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Supporting Information

Quantitative Evaluation of Accelerated Transdermal Drug Delivery by Electroosmosis via Frustoconical Porous Microneedles

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Experimental Details

Materials

Glycidyl methacrylate, triethylene glycol dimethacrylate, polyethylene glycol (PEG, 10 kDa), Irgacure 184, 2-acrylamido-2-methylpropanesulfonic acid (AMPS) (326-26695), 10 w/v% ammonium peroxodisulfate solution (APS) (019-15922), Rhodamine B (183-00122) and phosphate-buffered saline (PBS) were purchased from FUIJFILM Wako Pure Chemical Corporation, Ltd., Osaka, Japan. Trimethylolpropane trimethacrylate, N,N,N',N'-tetramethylethylene-diamine (TEMED), 3-(methacryloyloxy)propyl trimethoxysilane (MPS) (440159-500ML), fluorescein isothiocyanate-dextran (FITC-Dextran, 10 kDa and 40 kDa) and Proteinase K from Tritirachium album (P2308-25MG) were purchased from Sigma-Aldrich Co., St. Louis, MO, USA. (3-acrylamidopropyl) trimethylammonium chloride (APTAC) (655821-250, 75 wt%) and diethylene glycol monomethyl ether (DEG) was purchased from Tokyo Chemical Industry Co., Ltd, Tokyo, Japan. The precursor of poly(dimethylsiloxane) (PDMS, SILPOT 184) was purchased from Dow Corning Toray Co., Ltd., Tokyo, Japan. The pig skins (thickness: ~4 mm) with epidermis, dermis, and hypodermis (Landrace swine, 6-month-old, castrated males, not pigmented, DARD Corp., Tokyo, Japan) were transported with ice cooling at ~0 °C without freezing and stored in a refrigerator at 4 °C.

Preparation of frustoconical porous microneedles

PMNs can be fabricated using metals, ceramics, or a variety of organic materials including hard resins and biodegradable polymers The micro-molding is suitable for fabrication of needle shape of PMNs, which involves introducing a mixture of base material and a porogen (a sacrificial material that is later removed) into a needle-shaped mold, solidifying it, and then removing the porogen. In the case of metals or ceramics, the binder and porogen can be removed through sintering. For polymers, water-soluble solids such as salt and sugar can be used as porogen. The surface modification method we used, which combines silane coupling and graft polymerization, is a versatile method that can be applied to oxides and plastics.

The array of frustoconical porous microneedles (F-PMN) was prepared by molding (Supplementary Figure S1) as described in our previous paper. Briefly, the monomer stock solution (10 mL glycidyl methacrylate, 5.23 mL trimethylolpropane trimethacrylate and 15.7 mL triethylene glycol dimethacrylate) and the porogen stock solution (4.0 g PEG and 20 mL DEG) were mixed (11:9 in volume) with the addition of a photoinitiator (Irgacure 184). This precursor solution was poured on the female PDMS mold, and the degassing was performed under a vacuum to completely fill the microneedle-shaped cavities of the mold with the solution. The photopolymerization with 365 nm UV light irradiation and the elution of the PEG with water/ethanol (1:1 in volume) provides F-PMN with internal porous structures (porosity, ca. 45 %) made up of aggregated particles with interconnecting pores of ca. 1.0 μm diameter on average. If the ratio of porogen to the base material is too high (high porosity), the needle may lack the mechanical strength. Conversely, if the porogen ratio is too low (low porosity), the microchannel structure running through the needle is hard to form. Therefore, it is necessary to find the optimal ratio of base material to porogen. The substrate of the F-PMN array chip was 8 mm in diameter; the surface area was 0.5 cm^2.

The procedure used to modify a thin film of PAMPS and PAPTAC (< 20 nm thickness) onto the inner surface of the porous channels of the F-PMN also followed that reported in our previous papers [29,31]. The naked F-PMN was firstly modified with MPS monomer through a salinization reaction, followed by graft-polymerization using the AMPS aqueous solution (0.05 M AMPS, 1 vol% APS, and 0.1 vol% TEMED) or APTAC aqueous solution (0.4 M APTAC and 0.01 M VA-044) in an oven at 80℃ for 1 h.

