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International Journal of Pharmaceutics 316 (2006) 102–108
INTERNATIONAL JOURNAL OF PHARMACEUTICS
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Effects of pretreatment of needle puncture and sandpaper abrasion on the in vitro skin permeation of fluorescein isothiocyanate (FITC)-dextran

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Received 26 August 2005; received in revised form 5 January 2006; accepted 24 February 2006
Available online 4 April 2006

Abstract

Microneedle systems have gained attention as having many advantages over transdermal patches and hypodermic needles. The procedure provides adequate skin permeation rates without pain or severe infection. To obtain information for designing a microneedle system, macroneedles were used instead of microneedles to investigate the effects of pretreatment of needle puncture in the skin barrier stratum corneum on in vitro skin permeation of fluorescein isothiocyanate (FITC)-dextrans (4.3, 9.6 and 42.0 kDa) (FD-4, FD-10 and FD-40). The effect of sandpaper abrasion was also investigated for comparison. Both pretreatments on the skin barrier significantly increased the skin permeation of FDs. Lactate dehydrogenase (LDH) leaching was measured after pretreatment of macroneedle and sandpaper abrasion on the skin to evaluate the skin damage by these pretreatment methods. Lower leaching of LDH was observed after macroneedle puncture than after sandpaper abrasion. Next, a parallel permeation-resistance model of the skin barrier was established. Skin permeation of FD-10 was predicted by the model as a function of the number of pores in the skin barrier. Our results suggest that needle puncture may provide a safe, efficient and controllable alternative for increasing transdermal drug delivery.

Keywords: Microneedle; Macroneedle; Sandpaper abrasion; Skin permeation; Penetration enhancement; LDH leaching

1. Introduction

Oral formulations of drugs such as tablets and capsules and injections are two of the main drug administration techniques. However, the use of tablets and capsules is not always feasible due to easy drug degradation in the gastrointestinal tract and possible first-pass effects in the liver. Injections are limited by pain at the injection site, possible infection during and after injection, and difficult self-administration. To avoid these problems, transdermal drug delivery has gradually gained attention as a third route of drug administration. It is difficult, however, to efficiently deliver therapeutically effective doses of drugs through the skin into the systemic circulation due to the large barrier function of the stratum corneum, the outermost layer of skin. To increase or improve the skin permeation of drugs, a variety of approaches have been studied such as combined use of chemical enhancers (Williams and Barry, 2004) and physical means of iontophoresis (Kalia et al., 2004), electroporation (Denet et al., 2004) and sonophoresis (Prausnitz et al., 2004; Mitragotri and Kost, 2004). Among them, electroporation generates nanometer-scale disruptions in the stratum corneum, which creates drug pathways through the skin barrier.

Recently, as an attractive alternative, the use of microneedles, which have advantages over conventional needles and transdermal patches, have gained unprecedented attention for increasing the skin permeability of drugs. As compared to hypodermic needle injection, microneedles can provide a minimally invasive means of painless, precisely controlled and convenient delivery of therapeutic molecules into the skin, and the technique seldom causes infection (Kaushik et al., 2001). On the other hand, microneedles can create nanometer or micrometer-scale transport pathways sufficiently large enough to deliver macromolecules and even drug-loaded nanoparticles into the skin, which cannot be achieved by the conventional enhancing methods mentioned as above (McAllister et al., 2003).

Since Henry et al. (1998) first used microneedles as a transdermal drug delivery system, there has been a rapid increase of interest in microneedle systems (Prausnitz, 2004). The use of microneedles increased skin permeability of many molecules such as calcein (Henry et al., 1998), desmopressin (Cormier et

