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Time-Dependent Electrical Properties of Human Nail Upon Hydration *In Vivo*

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Abstract

The objectives of this study were to investigate the effects of hydration and solution ion concentration on the electrical properties of human nail *in vivo* and compare these *in vivo* results with those *in vitro*. *In vivo* electrical resistance measurements on the nail were conducted with a three-electrode system in phosphate buffered saline of 0.01–0.6 M. The effect of electric current on nail resistance and possible adverse effects were studied under 1.5- and 9-V iontophoresis *in vivo*. The electrical resistance of the nail plate was measured *in vitro* in side-by-side diffusion cells under the same conditions and compared with those *in vivo*. The *in vivo* electrical resistance decreased significantly upon 2-h nail hydration and then slowly decreased to a constant value, showing the same pattern as that *in vitro*. No significant effect of the applied voltage upon the nail electrical resistance was observed. Higher current densities caused moderate sensation and slight changes in nail appearance after iontophoresis. The observed decrease in nail resistance demonstrates the significance of nail hydration in transungual iontophoresis. The *in vitro* and *in vivo* correlation suggests that the *in vitro* nail plate can be a model in the research and development of transungual iontophoretic delivery.

Keywords

iontophoresis; human nail plate; constant voltage; hydration; electrical property

INTRODUCTION

The human nail plate is a resistive barrier to the penetration of large and lipophilic compounds, including antifungal agents commonly used to treat nail diseases.¹ Iontophoresis is a method to enhance the delivery of compounds across a membrane by means of an electric field.² Iontophoresis may also possess bacteriostatic and fungistatic properties.³ Since the first attempt of iontophoretic delivery of prednisolone across human nail was reported,⁴ the feasibility of iontophoresis to enhance topical transungual delivery of ionic compounds has been demonstrated *in vitro*.^{5,6} Likewise, iontophoretically enhanced delivery of antifungal agents like terbinafine⁷ and ciclopirox⁸ into and across the human nail plate *in vitro* was shown.

Understanding the electrical properties of nail can help scientists better interpret and model the transport behavior of ions in the nail for transungual iontophoretic delivery. The electrical properties of human nail plate under physiological conditions *in vivo* have not been systematically studied. The focus of our previous studies was to understand the mechanisms of constant current iontophoretic transport across fully hydrated human nail plate *in vitro* as well as the influencing factors namely pH, ionic strength, and chemical enhancers in

transungual iontophoresis.^{9–12} Iontophoretic transport across the human nail can be modeled as hindered transport across aqueous pathways in a hydrogel matrix. The hydration state of the nail plate was found to be important in determining the permeability of the nail.^{13,14} The effective diffusion coefficient of water in the nails could increase from $8 \times 10^{-10} \text{ cm}^2/\text{s}$ at 15% relative humidity to $3 \times 10^{-7} \text{ cm}^2/\text{s}$ at 100% relative humidity.¹⁵ Our preliminary hydration study with nail clippings showed that the nail approached 90% of complete hydration in an hour and then reached the fully hydrated state over 1 day. When the nail was completely hydrated in solutions *in vitro*, the steady-state nail electrical resistance decreased when increasing the ionic strength of the equilibrating solutions.¹⁰ It is expected that lowering the nail electrical resistance would reduce the power consumption of the iontophoresis power supply and increase the efficiency of transungual iontophoretic transport of ions. Therefore, understanding the time profile of the nail electrical resistance change upon hydration under different ion concentrations could assist the optimization of transungual iontophoretic delivery. For example, how much time is needed to lower the electrical resistance of the nail through hydration *in vivo* before effective transungual iontophoresis can be conducted? What is the electrical resistance of the nail that can be reached *in vivo* upon hydration and as a result of ion penetration from the iontophoresis formulations?

In addition to constant current transungual iontophoresis, constant voltage transungual iontophoresis has been investigated.⁸ In this previous study, a constant voltage transungual iontophoresis device powered by a 9-V battery was used to enhance the delivery of ciclopirox into and across partially hydrated human nail plate *in vitro*. The constant voltage iontophoresis system has several practical advantages over a constant current iontophoresis device, including a smaller device design and reduced manufacturing cost. However, the electric current applied on the nail could vary during constant voltage iontophoresis due to the changes in the electrical resistance of the nail during iontophoresis. Questions such as the electric current profiles in constant voltage transungual iontophoresis should be addressed. Finally, the safety of transungual iontophoresis has not been extensively studied in humans. The tolerance threshold of human skin to an electric current is suggested to be $0.5 \text{ mA}/\text{cm}^2$.¹⁶ Will human nail show similar electric current tolerability to human skin? What is the tolerable voltage range in constant voltage transungual iontophoresis?

