity of $61.20 \pm 2.34$ which was 0.55 times the value of fluorescent group ($109.67 \pm 12.29$) which also had statistic significance ($P<0.001$). It could be concluded that negative porous PTFE/LDPE/PP electret treated skin had lower permeation barrier and FINa could penetrate through stratum corneum to subcutaneous tissue, such as dermis and muscle, with quick diffusion rate.

Fig. 7 and Fig. 8 are the confocal micrographs of skin after FINa passive penetration or electret combined FINa penetration for 3 h. For fluorescent electret group, the fluorescent intensities in epidermis increased markedly and the arrangement of stratum corneum loosed as compared with that of fluorescent group. Besides, the CLSM imaging visualized stronger fluorescent intensity in hair follicles than in dermis and muscle for fluorescent electret group, certainly all stronger than that of in fluorescent group (images not showed here). Therefore, due to the looseness of epidermis structure and enlargement of hair follicles caused by electret, large amount of FINa penetrated into the epidermis not only via intercellular route, but also via hair follicles route. And the barrier function reducing resulted in a quick intercellular permeation rate of FINa.

4. Conclusion
Negative porous PTFE/LDPE/PP resulted in an apparent increase in transdermal drug delivery. SEM, TEM and CLSM studies had confirmed the above observations. Electret caused considerable loosening of cells with increased cell separation, resulted in very large intercellular spaces. In addition, electret enlarged the hair follicles, resulting drug permeation through appendages pathway. Hence, electret drives drug across the skin not only by intercellular route but also by porous route, resulting in enhanced permeation amount. Electret could be a promising strategy in transdermal drug delivery enhancement.
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