Structure of rat skin after application of electret characterized by DSC

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Abstract. Polypropylene (PP) electrets with surface potential of \(-500\text{V}, -1000\text{V}\) and \(-2000\text{V}\) were prepared by constant voltage corona charging. The electrets were applied to excised rat skin for 2 hours respectively and then the skin samples were analyzed with the differential scanning calorimetry (DSC) technique to study the alteration of lipid organization of the skin. There were three peaks at \(63^\circ\text{C}, 82.7^\circ\text{C}\) and \(115.1^\circ\text{C}\) in the DSC spectra for rat skin untreated, which have been assigned essentially to lipid, lipid-protein and protein alterations. For \(-500\text{V}\) electret treated-skin sample, only a single peak appeared at \(79.1^\circ\text{C}\). With the increase of electret surface potential from \(-500\text{V}\) to \(-2000\text{V}\), the transition temperature and peak areas at moderate decreased first and then increased. The negative electret could result in the transition of stratum corneum (SC) lipid from gel to liquid crystal and protein transition from \(\alpha\) helix structure to \(\beta\) folding structure. The regulation action of electret to skin microstructure presented an effect of “potential window”.

1. Introduction
An electret is a functional material which can keep space and polar charge for a long time. The external electric field of the electret can result in a series of biological effects, such as improving microcirculation, lowering blood viscosity, enhancing fracture and wound healing, regulating cell growth cycle and apoptosis, bacteriostasis and air cleaning [1-5], etc. With the development of studies on the biological effect of electrets, the regulation effect of electret on skin state and the electret transdermal drug delivery system have become a research focus [6-8], giving a novel formulation and route for clinical application.

Transdermal delivery has a variety of advantages over the oral route as avoidance of the first-pass metabolism, sustained therapeutic action and better patient compliance. However, its prevalent use is restricted due to excellent impervious nature of skin. Therefore, many approaches have been applied to overcome the skin barrier. Since 1980s, we have studied the enhancing effect of electret on transdermal drug permeation. Although our studies and those of Murthy et al. confirmed that an electret could be an excellent physical approach to enhance transdermal drug permeation [8, 9], the mechanism of electret action is still an attractive study area. To well understand the mechanism of electret action, this paper will use differential scanning calorimetry (DSC) and microstructure analysis to study the structure change of rat skin after application of an electret.
2. Materials and Methods

2.1. Electret preparation
The polypropylene (PP) film with a thickness of 13µm was from Toray Fan Co. Japan. All the materials were from the same rolls to keep the same morphometrics. The bare PP film was corona charged at constant voltage and 77% humidity for 5 minutes using a corona charging system (Dalian University of Technology, China) with the point voltages of -20kV and grid voltages of -500V, -1000V and -2000V, respectively. The surface potentials of the samples were measured with a surface potentiometer (ESR102A, Beijing Huajinghui Technology Co. Ltd. China).

2.2. Reagents
Solutions of PBS (0.2M, pH 7.2) and 20% urethane were prepared in our laboratory.

2.3. Animals and grouping
12 Male Sprague-Dawley (SD) rats were randomly divided into groups of control, -500V, -1000V and -2000V electret samples, respectively. The rats were anesthetized by 20% urethane (0.5ml/kg body weight). The abdominal hair of the rat was shaved with a hair clipper. Four test sites were selected and marked, each having an area of 2cm×2cm and an interval of 2cm. The test sites were treated for 2 hours with PP film (0V) and electrets with different surface potentials. Then the rats were killed. The tested skin was cut off quickly, wiped with PBS solution, then the excess fat adhering to the dermis side was removed using cotton, and the skin sample was kept for further study.

2.4. DSC analysis
The endotherm scans of the skin samples were obtained by DSC, using a scanning rate of 10°C/min from 25 to 350°C. A Diamond TG/DTA system (PerkinElmer, USA) was used.

2.5. Microstructure analysis
The skin was dehydrated and paraffin embedded, Hematoxylin and Eosin (HE) stained and sliced for microstructure analysis using the camera microscope (Olympus SF07103, Japan).

3. Results and discussion

3.1. DSC profiles of normal skin
Fig. 1 is the DSC profile of the normal rat skin. It demonstrated three typical endothermic transition temperatures at 63°C, 82.7°C and 115.1°C with the first peak being attributed to either lipid lamella phase transition from a crystalline to a gel like phase or melting of sebaceous lipids, the second peak corresponding to the transformation from a lamellar to disordered state in the lipid structure and protein associated lipid transition from gel to liquid state, respectively, the third peak being associated with the conformation change of protein [10].

3.2. Influence of negative electrets in skin structure
Figure 2 is the DSC profile of rat skin treated by -500V electret. There was only a single peak which appeared at 79.1°C with an increased peak height, decreased peak area and transition temperature. The electrostatic field and microcurrent produced by -500V electret could result in space structure alteration of lamellar lipid from gel to liquid state and resulting in an absence of transition temperature at 63°C. Besides, -500V electret increased the orderliness of SC lipid, reduced the resistance and thermodynamic energy of SC. Thus, the energy needed for lamellar lipid in the same or different SC layer to transform from the gel to liquid state decreased and demonstrated a trend of cooperation, which resulted in shifting of the transition peak to lower temperature and increase of peak height. Figure 2 further indicated an absence of transition temperature at 115.1°C as compared with control skin, because a large amount of protein in SC denatured from α helix to β fold after action of -500V.
electret. Since the secondary and tertiary space structure changes of some protein, both the proportion of protein to absorb heat and the heat absorbed for structure alteration reduced. Therefore, the endothermic transition temperature attributed to conformation change of protein the disappeared after -500V electret treatment.

Compared with the action of -500V electret, the peak area of DSC endotherms for -1000V electret-treated skin sample showed a further decrease (Figure 3). It suggested that with the increase of surface potential of negative electret the orderliness and fluidity of the SC lipid improved, the conformation proportion of liquid state increased, being help for drug transdermal permeation. For skin sample treated by -2000V electret, there was no significant change in peak shape and phase transition temperatures at 78.9°C and 113.7°C when compared to -1000V skin sample except for the obvious increases of peak areas. Besides, the phase transition temperature and peak area between 70°C–200°C were almost the same as that of the control skin (Figure 4), indicating a weaker regulating effect of -2000V electret than -1000V electret on orderliness and fluidity of SC lipid structure and both of the secondary and tertiary structures of the protein. The influence of negative electret in rat skin structure presented an effect of a “potential window” with the -1000V electret having a better regulating effect on skin structure.

3.3. Microstructure analysis
The regulating effect of -1000V electret on skin structure was further confirmed by microstructure analysis. Figure 5 and Figure 6 are the microphotographs of normal rat skin and -1000V electret-treated rat skin, respectively. A considerable change could be observed for the -1000V electret treated skin (figure 6). The structure of the rat skin changed obviously with a loosening and thickening of the SC, disengagement of SC lipid cells, and separation of part of the SC from the skin epidermals when compared to normal skin. Therefore, -1000V electret could result in alteration of the arrangement of SC lamellar lipid, reducing the barrier function of the skin.
4. Conclusion

Negatively charged electret can regulate the skin structure by alteration of the SC lipid organization and α helix to β fold of the protein, regulating the lipid fluidity by transformation of gel to liquid state of the lamellar lipid. The effect of negative electret on rat skin structure presented an effect of a “potential window”. Over the potential range from -500V to -2000V, -1000V the electret showed a stronger skin structure regulation effect. Electrets have an excellent effect on enhancing transdermal drug delivery by skin structure regulation.

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