The objectives of the present study were to investigate the electrical properties of human nail plate in solutions of different ion concentrations *in vivo*; identify the effects of nail hydration, solution ion concentration, and electric current upon the nail electrical resistance *in vivo*; and compare the *in vivo* data with those obtained *in vitro*. The electrical resistance of the nail was measured *in vivo* with a three-electrode system and *in vitro* with a side-by-side diffusion cell setup. Phosphate buffered saline (PBS) solutions of different concentrations (0.01–0.6 M) were used as the testing solutions. Time-dependent changes in the electrical resistance of the nail were determined by applying a constant current across the nail *in vitro* and *in vivo*. The nail electrical resistance *in vivo* upon applications of constant voltage (1.5 and 9 V) was monitored and the tolerability of human nail to electric current was examined.

EXPERIMENTAL

Materials

PBS of pH 7.4 (~0.15 M total ion concentration, consisting of 0.01 M phosphate buffer, 0.0027 M potassium chloride, and 0.137 M sodium chloride) was prepared by dissolving PBS tablets (Sigma–Aldrich, St. Louis, MO) in distilled, deionized water. Solutions of 0.01, 0.06, and 0.6 M PBS (total ion concentration) were prepared by either diluting 0.15 M PBS with distilled, deionized water or dissolving an appropriate number of PBS tablets in distilled, deionized water. Solution conductivity was checked with a pH/conductivity meter (PC510, Oakton Instruments, Vernon Hills, IL). All materials were used as received.

Nail Samples and Human Subjects

Nineteen human cadaver fingernail plates (male, age 24–90) were obtained from Science Care Anatomical (Phoenix, AZ). The frozen nail plates were thawed at room temperature ($20 \pm 2^\circ\text{C}$) and cleaned by removing adhering tissues with forceps. The nail plates were then rinsed with distilled, deionized water. The thickness of the nail plates, ranging from 0.4 to 0.9 mm (0.6 ± 0.1 mm, mean $\pm$ SD), was measured using a micrometer (Mitutoyo, Kawasaki, Kanagawa, Japan). The clean nail plates were mounted in side-by-side diffusion cells and allowed to air-dry overnight. The nails without pre-equilibration to full hydration in PBS were used in the *in vitro* electrical resistance measurements. Four healthy subjects (male and female, age 24–45) were recruited for the *in vivo* electrical resistance measurements. A total of 24 fingernails of the thumb, index, and middle fingers from both the left and right hands were randomly selected and used, with each fingernail of the subject only used once. The use of human subjects and tissues was approved by the Institutional Review Board at the University of Cincinnati, Cincinnati, Ohio.

Electrical Resistance Measurements *In Vivo*

A three-electrode system¹⁷ was utilized to measure the electrical resistance of human nail and underlying tissues *in vivo* (Fig. 1a). A custom-made electrode chamber (Fig. 1b) acting as the donor compartment with an Ag/AgCl electrode was placed on a fingernail. Specifically, a circular hole with a diameter of 9 mm was punched in the center of a piece of double-sided medical adhesive (~ 12 mm $\times$ 12 mm, thickness of 1.2 mm). The donor Ag/AgCl electrode was sandwiched between two pieces of the double-sided medical adhesive such that their circular holes aligned. A piece of Kimwipes® (~ 5 mm $\times$ 12 mm) was folded and placed under the electrode in the chamber to hold ~ 0.2 mL of the testing solution. To ensure that the concentration of the ions in the chamber was not contaminated by the Kimwipes® in the chamber, the ion content in the Kimwipes® was checked by equilibrating the Kimwipes® in distilled deionized water and determining the conductivity of the equilibrating solution. The low conductivity of the equilibrating solution indicated insignificant amount of ions in the Kimwipes®. The electrode chamber on the nail was sealed with Tegaderm™ roll (3 M Health Care, St. Paul, MN). Two other Ag/AgCl electrodes were embedded in PBS (0.15 M) saturated cotton squares (~ 3 cm $\times$ 3 cm, Target Corp., Minneapolis, MN) placed on the skin close to the tested nail. These electrodes served as the return electrode to complete the electric circuit and the reference electrode to measure the resistance of the nail and underlying tissues. The testing solutions were PBS at concentrations of 0.01, 0.06, 0.15, and 0.6 M. The procedure to set up the experiments was the following. The donor, return, and reference electrodes were first applied on the nail and skin of the subject, respectively. PBS was then added to the cotton squares embedded with the return and reference electrodes and subsequently the test solution was loaded into the donor compartment. Immediately after loading the test solution, connecting the wiring, and setting the parameter of the iontophoresis device (generally took <30 s), a 0.1-mA current was applied for 1 min with the iontophoresis device (Phoresor II Auto, Model PM 850, Iomed, Inc., Salt Lake City, UT) and with the donor electrode as the anode and the return electrode as the cathode. This device slowly ramped the electric current to the target value and could provide electrical potential up to 80 V. The electric current across the nail plate was monitored by the voltage drop across a fixed resistor (2.17 k Ω) connected in series to the nail plate in the electric circuit using Ohm's law. The voltage drop across the nail and underlying tissues was measured using a multimeter (Fluke 73III, Everett, WA) connected to the donor and reference electrodes with the assumption that the overpotential at the electrodes was negligible. The electrical resistance of the nail and the underlying tissues was determined by Ohm's law and normalized to the effective area of 0.64 cm² (i.e., resistance $\times$ area). The nail was left in contact with the solution in the chamber, and at 0.5, 1, 2, 3, 4, 5, and 6 h, the measurement was repeated. A preliminary study using 0.15 M PBS as the test solution showed essentially the same nail resistance results when the donor electrode was the cathode and the

