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Transdermal drug delivery using disk microneedle rollers in a hairless rat model

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Abstract

Background Transdermal drug delivery systems (TDDSs) represent more reliable and consistent methods of drug dosing than oral administration. However, TDDSs can administer only low molecular weight (MW) drugs and require a power source. Disk microneedle rollers facilitate the passage of low and high MW substances through the direct perforation of the stratum corneum and dermis, without stimulating dermal nerves.

Objectives We investigated *in vitro* whether disk microneedle rollers, developed for the Diskneedle Therapy System (DTS^{TM}) in South Korea, can deliver drugs effectively through the skin of hairless rats.

Methods The disk microneedle rollers used in the DTS^{TM} are metal and consist of several plates bearing microneedles of graded lengths (0.15 mm, 0.25 mm, 0.50 mm). To test *in vitro* permeation, the skin of a hairless rat was mounted in a Franz diffusion cell system and rolled with a disk roller without microneedles and with rollers fitted with microneedles of each size. Rhodamine B base (80 μl) was applied to the skin for 24 hours, 48 hours, and 72 hours, and dye permeation was detected at 543 nm. Dye binding to the skin was also confirmed using fluorescence microscopy at six hours after the application of rhodamine B.

Results Use of the disk microneedle roller increased the skin penetrance of rhodamine B base in hairless rats in accordance with microneedle length, as assessed using a fluorescence penetration test.

Conclusions Disk microneedle rollers, as designed for the DTS^{TM}, can be used for transdermal drug delivery. Microneedles can be selected according to the length appropriate for each application.

Introduction

Transdermal drug delivery systems (TDDSs) provide more reliable and consistent administration than oral routes because they avoid the effects of digestive degradation and first-pass liver metabolism. Transdermal delivery may be safer than intravenous injection and avoid associated pain. However, TDDSs can deliver only drugs of low molecular weight (MW) and require a power source. The use of microneedles in TDDSs, first reported in 1998, was shown to facilitate drug penetration through the stratum corneum[1–3] and hair follicles.[4–7] Since then, microneedles of various sizes, structures, and materials have been used in many types of drug delivery. Other methods to increase skin permeability include the use of chemical and lipid enhancers[8,9] and electric fields in iontophoresis and electroporation.[10,11] These methods mildly perturb the stratum corneum to create holes through which molecules can pass.[12] Microneedles perforate the stratum corneum and dermis without stimulating dermal nerves; therefore, drugs of low and high MW may permeate the skin with very little pain. Different types of microneedle, including solid, poke-with-patch, coat-and-poke, dip-and-scrape, and hollow types, may be selected according to the application. Poke-with-patch type needles are typically used to deliver oligonucleotides and insulin; coat-and-poke are used to deliver protein vaccines, and dip-and-scrape needles are used for DNA vaccines.[12–14]

Long microneedles (0.25 mm or 0.50 mm) deliver drugs effectively because they penetrate the epidermis; however, they may also stimulate the epidermal nerves. Therefore, we hypothesized that shorter microneedles (0.15 mm) might be effective without causing pain.

In this study, we tested the drug delivery effectiveness of disk microneedle rollers developed for the Diskneedle

Therapy System (DTS™ LAB Co. Ltd, Seoul, South Korea) with microneedles of varying lengths (none, 0.15 mm, 0.25 mm, 0.50 mm). Drug delivery was measured as the penetration of rhodamine B base through the skin of hairless rats using a Franz diffusion cell (FDC) system and fluorescence microscopy of skin sections.