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doi:10.1016/j.ijpharm.2006.02.046

al., 2004), diclofenac (Gardeniers et al., 2003), methyl nicotinate (Sivamani et al., 2005), bischloroethyl nitrosourea (Li et al., 2004), insulin (Gardeniers et al., 2003; Martanto et al., 2004; Davis et al., 2005; Teo et al., 2005), bovine serum albumin (Park et al., 2005), ovalbumin (Matriano et al., 2002), oligodeoxynucleotide (Lin et al., 2001), plasma DNA (Mikszta et al., 2002), and particles such as polystyrene latex nanospheres (McAllister et al., 2003; Chabri et al., 2004), and gene therapy vectors (Chabri et al., 2004), and so on. To our knowledge, however, little quantitative research has been performed for microneedle systems, such as those for determining a relationship between permeability and the number, size or length of microneedles. Unfortunately, it is difficult to determine the penetration-enhancing effect caused by each individual microneedle. We therefore used a macroneedle system instead of a microneedle one to investigate the effects of pretreatment of needle puncture in the skin barrier on the in vitro skin permeation of macromolecules. The final objective of this study was to obtain quantitative information for designing microneedle systems. The relationship between the number of pores created by needle puncture and the drug permeability through the pretreatment skin barrier was studied using fluorescein isothiocyanate (FITC)-dextrans (4.3, 9.6 and 42.0 kDa) (FD-4, FD-10 and FD-40) as model compounds. The effect of sandpaper abrasion (Scott et al., 1986) was also investigated for comparison. Moreover, LDH leaching from the pretreated skin, which is a marker of skin viability (Messager et al., 2003), was determined as an index of skin damage by the macroneedle system and sandpaper abrasion.

2. Materials and methods

2.1. Materials

FITC-dextrans (FD-4, FD-10 and FD-40; average molecular weight, 4.3, 9.6 and 42.0 kDa, respectively) were purchased from Sigma Aldrich (St. Louis, MO, U.S.A.). Other reagents were of analytical grade and used without further purification.

2.2. Preparation of macroneedles for skin puncture

In the needle puncture experiment in the skin barrier, a 27-gauge disposable hypodermic needle (i.d., 0.22 mm; o.d., 0.40 mm; Terumo Co., Tokyo, Japan) was used. The needle was covered with polyethylene tubing (PE-50; i.d., 0.58 mm; o.d., 0.97 mm; Hibiki, Tokyo, Japan) as a needle sheath to maintain constant insertion depth in the skin barrier, as shown in Fig. 1. The PE-50 cover allows only insertion of the needle tip (about 160 μm length) into the skin.

2.3. Experimental animals

Male hairless rats (WBM/ILA-Ht, 7–9 weeks-old, body weight: 180–250 g) were purchased either from Life Science Research Center, Josai University (Sakado, Saitama, Japan) or Ishikawa Experimental Animal Laboratories (Fukaya, Saitama, Japan). They were housed in temperature-controlled rooms (25 ± 2 °C) with a 12 h light–dark cycle (07:00–19:00 h). The rats were allowed free access to food (M.F. Oriental, Tokyo, Japan) and tap water for a week before experiments began. Every animal experiment was conducted under the guidelines of the Life Science Research Center at Josai University.

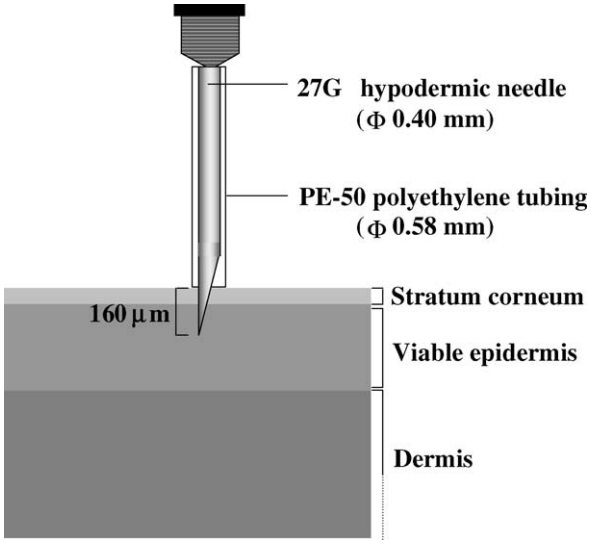

Fig. 1. Schematic representation of needle puncture on skin surface with a hypodermic needle.

2.4. Pretreatment of skin

Abdominal full-thickness skin of hairless rats was excised under anesthesia by i.p. injection of sodium pentobarbital (50 mg/kg) and excess subcutaneous fat was carefully eliminated.

2.4.1. Puncturing with a 27-gauge hypodermic needle

The excised skin was punctured with a 27-gauge hypodermic needle with the PE-50 sheath (see Fig. 1). The number of punctures with a depth of about 160 μm was set to be 1, 3, 9 or *n* in the stratum corneum.