return electrode was the anode versus that when the donor electrode was the anode and the return electrode was the cathode in the measurements ($<10\%$ difference, $n = 3$); this suggests that the electric field polarity had minimal effects on the nail resistance measurements. Due to the maximum voltage of the iontophoresis device and the high initial nail/skin resistance, the skin at the return electrode was electroporabilized by applying a 0.1-mA current between the return and reference electrodes for 1 min before the nail resistance study. This would reduce the resistance of the skin and avoid reaching the maximum voltage of the iontophoresis device while leaving the resistance of the nail unaffected before the experiment. The permeabilization pretreatment was carried out before loading the solution into the donor. In a few cases, the initial resistance across the nail was too high to be measured by the 0.1-mA constant current method (voltage across the system >80 V) even after the electroporabilization of the skin. Therefore, the nail had to be hydrated (<3 min) by leaving the testing solution in the donor chamber on the nail until a measurement could be performed; the initial nail resistance determined would be lower than the “real” initial nail resistance using this method.

In the study of the effect of electric current on the nail electrical resistance, a constant voltage (1.5 and 9 V) was applied between the anode on the nail and the cathode on the skin for 6 h using a portable alkaline battery. At 0, 0.5, 1, 2, 3, 4, 5, and 6 h, the voltage across the nail and underlying tissues was measured by the multimeter connected to the donor and the reference electrodes, and the electric current was determined by the voltage across a fixed resistor connected in series to the battery and the nail using Ohm's law. The nail and underlying tissue resistance was calculated from the voltage and the electric current across the nail using Ohm's law and normalized by the effective area of 0.64 cm^2 (i.e., resistance $\times$ area).

A questionnaire was provided to the human subjects at the end of the study to evaluate the level of sensation on the nail and skin during and after iontophoresis applications. The subjects were asked to rank and describe the sensation as well as changes in the physical appearance of the nail and skin. Scores ranging from 0 to 3, where 0 represented no sensation and 3 represented severe sensation, or 0 represented no change and 3 represented drastic changes in appearance, were used.

Water Uptake of Human Nail Plates

Water uptake studies were conducted in PBS (0.15 M) *in vitro*. PBS—rather than deionized water—was used because PBS can conduct current better than water in the electrical resistance measurements. In a previous study, it was concluded that the salts (i.e., from PBS) deposited in the nail after water evaporation did not affect the water uptake measurements because water uptake between nail hydrated in deionized water and in 0.15 M PBS were shown to be essentially the same.¹⁰ In the present study, clean cadaver nail plates were weighed and soaked in 1 mL PBS (0.15 M) in a sealed vial. At different time points, the nail plates were removed, blotted dry with Kimwipes®, and quickly weighed (i.e., wet weight). At the end of the equilibration period, the surface area of the nail plates was estimated by flattening the wet nail plates and tracing their shapes on calibrated graph paper. The nail plates were then oven dried at 60°C until a constant weight was achieved (i.e., dry weight). The amount of water uptake in the nail was expressed as the difference between wet and dry weights normalized by the surface area of the nail. The water uptake studies were performed at room temperature ($20 \pm 2^\circ\text{C}$).