Materials and methods

Materials

The disk microneedle rollers used in this study were supplied by DTS™ LAB Co. Ltd. The rollers contained either no microneedles or microneedles measuring 0.15 mm, 0.25 mm, or 0.5 mm in length (Fig. 1). Rhodamine B base ($C_{28}H_{31}ClN_2O_3$, MW = 479) analytical reagent was purchased from Sigma-Aldrich Co. (St Louis, MO, USA) and prepared by dissolving in deionized water at 0.005 M.[15] Rhodamine B base can be easily detected in a lotion or cream used to deliver a model drug. The FDC system (PermeGear, Inc., Hellertown, PA, USA) used in this study consisted of a donor port, donor chamber, membrane holder, sampling port, and receptor chamber. Skin samples to be treated with the disk microneedle rollers were fixed in the membrane holder of the FDC system. Full-thickness back skin was removed from hairless rats, carefully freed of the fatty layer, washed with a cotton ball saturated in phosphate-buffered saline (PBS), and stored at −70 °C until each experiment. The skin of the back represents >70% of the skin of the rat and was easy to treat with disk microneedle rollers.

In vitro penetration studies

Skin samples were thawed in air, cut into 2 × 2-mm squares, and placed on the FDC, thereby exposing the epidermis to air and the dermis to PBS (pH 6.8 at 37 °C). The system was equilibrated by stirring for 30 minutes. The skin samples were then removed from the FDC and perforated with disk microneedle rollers containing no microneedles or microneedles measuring 0.15 mm, 0.25 mm, or 0.50 mm in length. All treatments were performed using the same force in all directions. The prepared skin was then transferred into the donor chamber of the FDC system, and 0.005 M rhodamine B base was added to the donor compound compartment. At specific time-points (12 hours, 24 hours, 48 hours, and 72 hours), samples of PBS (200 μl) were transferred from the receptor compartment to 96-well plates, and rhodamine B base penetration was measured in a microplate reader at an optical density of 543 nm. For each PBS sample removed, the same volume (200 μl) of buffer solution was added to the receptor compartment.

Fluorescence microscopic studies

Skin was washed with PBS, cut into 2 × 2-mm squares, and placed on the membrane of the FDC, thus exposing the epidermis to air and the dermis to PBS (pH 6.8 at 37 °C) in the receptor compartment. The cell was stirred and allowed to equilibrate for 30 minutes. The skin was removed from the FDC, perforated with each of the disk microneedle roller sets, and exposed to 0.005 M rhodamine B base as for the *in vitro* penetration study. When the rhodamine B base had been allowed to penetrate for six hours, skin samples were washed with PBS to remove residual rhodamine B, embedded in optimal cutting temperature compound and cut in a cryomicrotome for examination by fluorescence microscopy.

Statistical analysis

Results are presented as the mean ± standard deviation (SD) of results in three identical experiments. Data were evaluated using Student's *t*-test, and a *P*-value of < 0.05 was accepted as statistically significant.

Results

In vitro penetration studies

The *in vitro* penetration studies using rhodamine B base showed that drug delivery into the skin increased accord-

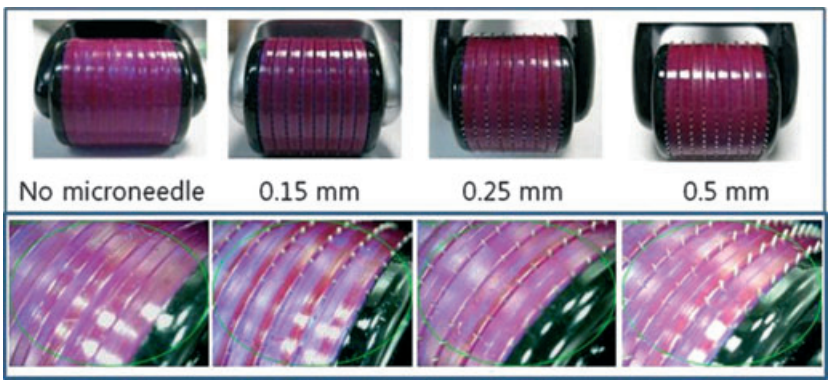

Figure 1 Disk microneedles as used in the study. Microneedles were arranged in a disk and disks were stacked for dense perforation