2.4.2. Abrading with sandpaper

The excised skin surface was gently abraded once with sandpaper of No. 600 (Sankyo Rikagaku Co., Okegawa, Saitama, Japan). In order to maintain consistent abrasion strength, the technique was practiced at length until an acceptable repeatability was reached.

2.4.3. Stripping the stratum corneum with adhesive tape

The stratum corneum of the excised skin was stripped with adhesive tape (Scotch Magic Transparent Tape®, 3M Co., Minneapolis, MN, U.S.A.) about 20 times, until the stratum corneum was entirely removed from the skin.

2.5. In vitro skin permeation study

The skin pretreated with needle puncture or sandpaper abrasion was mounted in a vertical diffusion cell with an effective diffusion area of 1.77 cm^2 (Tokudome and Sugibayashi, 2003).

Intact full-thickness skin and stripped skin were also used for comparison. A test solution (1.0 mL) containing 0.25 mM FD-4, FD-10 or FD-40 was placed on the stratum corneum side of the skin. The receiver solution was 6.0 mL of pH 7.4 phosphate buffered saline (PBS), which was maintained at 32 °C using a thermo-regulated water bath. A magnetic stirrer bar was added in the receiver compartment, which stirred at about 1200 rpm throughout the experiment. The receiver solution (0.4 mL) was withdrawn every 1 h, and the same volume of PBS was added to the receiver compartment to keep the volume constant. Every permeation-run was repeated 3–5 times.

2.6. Assay of FITC-dextrans

The concentration of FD-4, FD-10 and FD-40 in each receiver sample was determined using a spectrofluorophotometer (RF 5300PC, Shimadzu, Kyoto, Japan) at an excitation wavelength of 495 nm and fluorescent emission wavelength of 515 nm.

2.7. Measurement of lactate dehydrogenase leached from pretreated skin

Skin that had been pretreated with needle puncture or sandpaper abrasion was loaded into a vertical diffusion cell, with the stratum corneum side facing the receiver compartment. The receiver compartment was filled with PBS, which was continuously stirred by a magnetic stirrer bar and was thermo-regulated with a water jacket at 32 °C. The upside compartment (dermis side) was also filled with PBS to avoid the dermis side becoming dry. Samples were withdrawn from the receiver compartment at predetermined time intervals. LDH leached from the stratum corneum side was determined by an assay kit (Lactate Dehydrogenase Kit, Wako Pure Chemicals, Osaka, Japan). All experiments were carried out at least in triplicate.

3. Theoretical

A parallel skin permeation-resistance model was established to evaluate a relationship between skin permeability and the number of pores made by needle puncture. According to skin anatomy and barrier function, skin is generally divided into two or three different layers: stratum corneum, viable epidermis and dermis. The most upper epidermis, the stratum corneum, is considered to be the primary barrier to drug transport through the skin. Then the total skin resistance (R_{tot}), where permeation resistance, R, is represented as the reciprocal of the permeability coefficient ($1/P$), and may be composed of resistances in the stratum corneum (R_{sc}) and viable epidermis and dermis (R_{ved}), as shown in Fig. 2a and in the following equation:

$$R_{tot} = R_{sc} + R_{ved} \tag{1}$$

where R_{tot} and R_{ved} are obtained from permeation experiments using intact skin and stripped skin, respectively. Thus, R_{sc} is the difference between R_{tot} and R_{ved}. Furthermore, R_{sc} can be considered to be the sum of n numbers of the small and same resistance r_{sc} by a parallel connection, as illustrated in Fig. 2b.