Electrical Resistance Measurements *In Vitro*

Nail plates were mounted between side-by-side diffusion cells (HazelGlas, Cincinnati, OH) with custom-made nail adapters to fit the curvature of the nail plates. The dorsal side of the nail plates faced the donor chamber and the ventral side of the nail plates faced the receptor chamber. The diffusion cells had an effective diffusion area of $\sim 0.64 \text{ cm}^2$ and a cell volume

of 2 mL. The adapters, which had circular openings of 0.64 cm^2 in the center, were constructed from silicone elastomer (MED-6033, NuSil Silicone Technology, Carpinteria, CA). PBS solutions of 0.01, 0.06, 0.15, and 0.6 M were in the donor chamber, and PBS of 0.15 M was in the receptor chamber. The 0.15 M PBS on the ventral side of the nail plate was intended to mimic the extracellular fluid in the tissues underneath the nail plate, which was believed to be similar to saline under normal physiological conditions. In order to minimize the initial nail resistance measurement error caused by nail hydration, the receptor solution was first added into the receptor. The initial nail resistance was measured immediately after the donor testing solution was added. A constant current of 0.1 mA was applied across the nail plate for 1 min at 0, 0.5, 1, 2, 3, 4, 5, and 6 h with the Phoresor II iontophoresis device using Ag (anode, in the donor chamber) and Ag/AgCl (cathode, in the receptor chamber) as the driving electrodes. The voltage drop across the nail plate was measured using the multimeter. The electric current across the nail plate was monitored by the voltage drop across a fixed resistor ($2.17\text{ k}\Omega$) connected in series to the nail plate in the electric circuit using Ohm's law. The total electrical resistance of the nail and solutions were estimated using Ohm's law with the assumption that the overpotential at the electrodes was negligible. The solution resistance was measured using the same experimental setup but with a MF-Millipore™ membrane filter (HAWP, $0.45\text{ }\mu\text{m}$, Millipore Corp., Billerica, MA) sandwiched between the diffusion cells. The nail resistance was calculated by subtracting the solution resistance measured for each diffusion cells from the total resistance of the corresponding diffusion cells and then normalizing to the effective area of 0.64 cm^2 (i.e., resistance $\times$ area). The solution resistance ranged from 0.41 to $2.4\text{ k}\Omega\text{cm}^2$, which was $<30\%$ of the measured nail resistance. The electrical resistance measurements *in vitro* were performed at room temperature ($20 \pm 2^\circ\text{C}$).

Statistical Analysis

The Student's *t*-test for two-tailed distribution was used to evaluate the significance of resistance changes upon nail hydrated in different concentrations of PBS. Differences were significant at a level of $p < 0.05$. The means $\pm$ SDs of the data are presented.

RESULTS

Hydration Effect *In Vivo*

Figure 2 compares the conductance of the human nail (i.e., the reciprocal of the nail resistance) *in vivo* and the kinetics of water uptake into human nail plates *in vitro*. With nail hydration, the nail conductance *in vivo* increased quickly after the application of 0.15 M PBS and approached a constant value of 0.082 mS. The nail water uptake amount was $\sim 0.02\text{ g/cm}^2$ after 2-h hydration and remained relatively constant over 6-h hydration. This suggests that the nail approached its fully hydrated state after ~ 2 -h hydration. The decrease in the nail resistance *in vivo* was consistent with the hydration kinetic profile observed with the nail plate in the water uptake studies.

Solution Ion Concentration Effect *In Vivo*

Figure 3 shows the time-dependent nail resistance profiles *in vivo* under the solution conditions of 0.01, 0.06, 0.15, and 0.6 M PBS. Similar to the electrical resistance profile of the nail in 0.15 M PBS, the nail resistance in PBS solutions of different concentrations decreased drastically in the first hour and then slowly approached the pseudo steady-state values from 1 to 2 h. After 2-h hydration, the resistance was relatively constant, suggesting complete hydration of the nail and equilibration of the ions into the nail. The resistance of the nail after 6-h hydration was plotted against the concentration of the testing solutions (Fig. 4). The nail resistance at pseudo steady state decreased when the solution concentration increased from 0.06 to 0.6 M.

In Vitro–In Vivo Comparisons

Similar nail conductance profiles were observed *in vitro* and *in vivo* in 0.15 M PBS, despite the higher absolute nail conductance values *in vitro* compared to those *in vivo* (e.g., 0.19 ± 0.08 mS vs. 0.082 ± 0.059 mS after 6-h hydration, respectively, Fig. 2). To further investigate a possible *in vitro–in vivo* correlation of the nail barrier properties based on its electrical resistance, the electrical resistance of the nail plate was determined *in vitro* under experimental conditions similar to those used in the *in vivo* study and compared to the *in vivo* data. Figure 5 shows the *in vitro* electrical resistance of the nail plate with 0.01, 0.06, 0.15, and 0.6 M PBS on the dorsal side and 0.15 M PBS on the ventral side. The electrical resistance of the nail plate decreased during the first 2 h of the study and then reached the pseudo steady-state values after ~2–3 h. As shown in Figure 5, the time-dependent resistance profiles observed *in vitro* were affected by the solution concentration similar to those obtained *in vivo*. Like the *in vivo* resistance studies, the pseudo steady-state resistance *in vitro* decreased with increasing the concentration of solutions on the dorsal side of the nail from 0.06 to 0.6 M (Fig. 4). Figure 6 shows the resistance ratios, expressed as the ratio of the resistance at time t during nail hydration in PBS at different concentrations to the initial resistance. The rates of resistance change *in vivo* were more dramatic than those observed *in vitro* in the first 2 h ($p < 0.05$) but were less significant thereafter ($p > 0.05$). It is speculated that the initial nail barriers *in vivo* might be different from those *in vitro* possibly due to the perturbation of the *in vitro* nail during excision and/or preparation, but the *in vivo* and *in vitro* nail barriers were quite similar after the *in vivo* nail became hydrated.