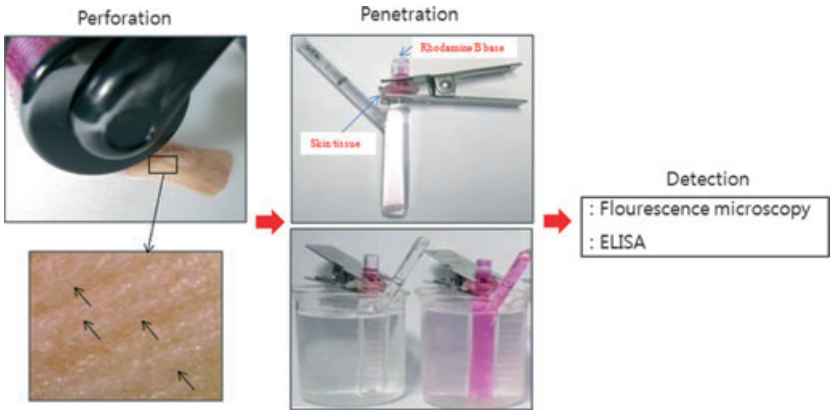

Figure 2 Penetration of hairless rat skin *in vitro* using the Franz diffusion cell system. Rhodamine B base was applied to hairless rat skin using a disk microneedle roller and an *in vitro* penetration test was performed. Dye penetration was detected by fluorescence microscopy and by enzyme-linked immunosorbent assay (ELISA)

ing to the length of the needles in the disk microneedle rollers (Figs. 2 and 3). Thus, we expect that these disk microneedle rollers will be effective in therapeutic drug delivery.

Although microneedles measuring 0.25 mm, 0.50 mm, 1.00 mm, and 1.50 mm all increase drug passage through the stratum corneum and dermis, rollers with microneedles measuring 0.50 mm, 1.00 mm, and 1.50 mm cause injury to the skin and stimulate dermal nerves. Thus, disk rollers with microneedles measuring 0.15 mm and 0.25 mm proved to be most appropriate for drug delivery under these conditions.

Fluorescence microscopy studies

Microscopic examination showed that treatment with disk microneedle rollers, using various lengths of microneedle, enhanced the passage of rhodamine B base through hairless rat skin. Rhodamine B delivered using a roller with no microneedles did not penetrate the hairless rat skin. As in the *in vitro* penetration studies, rhodamine penetration increased in line with microneedle length (0.15 mm, 0.25 mm, 0.50 mm) (Figs. 2 and 4). In particular, we compared the efficacy of drug delivery using 0.15 mm, 0.25 mm, and 0.50 mm microneedles, respectively, and found that the shortest microneedle was able to effectively perforate the stratum corneum with minimal injury to tissue.

Conclusions

Use of a TDDS may circumvent problems such as first-pass hepatic metabolism, the poor bioavailability provided by the oral route, and the pain of hypodermic injection. Transdermal delivery systems may also reduce the expense and inconvenience associated with drug delivery.

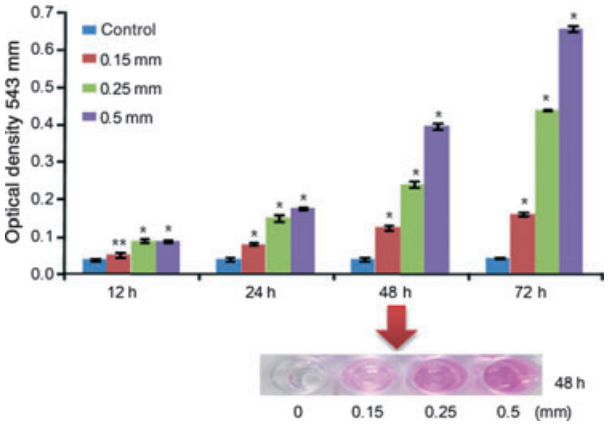

Figure 3 Microneedle length affects penetration depth using the DTS™ disk microneedle roller in hairless rat skin. Rhodamine B base was delivered across the stratum corneum by disk needling, and penetration depth was proportional to the microneedle length (control = no microneedle).
$^{*}P < 0.01$; $^{\dagger}P < 0.05$