Measurement of transdermal DC resistance

The F-PMN chips filled with PBS solution were placed on the skin of subject's arm. Two KCl-saturated Ag/AgCl electrodes were connected to the F-PMNs through the tubular salt bridges (agarose gel with PBS), and 1 μA was applied by a current source (ALS 7082E; BAS Inc., Tokyo, Japan) to measure the voltage drop generated. For evaluation of the penetration of needles, 50 μL of 0.4 w/v% aqueous solution of Trypan blue was dropped onto the skin surface for staining, followed by observation by a digital microscope (RH-8800; Hirox Co., Ltd., Tokyo, Japan).

All procedures performed in studies involving human participants were in accordance with the standards of Ethics Committee of Graduate School of Engineering, Tohoku University (23A-14) and with the 1964 Helsinki declaration and its later amendments. Before experiments, the purpose of this study was explained to subjects who signed the university institutional approved informed consent.

Quantitative evaluation of transdermal delivery

The 0.5 mg/mL Rhodamine B solution (molecular weight: 479 Da) and 5 mg/mL FITC-dextran solutions (molecular weight 4,000 Da and 10,000 Da) were prepared using PBS and loaded to the F-PMNs (or flat porous monolith) by immersing overnight. The PMN (or flat porous monolith) was pressed onto the center of the pig skin (ca. 50 mm x 50 mm x 2.5 mm) at 15 N, followed by connecting to the source-meter (GS820) via tubular agarose salt bridges and Ag/AgCl electrodes. A constant current density of 0.5 mA/cm^2 (or 0 mA/cm^2 as the control group) was applied to accelerate penetration of the drug solutions.

The skin area under the F-PMN (ca. 10 mm x 10 mm) was excised using a scalpel and digested with Proteinase K at 55 °C for at least 5 hours. The digested solution was centrifuged with a filtered tube at 5,000 rpm for 10 min, and 100 μL of the supernatant was transferred to a plate for fluorescence intensity measurement. The amount of excised porcine skin did not significantly affect the fluorescence measurement, as confirmed in the process of creating a calibration curve for 10 kDa dextran (Supplementary Figure 3).

Statistical analysis

All experiments were carried out at least three independent batches under identical conditions ($n \geq 3$), and the data were presented as mean ± standard deviation and presented without further preprocessing. Statistical analysis was carried out using Microsoft 365 Excel.

Supplementary Figures

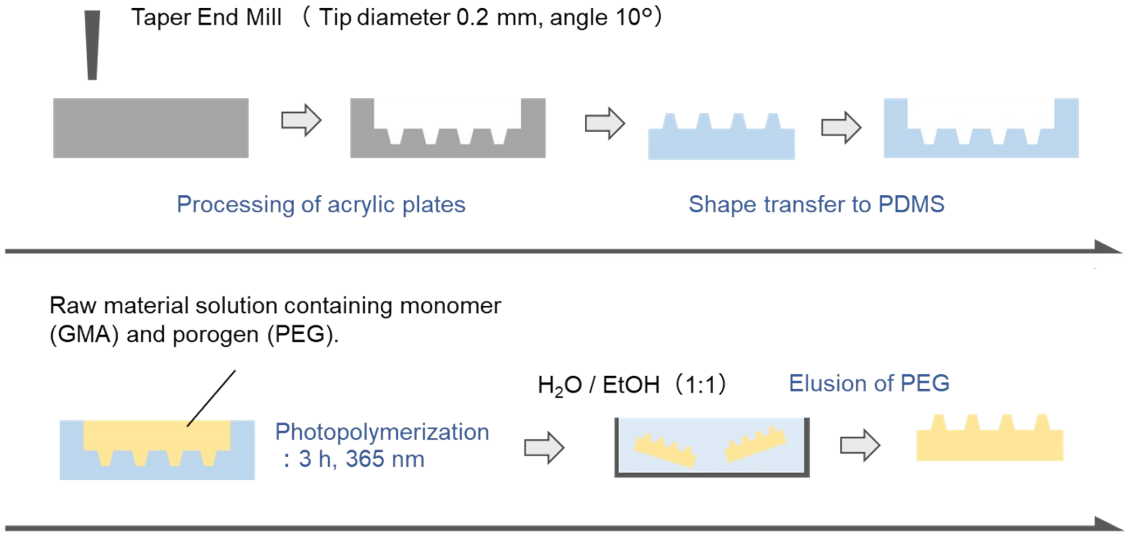

Figure S1. Illustration of the molding-based fabrication process of F-PMN: pouring of the stock solutions of monomer and the porogen, photopolymerization with 365 nm UV light irradiation, and the elution of the PEG with water/ethanol.