Electric Current Effect *In Vivo*

Constant voltages of 1.5 and 9 V were applied on the nail *in vivo* for 6 h, and the resulting nail resistance profiles are shown in Figure 7. The nail resistance decreased promptly from as high as 900 to $23 \text{ k}\Omega\text{cm}^2$ for 1.5-V iontophoresis and to $16 \text{ k}\Omega\text{cm}^2$ for 9-V iontophoresis in the first 2 h of treatment and then fluctuated around a relatively constant value of $19 \text{ k}\Omega\text{cm}^2$ for 1.5-V iontophoresis and $12 \text{ k}\Omega\text{cm}^2$ for 9-V iontophoresis. This corresponds to an increase of electric current density from ~0.001 to 0.08 mA/cm^2 and 0.01 to 0.8 mA/cm^2 for 1.5- and 9-V iontophoresis, respectively. Aside from the initial nail resistance determined in the experiments, which might be affected by the different experimental methods used, the electrical resistance profiles during constant voltage iontophoresis in Figure 7 were essentially the same as those in the experiments without continuous electric current application in Figure 3. The voltage and electric current had no significant effects upon the nail electric resistance in the present study.

Transungual Iontophoresis Safety and Sensation

In the nail hydration and ion concentration studies, no adverse effect was observed when a 0.1-mA constant current was applied during resistance measurements, probably due to the short transungual iontophoresis applications (data not shown). In the 1.5-V iontophoresis study, no sensation or color change of the nail (i.e., score ~0) were reported. In the 9-V iontophoresis study, high electric current and subsequent high electric current density ($>0.5 \text{ mA/cm}^2$) across the nail was observed. The applied electric current caused moderate to severe sensation (Fig. 8). The subjects felt an electric shock-like sensation when the current was switched on and off during the measurements. After the treatments, yellowish to brownish stains were found on the nail surface. These stains could be removed easily by rubbing a file/sandpaper several times on the nail surface and were believed to be related to the precipitation of Ag ion on the nail due to the depletion of chloride ion in the electrode chamber during iontophoresis. The subjects also reported soreness when the nail was pressed after the treatments. This might be related to possible edema/swelling of the tissues underneath the nail plate. The soreness went away in 2–3 days.

DISCUSSION

Electrical Resistance *In Vivo*

Human nail plate consists of 80% protein (mainly α -keratins), 10–30% water, 0.1–1% lipids, and trace amounts of minerals and electrolytes.^{1,18} The main electrolytes in the nail plate are similar to those circulating in the plasma and other body fluids, such as K, Na, Mg, and Ca.¹⁸ The amounts of Na in the nail of adults have been reported to be on average in the order of 1 mg/g nail.¹⁸ Trace metals like Cu, Fe, Zn, Au, Cr, Se, and Ag have also been reported in human nails.^{18,19} Ions and drugs (e.g., itraconazole) are incorporated into nails by both nail matrix and nail bed mechanisms.²⁰ The polar and highly disulfide-linked keratins in the nail facilitate water penetration into the nail when it is in contact with water. Under physiological conditions, the nail plate is partially hydrated and a water gradient exists from the nail bed to the nail surface in the nail plate.¹³ The nail plate is negatively charged at the physiological pH of 7.4.^{1,19} As such, the electrical resistance of the nail depends on the concentrations of both the counterions along the surface of the pore walls and the bulk ions in the pores of the nail. The equilibrium concentrations of the ions in the nail are related to the ionic strength of the surrounding solution. The transient concentrations of bulk ions in the nail are related to the kinetics of water uptake and ion penetration into the nail from the surface.

