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Letter

Influence of skin condition on the skin penetration of dextran 4000 and ovalbumin

Makiko Fujii, Miko Imai, Hiroki Kuwabara, Minori Awano, Kaname Hashizaki and Hiroyuki Taguchi

School of Pharmacy, Nihon University, 7-7-1, Narashinodai, Funabashi-shi, Chiba 274-8555, Japan

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ABSTRACT — Transdermal sensitization by Glupearl 19S, which is a hydrolyzed wheat protein with a molecular weight of tens of thousands and present in a facial soap, has been reported. Intact skin is considered to function as a barrier; thus, a substance with molecular weight as high as that of Glupearl 19S cannot penetrate to the skin and cause sensitization. We studied whether moderate- or high-molecular-weight materials could penetrate into the skin by using intact and injured Yucatan micropig skin *in vitro*. Fluorescein isothiocyanate (FITC)-labeled dextran (MW 4000, FD4) and ovalbumin (FITC-OVA) were applied as 1% aqueous solutions with or without 1% sodium dodecyl sulfate (SDS) as a surfactant. FD4 penetrated in the stratum corneum of intact and delipidized skin, especially when combined with SDS. It also penetrated into the dermis of stripped skin (the stratum corneum was removed by tape stripping). FD4 was observed in some scratches of the skin and diffused into the epidermis. FITC-OVA was partially observed in the stratum corneum of intact and delipidized skin. In the case of stripped skin, FITC-OVA did not penetrate in the viable epidermis or dermis because it could not pass through tight junctions even if they were open. FITC-OVA was observed in every scratch 2 min after the application and did not diffuse into the surrounding area as FD4 did. It is considered that FITC-OVA penetrates skin defects and remains at the site, which increases the possibility of sensitization by Langerhans cells.

Key words: Skin penetration, Ovalbumin, Scratched skin, Delipidized skin, Stripped skin, *In vitro*

INTRODUCTION

The skin functions as a barrier to protect the internal organs of the body. It consists of two layers, the epidermis and dermis. The epidermis consists of keratinocytes, which multiply in the basal layer, and differentiates into the stratum spinosum epidermis, granular layer, and stratum corneum. The stratum corneum, which is the outermost layer of the skin, prevents substances from penetrating the skin. It is made up of keratinocytes consisting of densified keratin and intercellular lipids, in the so-called brick/mortar model. Materials with low molecular weight and appropriate lipophilicity are partitioned into the lipid layer between the stratum corneum and diffuse into deeper sites in the skin (Benson, 2005). However, hydrophilic materials are hardly partitioned in the lipid layer. Materials with a molecular weight higher than 500 are considered to never penetrate into the skin because the diffusion rate in the stratum corneum is lower than the desquamation rate (Bos and Meinardi, 2000). In addition, a tight junction in the granular layer prevents the penetration of materials into the viable epidermis, even if they penetrate through the stratum corneum. Thus, high-molecular-weight hydrophilic materials, such as polysaccharides or proteins, can never penetrate in the viable epidermis of intact skin.

Correspondence: Makiko Fujii (E-mail: fujii.makiko@nihon-u.ac.jp)

In Japan, transdermal sensitization by Glupearl 19S, a hydrolyzed wheat protein present in a facial soap, was reported (Teshima, 2014). Glupearl 19S is hydrophilic with a molecular weight of tens of thousands, and it is considered that this substance cannot easily penetrate in the skin. There are many reports about sensitization characteristics, and it has been revealed that sensitization characteristics become stronger by hydrolysis under acidic conditions. However, even if Glupearl 19S has sensitization activity, it would not be established that Glupearl 19S penetrated in the skin and was recognized as an antigen. Sensitization with a high-molecular-weight material, such as tick antigens, has been reported in atopic dermatitis (Gittler *et al*., 2013). However, thus far, atopic dermatitis has not been reported in all patients sensitized to Glupearl 19S.

Thus, we studied the conditions that affect the penetration of high-molecular-weight materials into the skin. To this end, we evaluated whether moderate- or high-molecular-weight materials could penetrate the skin by using intact and injured Yucatan micropig (YMP) skin *in vitro*.

MATERIALS AND METHODS

Materials

Fluorescein isothiocyanate (FITC)-dextran (FD4, MW 4,000) and FITC-ovalbumin conjugate (FITC-OVA, MW45,000) were obtained from Sigma-Aldrich Japan (Tokyo, Japan) and Thermo Fisher Scientific (Tokyo, Japan), respectively. OVA was obtained from FUJIFILM Wako Pure Chemicals (Osaka, Japan). Sodium dodecyl sulfate (SDS) was obtained from Kanto Chemicals (Tokyo, Japan).