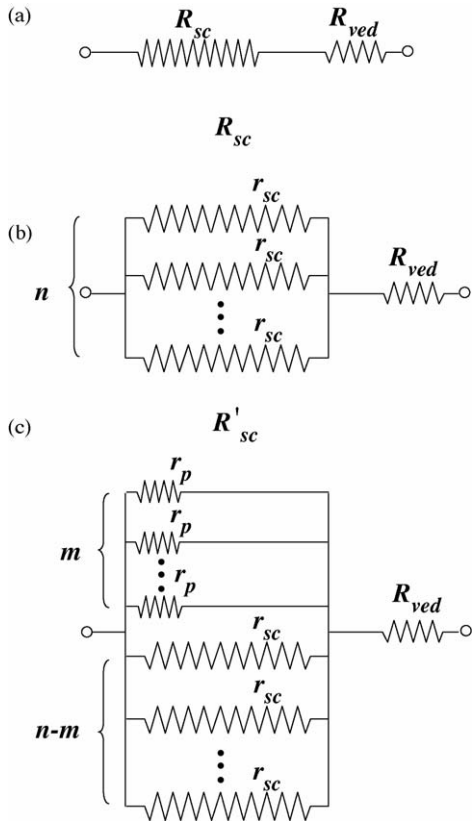

Fig. 2. Schematic representation of a skin permeation-resistance model. (a) Resistance model for intact skin; (b) parallel resistance model for intact skin; (c) resistance model for punctured skin.

The relationship between R_{sc} and r_{sc} is:

$$\frac{1}{R_{sc}} = \frac{1}{r_{sc}} + \frac{1}{r_{sc}} + \cdots + \frac{1}{r_{sc}} = \frac{n}{r_{sc}} \tag{2}$$

where both r_{sc} and n are dependent on pore properties, i.e., size and depth. When skin is punctured m times, the resistance of the stratum corneum treated, R'_{sc}, can be represented as follows (see Fig. 2c):

$$\frac{1}{R'_{sc}} = \frac{1}{r_{sc}} + \frac{1}{r_{sc}} + \cdots + \frac{1}{r_{sc}} + \frac{1}{r_p} + \frac{1}{r_p} + \cdots + \frac{1}{r_p} = \frac{n-m}{r_{sc}} + \frac{m}{r_p}. \tag{3}$$

Then R'_{sc} is

$$R'_{sc} = \frac{r_{sc} r_p}{(n-m) r_p + m r_{sc}} \tag{4}$$

where r_p is the permeation resistance of one pore. The resistance r_p can be experimentally determined by skin permeation study using punctured skin ($m = 1$). A r_{sc} can be replaced using R_{sc} and the R'_{sc} becomes

$$R'_{sc} = \frac{n R_{sc} r_p}{(n-m) r_p + nm R_{sc}}. \tag{5}$$

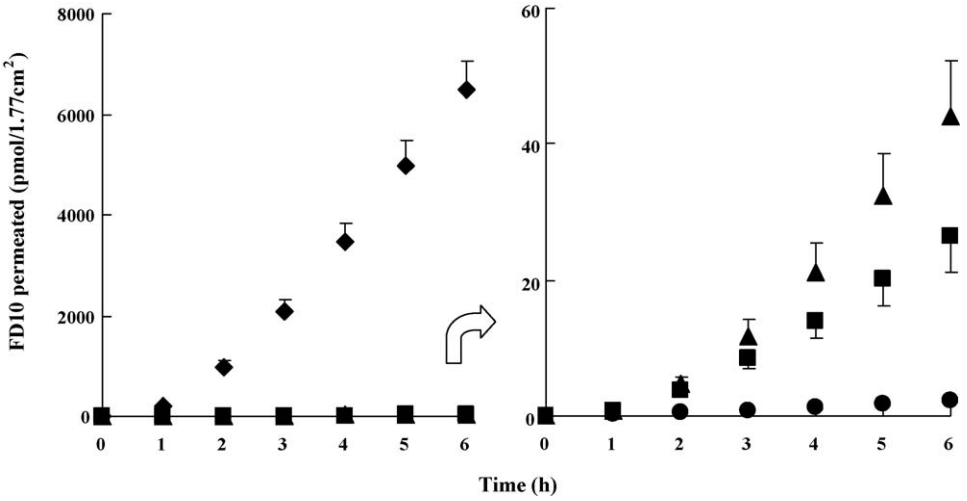

Fig. 3. Effect of different pretreatments on skin permeation of FD-10. Symbols: ●, intact skin; ■, needle puncture (pore-3); ▲, sandpaper abrasion (sandpaper-1); ♦, tape stripping. Each point represents the mean ± S.E. of three or four experiments.