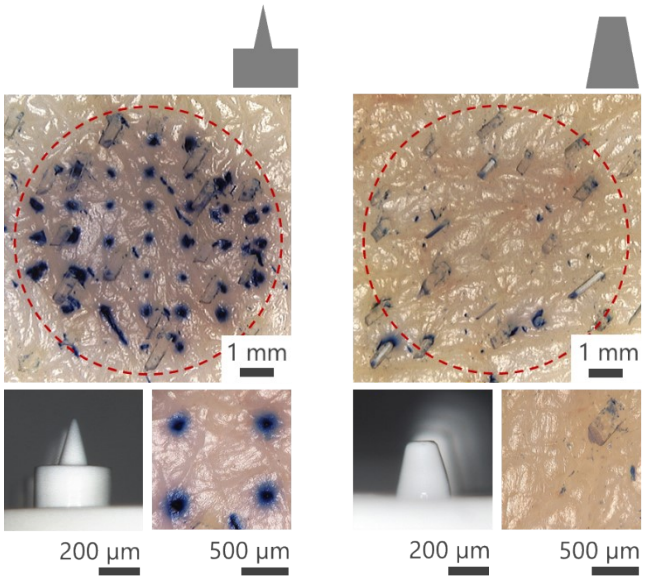

Figure S2. The PMN substrates were pressed onto the pig skins for 1 min with a load of 15 N, and then 50 µl of 0.4 w/v% aqueous solution of Trypan blue was dropped onto the skin surface for staining, followed by observation by a digital microscope.

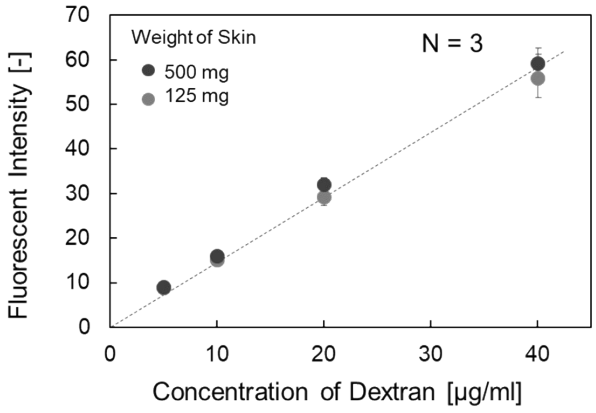

Figure S3. Fluorescent calibration curves for 10 kDa dextran PBS solutions containing the enzymatically digested skin specimen (125 mg and 500 mg). N = 3 independent experiments; mean ± SD.

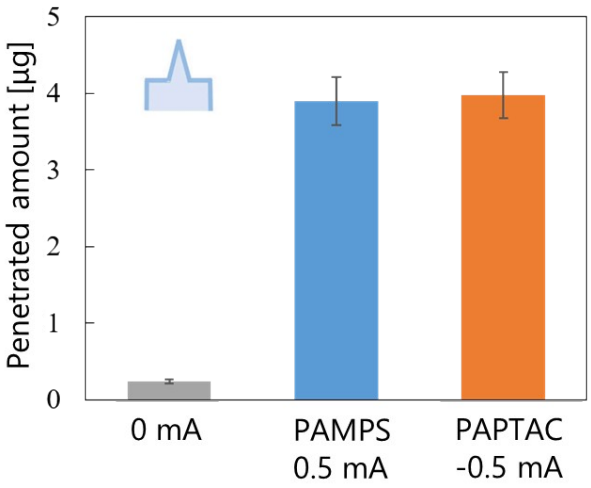

Figure S4. The amount of FITC-dextran (10 kDa) penetrated into the skin by 120 min application of ± 0.5 mA/cm^2 (or 0 mA/cm^2) to the sharp PMNs modified by PAMPS and PAPTAC. N = 3 independent experiments; mean ± SD.

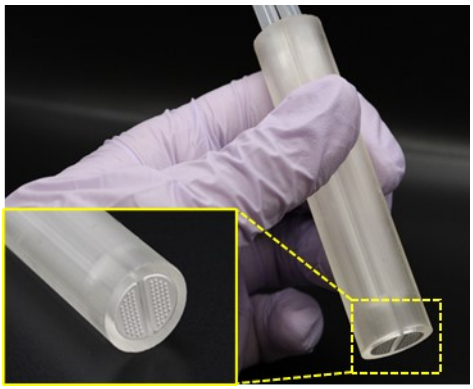

Figure S5. Typical design of a compact dual-mode iontophoresis system with the integrated anodic and cathodic F-PMNs in closed proximity.