Preparation of the skin

YMP skin (skin set obtained from female animals aged 5 months, Charles River, Japan, Kanagawa, Japan) was used as the model skin. Frozen YMP skin was thawed at room temperature and subcutaneous fat was removed. The skin was stripped with tape (3M Japan, Tokyo, Japan) twice to obtain surface free energy similar to that of human skin (intact skin) (Fujii *et al*., 2019). Intact skin was stripped 50 times with adhesive tape to obtain stripped skin. Delipidized skin was prepared by application of 1 mL of an ethyl ether-acetone (1:1) mixture to intact skin set for 40 min on Franz-type diffusion cells. The skin was washed with water and wiped with a Kim-wipe. Intact skin was attached with electronic balance by adhesive tape with the stratum corneum upside. Then, the skin was scratched with a needle for injection (19G × 1 ½" S/B, Terumo, Tokyo, Japan) with a force of 0.2 N (balance showed 20 g) (scratched skin). In the case of application for 2 min, a lancet (Smart Practice, Japan, Kanagawa, Japan) was used to prepare scratched skin.

Skin penetration of FD4 or FITC-OVA

FD4 or FITC-OVA was dissolved in injectable water at a concentration of 1%. For the co-administration of SDS, 1% SDS solution was used instead of injectable water. The solution was applied to various types of the skin at 10 μL/cm^2. The skin was placed on a wet paper towel and kept at 32°C for 4 hr in an air incubator. Then, the skin surface was wiped with a Kim-wipe and stripped once with adhesive tape (Scotch®, Cho-Tomei Tape S, 3M, Japan) to remove skin surface residues. The treated area of the skin was cut to a size of ca. 5 × 5 mm, embedded in a freezing medium (Tissue-Tek®, O.C.T. compound, Sakura Finetek Japan, Tokyo, Japan), and frozen. Embedded and frozen skin was sliced at a thickness of 20 μm by using a cryostat (Sakura Finetek Japan) and observed with a confocal laser microscope (CLSM). Three slices were obtained continuously, 20 slices were abandoned (0.4 mm), and three slices were obtained again. Consequently, three continuous slices were obtained three apart parts, *i. e.* nine slices were observed under the CLSM.

CLSM (LSM510 or LSM710, ZEISS Japan, Tokyo, Japan) with a 488 nm Ar laser was used to observe the green fluorescence of FITC. Microscope examination of the untreated skin sensitivity set showed no fluorescence.

Measurement of the surface tension of FD4 and OVA solutions

The surface tension of the solutions was measured with optical contact angle measuring instruments (DataPhysics, OCA20, Eko Instruments, Tokyo, Japan) by using the pendant drop method. A syringe needle (22G × 1 ½", non-bevel type, Terumo) was used for the drop.

Measurement of the contact angle of the solution on YMP intact skin

Intact YMP skin was dermatomed to a thickness of 300 μm, the hair on the skin was removed, and the skin was spread on a glass plate. One microliter of the solution was placed on the skin and its image was captured immediately. The contact angle was measured with a curve-fitting method using optical contact angle measuring instruments (DataPhysics, OCA20, Eko Instruments).

RESULTS AND DISCUSSION

Figure 1 shows CLMS images of the skin section after the application of the FD4 aqueous solution. In the case of intact skin, the fluorescence of FD4 was observed partially in the stratum corneum; however, some of the skin sections showed no fluorescence. When FD4 was used with SDS, it distributed thoroughly in the stratum corneum. In the case of delipidized skin, FD4 distributed thoroughly in the stratum corneum regardless of the presence of SDS. It is considered that intercellular lipids inhibit the penetration of FD4 into the stratum corneum. In the case of stripped skin, FD4 penetrated the viable epidermis and dermis regardless of the presence of SDS. It is thought that the barrier function was lost when the stratum corneum was removed. There is a tight junction in the granule layer under the stratum corneum. The function of the tight junction might be lost by the physical overstimulation caused by stripping (Svoboda *et al*., 2016). In the case of scratched skin, FD4 without SDS penetrated some scratches but not some scratches, while FD4 with SDS penetrated all scratches. When FD4 enters scratches, it penetrates the deeper parts of the skin.