Generally, n is much higher than m, then R'_{sc} can be simplified as follows:

$$R'_{sc} = \frac{r_p R_{sc}}{r_p + m R_{sc}}. \quad (6)$$

Finally, total resistance of skin, R'_{tot}, becomes

$$R'_{tot} = R'_{sc} + R_{ved} = \frac{r_p R_{sc}}{r_p + m R_{sc}} + R_{ved}. \quad (7)$$

When m is large enough, $m \times R_{sc}$ becomes much larger than r_p, and then Eq. (7) is

$$R'_{tot} = \frac{r_p}{m} + R_{ved}. \quad (8)$$

4. Results and discussion

4.1. Effect of pretreatment (needle puncture and sandpaper abrasion) on the skin permeation of FD-10

To assess the ability of physical pretreatments of needle puncture, sandpaper abrasion and tape stripping for increasing skin permeation of drugs, the permeability of FD-10 through excised hairless rat skin was determined using a vertical diffusion cell. The obtained results are shown in Fig. 3. The cumulative amounts of FD-10 that permeated through skin showed a typical time course, i.e., short lag time and a following pseudo-steady state flux, independent of the pretreatment methods.

The passive permeability coefficient of FD-10 across intact skin was 2.67×10^{-10} cm/s. Puncturing three holes across the surface of skin barrier (pore-3) enhanced the permeability by more than 10-fold (3.70×10^{-9} cm/s). In this needle puncture experiment, the skin barrier was partly damaged by three times of local puncture. The permeability coefficient of FD-10 through the skin was also significantly increased by sandpaper abrasion (sandpaper-1) (6.65×10^{-9} cm/s). However, the stripped skin permeability (8.25×10^{-7} cm/s) was higher. Thus, the permeations of FD-10 through skin treated with needle puncture and sandpaper abrasion were much higher than the passive permeation through intact skin, but was much lower when compared with stripped skin. These results suggest that the present physical penetration-enhancing methods have an effect mainly on the barrier function of the stratum corneum.

4.2. Relationship between P and MW

In order to further study the effect of needle puncture, sandpaper abrasion and tape stripping on the macromolecular permeation through hairless rat skin, a relationship was investigated between the permeability coefficient (P) through the treated skin and molecular weight (MW) of the penetrants. Skin permeations of FD-4 and FD-40 were also determined.

In all experiments, P after these pretreatments decreased with an increase in MW of the penetrants as shown in Fig. 4.

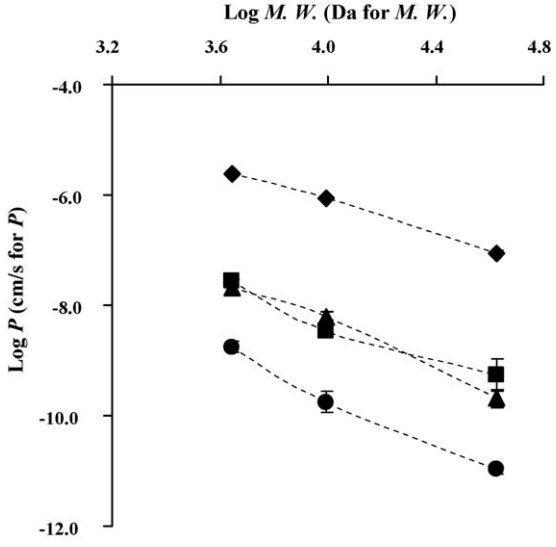

Fig. 4. Relationship between Log P and Log MW of FD-4, FD-10 and FD-40. Symbols: the same in Fig. 3. Each point represents the mean ± S.E. of three or four experiments.

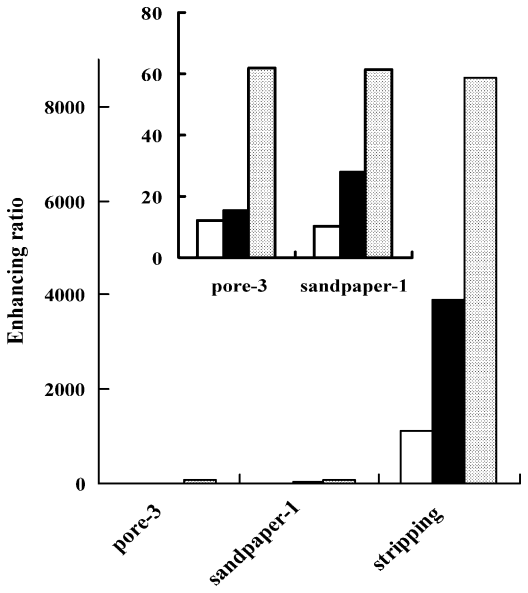

Fig. 5. The effect of difference pretreatments on the enhancing ratio of skin permeation of FD-4, FD-10 and FD-40. Symbols: open column, FD-4; close column, FD-10; dotted column, FD-40. Each column represents the mean value (the mean permeability of pretreatment against the mean permeability of control).