At the beginning of the study, the nail was partially hydrated and the ions in the nail were not highly conductive due to hindered transport under the partial hydration state of the nail. Therefore, the resistance of the nail and the underlying tissues *in vivo* was generally high. When water permeated into the nail, the nail swelled, the porous network in the nail enlarged, the effective mobilities of the ions in the nail increased, and the nail electrical resistance decreased. In the present study, the electrical resistance of the nail drastically decreased within the first 2 h of the studies. At 2 h, the nail is believed to approach its fully hydrated state, and the nail electrical resistance reached a relatively constant value. This resistance profile is consistent with the nail hydration kinetic profile *in vitro*, suggesting the importance of nail hydration in lowering the nail electrical resistance. It should be pointed out that the initial nail resistance determined in the nail hydration study *in vivo* ($\sim 100 \text{ k}\Omega \text{ cm}^2$) was significantly lower than that obtained in the constant voltage iontophoresis study *in vivo* ($\sim 900 \text{ k}\Omega \text{ cm}^2$). The difference of these early resistance results may be related to the different procedures such as the power supply used in the studies. In the constant current electrical resistance measurements, due to the voltage limit of the iontophoresis device (maximum voltage of 80 V), the initial resistance of the nail could be underestimated because the measurements could not be performed until the iontophoresis voltage was below this limit; while the measurements were attempted, nail hydration would occur and likely lower its resistance. In order to obtain more accurate information on the initial nail resistance, a separate study was conducted in which the nail resistance was closely monitored in the initial stage (0–2 min) of nail hydration in 0.15 M PBS using the 1.5-V constant voltage method (unpublished data). In this study, the nail resistance determined was as high as $\sim 5.6 \text{ M}\Omega \text{ cm}^2$ immediately after the application of the PBS solution and dropped to $\sim 0.5 \text{ M}\Omega \text{ cm}^2$ ($n = 4$) after 2-min hydration. This implies that the experimental errors in measuring the initial nail resistance both with the constant current and constant voltage methods employed in this study could be significant even when the delay between the application of the test solution and the first measurement was relatively short (see Experimental Section). Although these errors do not affect the conclusions of the present study, researchers should exercise caution when using these early time results in the development of a transungual iontophoresis system.

The concentration of the ions in the solution in contact with the nails *in vivo* affected the rate of the decrease in nail electrical resistance over the first 2 h of the study and the pseudo steady-state resistance of the nail when the system approached equilibrium: the rate of the decrease in nail resistance was generally faster (Figs. 3 and 6) and the pseudo steady-state resistance

was lower with increasing the concentration of the testing solutions (Fig. 4). Interestingly, iontophoresis did not significantly affect the nail resistance in the present study even though ion transport into the nail was expected to be enhanced by the electric field applied across the nail during iontophoresis.⁹ Compared to the effect of hydration, the effects of ion penetration and the iontophoretic electric field were less significant (Figs. 2, 3, and 7). These findings are likely related to the presence of the endogenous ions in the nail plate and nail bed,²¹ which could play an important role in determining the resistance of the nail under hydration. The nail endogenous ions could have masked the effects of ion penetration upon nail electrical resistance. Therefore, the transport of ions into the nail from the testing solution is believed not to be the main mechanism causing the drastic decrease in nail resistance at the initial stage of the study. This is also based on the evidence that the effective diffusion coefficient of water in the fully hydrated nail¹⁵ is at least an order of magnitude higher than those of Na and Cl ions (data not shown). In the partially hydrated nail at the initial stage of the study, the effect of transport hindrance could be magnified, leading to an even larger difference between the diffusion coefficient of water and those of Na and Cl ions in the partially hydrated nail than that in the fully hydrated nail. Another effect that could mask the effects of passive and iontophoresis driven ion penetration into the nail was water driven ion penetration into the nail by convection during nail hydration. Together, all these factors could have contributed to the lack of significant dependence of the nail electrical resistance on the concentration of the solution in contact with the nail and the voltage applied across the nail during transungual iontophoresis.