Figure 2 shows the CLMS images of the skin sections after application of the FITC-OVA aqueous solution. Fluorescence of FITC-OVA without SDS was observed partly in the surface of the stratum corneum of intact skin. The use of SDS and delipidization of the stratum corneum resulted in slightly enhanced penetration of OVA in the stratum corneum; however, this was not obvious. The molecular weight of OVA is more than 10-fold that of FD4. Thus, the imperfect intercellular lipid layer or effect of SDS was smaller than on FD4 penetration. FITC-OVA showed no penetration even when the stratum corneum was stripped off. When the tight junction is open, macromolecules larger than 10 kDa cannot pass the gaps of cells (Krug *et al*., 2013). Thus, FITC-OVA could not penetrate the viable epidermis. In the case of scratched skin, FITC-OVA was observed in every scratch regardless of the presence of SDS. The fluorescence was detected in the scratch alone and did not spread as deep as in the case of FD4.

The penetration of FD4 and FITC-OVA is summarized in Table 1. Both FD4 and OVA did not penetrate the viable epidermis when they were applied to intact and delipidized skin. The addition of SDS and delipidization enhanced penetration into the stratum corneum and fluorescence was observed in the deep stratum corneum. Kubo *et al*. (2009) reported that Langerhans cells take up antigens near tight junctions. Thus, there is a possibility of sensitivity when an antigen penetrates the deep part of the stratum corneum. Thus, the use of a surfactant

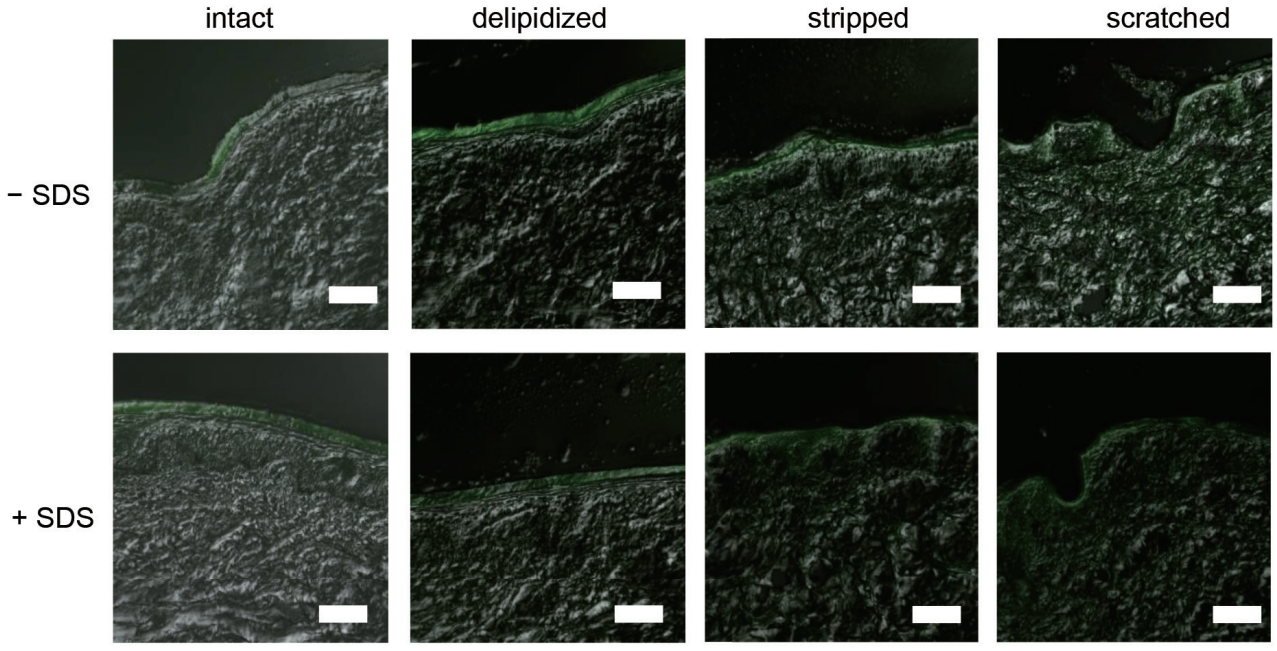

Fig. 1. Penetration of FD4 in the skin under various conditions with or without SDS. Application period: 4 hr. Each bar scale represents 100 μm.