However, certain drugs and pharmaceuticals do not readily permeate the stratum corneum, epidermis, and dermis, which, in typical human skin, span 10–20 μm, 50–100 μm, and 1–2 μm, respectively.[12]

Studies show that microneedles facilitate the delivery of drugs that pass through the stratum corneum and that a microneedle length of <0.2 mm causes little pain. Microneedles for use in drug delivery systems have been tested at lengths ranging from millimeters to micrometers.[12,16–18]

In this study, we confirmed that disk microneedle rollers enhance drug delivery through the skin. Using rhodamine B base as a test compound, we detected a difference in the time required for penetration as determined

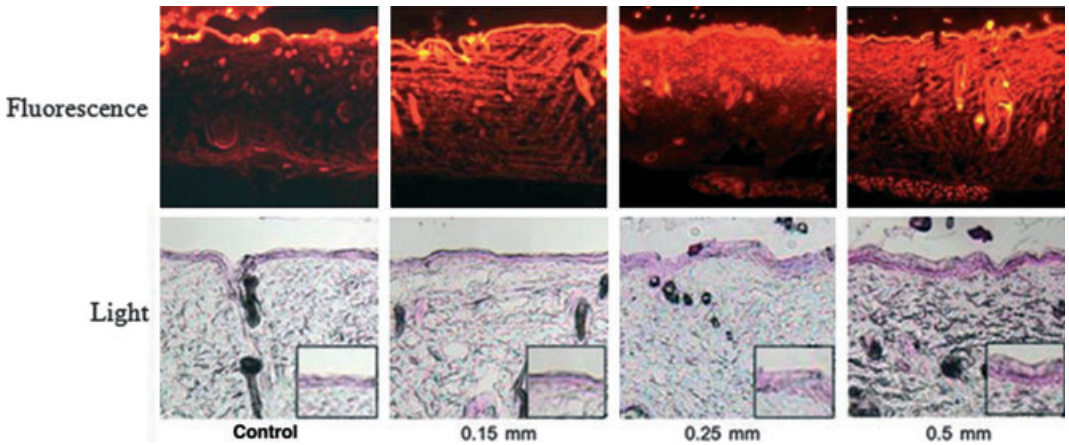

Figure 4 Microscopic images for rhodamine B base skin penetration at 6 h. Rhodamine B base penetration of hairless rat skin was tested following application of the dye using disk rollers with microneedles of varying length. And Rhodamine B base as a staining fluorescence dye can be detected easily in Light phase of fluorescence microscopy (control = no microneedle). (Original magnification: ×100)

by fluorescence microscopy in skin sections (six hours) and by the spectroscopic measurement of dye concentration in the receptor compartment of the FDC (24 hours). We determined that the effectiveness of drug penetration increased with microneedle length. A roller with no microneedles provided no penetration. Microneedles measuring 0.25 mm and 0.50 mm in length permitted penetration to the dermis, although the mechanism that determines penetration depth has not been identified.[19] A roller loaded with 0.15-mm microneedles can facilitate drug passage through the stratum corneum but not the epidermis and dermis. Use of this shorter needle length may reduce the injury and consequent pain caused by longer microneedles. The effectiveness of disk microneedle rollers loaded with 0.15-mm needles was increased by controlling the insertion time and the force with which rolling was performed and by the repetitive application of the roller. This study represents the first to investigate the efficacy of drug delivery by disk microneedle rollers using short microneedles (0.15 mm).

In conclusion, disk microneedle rollers can be used to facilitate drug delivery, and the effectiveness of the roller may be increased by matching the microneedle length to the application.[20,21] Further studies are necessary to determine how the use of a TDDS influences drug clearance by circulating blood.

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