In Vitro–In Vivo Correlation

The electrical conductance of the nail increased significantly upon nail hydration *in vivo* and *in vitro*, and the *in vivo* conductance pattern was similar to the nail hydration profile *in vitro* (Fig. 2). Similar nail behaviors were also observed *in vivo* and *in vitro* that the pseudo steady-state nail resistance both *in vivo* and *in vitro* decreased with increasing the concentration of the solutions in contact with the nail from 0.06 to 0.6 M (Fig. 4). Despite these similarities, the nail electrical resistances *in vivo* were different from those *in vitro* with the following aspects. First, the nail resistances were not exactly the same *in vivo* and *in vitro*: in particular, the initial nail resistances *in vivo* were significantly higher than those *in vitro* (Figs. 3 and 5). The decrease in the nail resistance *in vivo* was also found to be faster than that *in vitro* in the first 2 h of the study (Fig. 6). Figure 9 shows a correlation between the resistances *in vivo* and *in vitro* observed in the present study. The data points at 0.01 M deviated from the trendline, indicating that the *in vivo* nail resistance was lower than that *in vitro* at this low ion concentration. Our previous studies¹⁰ under symmetric conditions *in vitro* (i.e., the same concentrations of solutions on the both sides of nail plate) showed that the bulk ions in the pores became the dominant conducting ions at ion concentrations of higher than 0.06 M and the resistance decreased with increasing the ion concentration. Below 0.06 M, the resistance of the pores was dominated by the counterions of the fixed charges on the pore walls in the nail and was relatively independent of the ion concentration of the bulk solution.¹⁰ The results obtained in the present study are consistent with the previous results. Also observed in Figure 9 were the deviations of the data point at 0.06 M and to a smaller extent the data point at 0.15 M from the trendline at 0.5-h hydration, indicating that the correlation was worse with the partially hydrated nail. With further nail hydration at 1 h and thereafter, the data points were close to the trendline. The slope in Figure 9 indicates consistently 2.4 times higher resistance *in vivo* than *in vitro*. The higher resistance *in vivo* could be due to the resistance of tissues below the nail plate; the electrical resistance and ion content in this tissue layer could be affected by the concentration of the solution in contact with the nail. Overall, the observed correlation between the *in vitro* and *in vivo* nail resistance as well as the similar nail resistance behaviors upon nail hydration and ion penetration in the *in vitro* and *in vivo* studies suggest that the *in vitro* nail plate can serve as a model to study transungual iontophoresis and to develop an iontophoresis device for

transungual iontophoretic delivery *in vivo*. However, caution must be exercised when using *in vitro* human nail plates to study the properties of the *in vivo* nail barrier in the early stage of nail hydration due to the differences observed between the nail *in vivo* and *in vitro* in the present study.

Transungual Iontophoresis Safety and Sensation

While 1.5-V iontophoresis for 6 h had no adverse effect on the nail and underlying tissues, 9-V iontophoresis caused moderate sensation during the application. The higher voltage study also changed the color of the nail surface and caused moderate sensation to the underlying tissues of the nail plate post-iontophoresis. It is believed that the adverse effects are related to the current density, which increased to a level higher than that tolerable by the epidermis (e.g., $>0.5 \text{ mA/cm}^2$) when the nail resistance dropped below $20 \text{ k}\Omega/\text{cm}^2$ during 9-V iontophoresis. This condition occurred after 2 h in the 9-V treatments in two of the four subjects and should be avoided. In summary, the present results show that the low voltage of 1.5 V would be safe in transungual iontophoresis. Short duration application of 0.1-mA constant current iontophoresis during measurements was also safe and exerted little sensation or physical changes to the nail and underlying tissues. In long duration constant voltage iontophoresis, the voltage applied should not be higher than 9 V as unpleasant nail conditions and sensations may occur. Even under long duration of 9-V iontophoresis and with electric current higher than 0.5 mA/cm^2 , the changes in the nail and tissues were reversible. There was no significant long-term effect on the nail. As this study was to investigate the effect of a single iontophoresis application, the influence of repeated iontophoresis applications remains to be investigated.

Transungual Iontophoretic Delivery

The present *in vivo* human nail data suggest that nail hydration significantly affected the electrical resistance of the nail. The electrical resistance of the nail rapidly decreased from the initial resistance of above $100 \text{ k}\Omega \text{ cm}^2$ to below $10 \text{ k}\Omega \text{ cm}^2$ within 2-h hydration. The initial electrical resistance of partially hydrated nail *in vivo* implies that effective iontophoresis on the nail without hydration might not be feasible due to safety issues and iontophoresis efficiency. In constant current iontophoresis, the voltage required to maintain a reasonable electric current (e.g., 0.3 mA/cm^2) will be very high due to the high initial resistance, and adverse effects associated with the high voltage on the nail and underlying tissues might occur. In constant voltage iontophoresis, the amount of ions delivered will be too small to be effective due to the initial low electric current. Therefore, short duration iontophoresis without any prehydration may not be effective to deliver a sufficient amount of ions across the nail in clinical situations. The present data suggest that nail hydration would be preferred to lower the nail resistance before or concurrent with the iontophoresis treatment. Increasing ion concentration of the solution in the device decreased the nail resistance but its effect was not as significant as that of hydration. In addition, increasing the concentration of a drug ion might not be feasible because the drug concentration is likely limited by its solubility. Furthermore, the incorporation of ions other than the drug ion in the device to lower the nail resistance would have unfavorable ion competition and partitioning effects on drug transport.¹² Nail hydration pretreatment and/or long iontophoresis treatment to allow adequate nail hydration could be an effective, economic, and feasible approach in decreasing the nail resistance for transungual iontophoretic delivery.