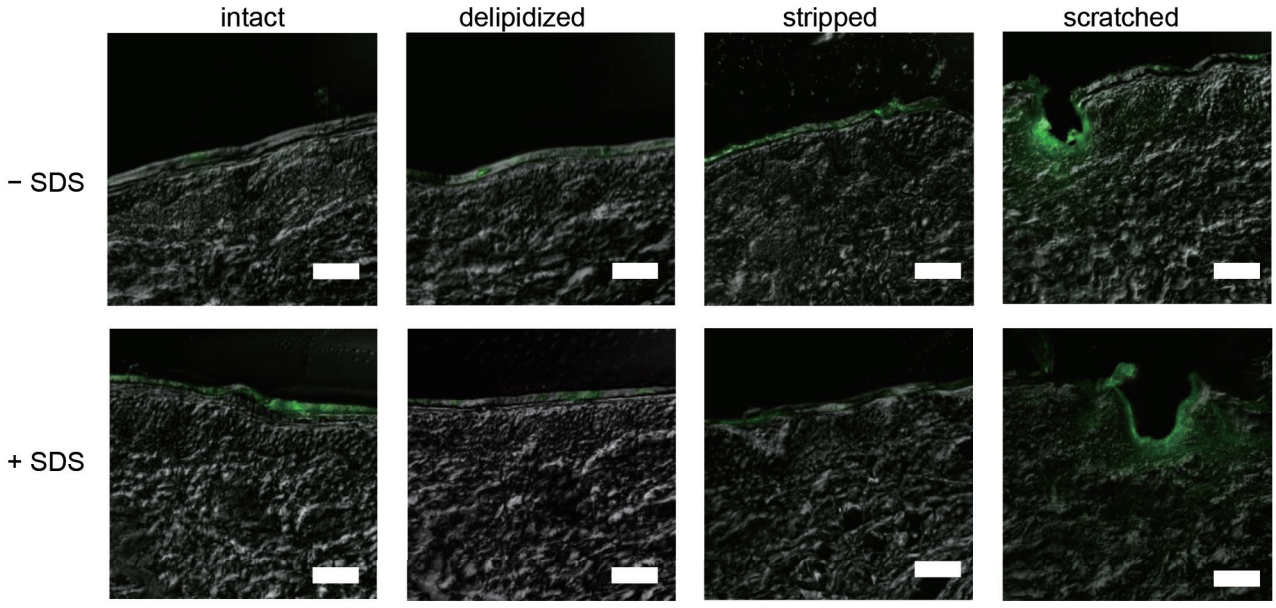

Fig. 2. Penetration of FITC-OVA in the skin under various conditions with or without SDS. Application period: 4 hr. Each bar scale represents 100 μm.

and delipidized skin conditions might increase the sensitization to an antigen of a moderate molecular weight. In the case of stripped skin, FD4 or OVA was applied on the granule layer; therefore, the risk of sensitivity was higher than that in the case of intact or delipidized skin. However, individuals may become aware of severe defects of the skin, such as entirely stripped skin, by themselves and thus the risk of sensitization can be avoided. In the case of scratched skin, some skin sections showed no FD4 fluorescence in scratches. If FD4 penetrated scratches, fluorescence was observed in the viable epidermis and dermis. FITC-OVA penetrated every scratch, although its molecular weight was greater than that of FD4. When the FD4 solution was applied on the skin, it was difficult to spread it uniformly. Figure 3 shows the change in the surface tension of the solution with concentration. The surface tension of the FD4 solution was the same as that of water and was not affected by the concentration of the

Table 1. Summery of observation with CLSM.

		intact	delipidized	stripped	scratched
FD4	-SDS	±	+	+++	±, +++
	+SDS	+	+	+++	+++
OVA	-SDS	±	±	+	++
	+SDS	±	±	+	++

Fluorescence was observed only partially in stratum corneum (±), in stratum corneum (+), in epidermis (++) and in both epidermis and dermis (+++).

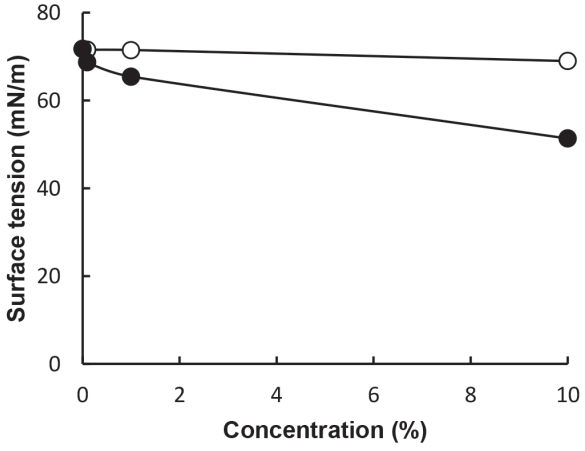

Fig. 3. Surface tension of FD4 (○) and OVA (●) solution.