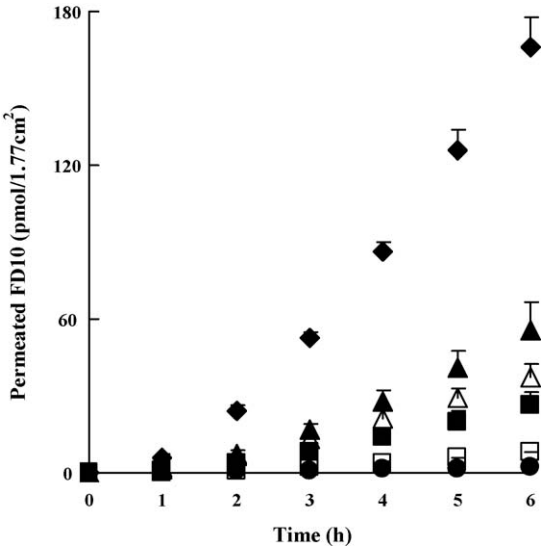

Fig. 6. Effect of pore number on the skin permeation of FD-10. Symbols: ●, intact skin; □, pore-1; ■, pore-3; △, pore-6; ▲, pore-9; ♦, pore-25. Each point represents the mean ± S.E. of three or four experiments. Pore-number shows the number of needle puncture into the skin surface.

In addition, relatively good linear correlations were observed between Log MW and Log *P*, which coincided with the previous report by Baker and Lonsdale (1974). Interestingly, a similar enhancement effect was observed by pretreatments with pore-3 and sandpaper-1.

Fig. 5 summarizes the enhancing ratio of skin permeation of FDs by the present pretreatments. It is very important to consider that the enhancing ratio for the skin permeation of FDs by the present pretreatments was more marked for higher MW FDs. This is probably due to different permeation pathways that exist for penetrants with different molecular sizes. Low molecular compounds primarily permeate the actual stratum corneum (probably intercellular lipid domain between corneocytes), whereas high molecular compounds may diffuse the pore region (containing hair follicles), as initially stated by Scheuplein (1967). Only a few pathways are available for the permeation of the largest model compound in the present study, FD-40 through the intact skin, whereas relatively more pathways are available for the skin permeation of FD-4. When decreasing the skin barrier function by physical means such as needle puncture and sandpaper abrasion, the permeation pathway, especially for high molecular weight compounds, may be produced in the stratum corneum.

4.3. Effect of number of pores made by needle puncture on the skin permeability of FD-10

Needle puncture and sandpaper abrasion were shown to effectively improve permeability of high molecular weight compounds through hairless rat skin. They were insufficient, however, compared to tape stripping (*P* for pore-3 or sandpaper-1 was about 1/100 of stripped skin). Thus, the skin permeation of FD-10 was further examined by increasing the number of pores made by needle puncture. The experimental results are shown in Fig. 6. Skin permeation increased as the number of pores increased. However, the skin permeation was not strictly proportional to the number of pores.

We then established a parallel resistance model showing skin permeation to predict skin permeation of drugs using microneedle (see Section 3). Fig. 7 shows relationship between R'_{tot} (calculated by skin permeation data of FD-10) and the number of pores made in the stratum corneum. Predictions using the skin permeation-resistance model were in agreement with the experimental data. In this calculation, R_{sc} and R_{ved} are fixed to 4.20×10^9 and 1.07×10^6 s/cm, respectively, from the experimental data. The parameter r_p was calculated to be 1.02×10^9 s/cm, by curve-fitting of a set of the obtained R'_{tot} and *m* to Eq. (7) using the least-squares method (Microsoft Excel,