CONCLUSION

The factors that affect the nail electrical resistance *in vivo* were investigated. The *in vivo* nail electrical resistance profile was consistent with the water uptake kinetics of the nail plate. The effect of solution ion concentration on lowering the nail resistance was relatively small, possibly due to the presence of endogenous ions in the nail plate. No significant effect of the

applied electrical potential upon the nail electrical resistance profile was found under the conditions studied. Together, these findings demonstrate the effect of nail hydration upon its electrical resistance and the importance of nail hydration in transungual iontophoretic delivery. The present study shows a correlation between the nail electrical resistances *in vivo* and *in vitro* after the nail becomes hydrated. This suggests that the *in vitro* nail plate can be used as a model to study transungual iontophoresis and to develop an iontophoresis device for transungual iontophoretic delivery *in vivo*. In low voltage application (low electric current), transungual iontophoresis was found to be relatively safe. The higher voltage application of 9 V caused moderate sensation during long constant voltage iontophoresis and could change the appearance of the nail surface post-iontophoresis, but these changes were reversible.

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Figure 1.

a: The electric circuit of the three-electrode system used in the *in vivo* electrical resistance measurements. b: The schematic diagram of the donor chamber used in the *in vivo* study. The cylindrical donor chamber had a diameter of 9 mm and thickness of 2.4 mm. The volume of the testing solutions in the donor chamber was ~0.2 mL.

Figure 2.

Nail conductance *in vivo* (squares) and *in vitro* (diamonds) measured by the application of 0.1-mA constant current for 1 min, and the kinetics of water uptake (triangles) into human nail plate in 0.15 M PBS. Nail conductance (in mS or mmho) is the reciprocal of nail resistance (in kohm). The amounts of water uptake in the nail were normalized by the nail surface area. Data represent the means and standard deviations of four samples.

Figure 3.

Time-dependent nail resistance *in vivo* upon hydration in PBS of 0.01 (diamonds), 0.06 (squares), 0.15 (triangles), and 0.6 M (circles). Nail resistance was measured by the application of 0.1-mA constant current for 1 min. Data represent the means and standard deviations of four samples.

Figure 4. Comparisons of the *in vivo* (squares) and *in vitro* (diamonds) pseudo steady-state nail resistance (i.e., resistance at 6 h) upon hydration in PBS of different concentrations. Nail resistance was measured by the application of 0.1-mA constant current for 1 min. Data represent the means and standard deviations of four samples.

Figure 5. Time-dependent nail resistance *in vitro* with 0.01 (diamonds), 0.06 (squares), 0.15 (triangles), and 0.6 M (circles) PBS in the donor chamber and 0.15 M PBS in the receptor chamber. Nail resistance was measured by the application of 0.1-mA constant current for 1 min. Data represent the means and standard deviations of four samples.

Figure 6.

The *in vitro* (closed symbols) and *in vivo* (open symbols) resistance ratios (R_t/R_0 , R_0 = initial nail resistance, R_t = nail resistance at time t) during nail hydration in PBS of 0.01 (diamonds), 0.06 (squares), 0.15 (triangles), and 0.6 M (circles). Nail resistance was measured by the application of 0.1-mA constant current for 1 min. Data represent the means and standard deviations of four samples.

Figure 7. Nail resistance *in vivo* during 1.5- (diamonds) and 9-V (squares) constant voltage iontophoresis applications with 0.15 M PBS. Data represent the means and standard deviations of four samples.

Figure 8.

Questionnaire scores of the sensation and changes in the physical appearance of the nail (gray bars) and skin (open bars) during and after constant 9-V iontophoresis applications. Data represent the means of scores obtained from four subjects on a scale of 0 to 3, where 0 = no sensation experienced or no difference observed in the tissues, 1 = slight sensation (e.g., tingling) or slight difference observed in the tissues, 2 = moderate sensation or moderate difference observed in the tissues, and 3 = severe sensation or drastic difference observed in the tissues.

Figure 9.

Correlation between nail resistance *in vivo* and *in vitro* upon 0.5- to 6-h hydration in PBS of 0.01 (diamonds), 0.06 (squares), 0.15 (triangles), and 0.6 M (circles). Nail resistance was measured by the application of 0.1-mA constant current for 1 min. All the data points under the specified PBS concentration conditions were included in the plot. Each data point represents the mean of four samples. The trendline was calculated using the 0.5- to 6-h resistance data but excluding the nail resistance in 0.01 M PBS and at 0.5 h in 0.06 M PBS.