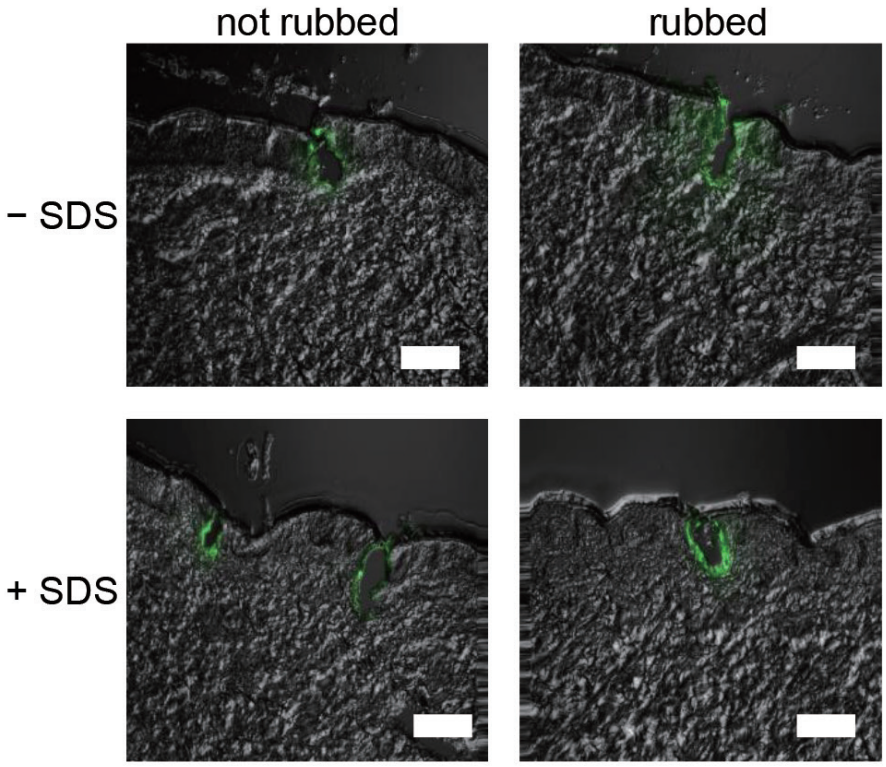

Fig. 4. Penetration of FITC-OVA with or without SDS in scratched skin after application for 2 min with or without rubbing. A lancet was used to make scratches. Each bar scale represents 100 μm.

solution. The contact angles of water, 1% FD4, and 1% OVA to YMP skin were 84.8°, 84.2°, and 67.5°, respectively. When the contact angle is greater than 90°, the solution does not penetrate the capillary. The contact angles for all the solutions were less than 90°; however, it is known that the contact angle increases when the solid surface is rough. Dermatomed skin was used to measure the contact angle to prevent the effect of surface roughness. Full-thickness skin is rough; hence, the FD4 solution could not penetrate some scratches, and the addition of SDS to FD4 lowered the surface tension, allowing improved penetration.

It was suggested that FITC-OVA penetrated the scratches. The application period was 4 hr in the cases shown in Fig. 2. Soap may be in contact with the skin for a few minutes; thus, FITC-OVA solution was applied for 2 min, with or without rubbing, followed by washing with water. The scratches made using needles for injection (19G × 1 ½" S/B) were relatively large, and a lancet was used to make smaller scratches. Figure 4 shows the penetration of FITC-OVA with or without SDS and with or without rubbing. In all cases, FITC-OVA was observed in every scratch but not in the stratum corneum. Rubbing the skin had some effect on penetration; however, it was not necessary for penetration.

In conclusion, penetration of FITC-OVA in the deeper layers of the stratum corneum tended to increase in the case of delipidized skin. In the case of small scratches on the skin surface, FITC-OVA immediately penetrated the scratches and remained in the scratches for a long time. Perfect intact skin would prevent the penetration of high-molecular-weight antigens; however, some defects, such as small scratches, of which individuals may be unaware, increase the risk of sensitization by an antigen even if it has a high molecular weight.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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