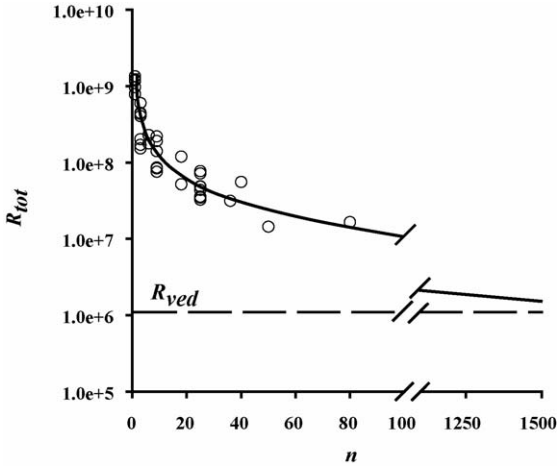

Fig. 7. Observed and calculated log (R_{tot}) of FD-10 with different number of pores. Symbols: ◯, observed value; —, curve-fitting line.

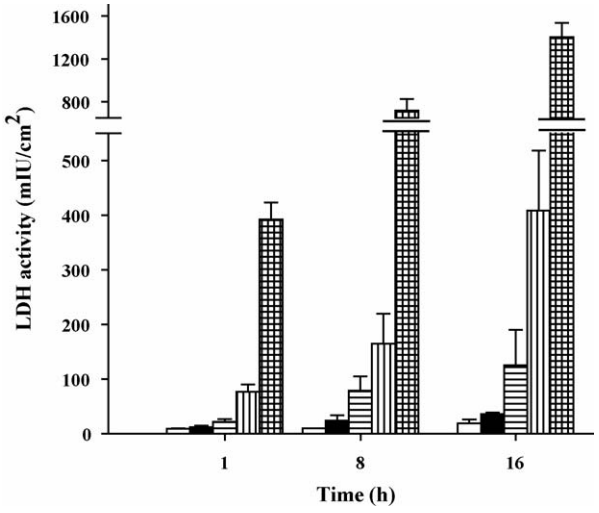

Fig. 8. Effect of different pretreatments on LDH leaching of the skin surface. Symbols: □, intact skin; ■, needle puncture (pore-3); =, needle puncture (pore-50); ▥, sandpaper abrasion (sandpaper-1); ▦, tape stripping. Each point represents the mean ± S.E. of three or four experiments.

Solver: algorithm, quasi Newton method). The obtained r_p was about one-fourth of R_{sc}, and when using Eq. (8) instead of Eq. (7), the curve-fitting line was almost the same. Although additional studies are necessary to clarify the effects of size, length and other properties of needle puncture on the skin resistance, the obtained results suggest a strong possibility of this resistance model to predict skin permeability. It is expected from the present data that microneedle-treated skin permeability can also be predicted by extrapolation from macroneedle-treated skin permeability through modifying the parameters in Eq. (7).

4.4. LDH activity of pretreated skin

LDH activity leached from the skin barrier pretreated by different physical enhancing methods was evaluated to assess skin viability (Messager et al., 2003). Fig. 8 shows the obtained data. In all pretreatment methods, LDH activity leached from the skin surface increased with the passage of time. Comparison of pore-3 to sandpaper-1, where the enhancing effect by both methods was almost the same, showed that LDH activity for the pore-3 was significantly lower than that for sandpaper-1, suggesting that skin damage caused by needle puncture was much lower compared to the sandpaper abrasion, and that needle puncture must be more effective and safe to enhance skin permeation of drugs.

5. Conclusion

The present study demonstrated that physical pretreatments, needle puncture and sandpaper abrasion, are capable of effective delivery of macromolecules through skin. Measurement of LDH leaching shows that needle puncture to the stratum corneum is much safer than sandpaper abrasion. These results may indirectly support the concept that microneedles provide a safe and efficient alternative for minimally invasive transdermal drug delivery. Using a parallel resistance model, skin permeability after needle puncture can be predicted as a function of pore number. Although further studies are needed concerning the resistance model, these preliminary results show a strong possibility of precise prediction and control of skin permeability with macroneedle or microneedle systems.

Acknowledgement

The authors would like to thank Mr. Takao Aoki, Toray Co. Inc., for his helpful discussion.

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