Expert Opinion

Iontophoretic drug delivery across the nail

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Introduction: Topical drug delivery to treat nail diseases such as onychomycosis and psoriasis is receiving increasing attention. Topical nail delivery is challenged by the complicated structure of the nail and the low permeability of most drugs across the nail plate. Considerable effort has been directed at developing methods to promote drug permeation across the nail plate. Iontophoresis efficiently enhances molecular transport across the skin and the eye and is now being tested for its potential in ungual delivery.

Areas covered: This review covers the basic mechanisms of transport (electroosmosis and -migration) and their relative contribution to nail iontophoresis as well as the key factors governing nail permselectivity and ionic transport numbers. Methodological issues concerning research in this area are summarized. The data available *in vivo* on nail iontophoresis of terbinafine specifically are reviewed in separate sections.

Expert opinion: Our understanding of nail iontophoresis has improved considerably since 2007; most decisively, the feasibility of nail iontophoresis *in vivo* has been clearly demonstrated. Future work is required to establish the adequate implementation of the technique so that its clinical efficacy to treat onychomycosis and nail psoriasis can be unequivocally determined.

Keywords: iontophoresis, nail, onychomycosis, terbinafine, topical

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1. Introduction

1.1 Topical drug delivery to the nail: relevance and challenges

Topical nail drug delivery is primarily targeted to the treatment of nail psoriasis and onychomycosis, two diseases which cause significant distress, morbidity and reduced quality of life [1-3]. Onychomycosis represents ~ 50% of all nail ailments; its prevalence is 2 – 10% in the general population, and much higher in specific high-risk groups [2,4]. Nail involvement affects 80 – 90% of psoriatic patients at some point of their lives [1,3,5]. Topical treatment would be, *a priori,* an advantageous alternative to avoid the systemic side effects and drug interactions associated with antifungal drugs and with the intralesional injections used to treat psoriasis [2,4,6]. Unfortunately, current topical treatments have limited efficacy [1-3,7] explained, at least partially, by their low competence in delivering drug efficiently to the different parts of the nail apparatus. Further, the nail unit is a relatively complex structure [8] and drug access to the target site, typically the nail bed, is not easy due to the important barrier to penetration offered by the nail plate.

It is not surprising, therefore, that this area of research has received increasing attention in recent years as detailed in excellent reviews [9-11]. Different strategies have been tested to enhance topical nail drug delivery, including iontophoresis, which constitutes the subject of this review.

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Article highlights.

- Current knowledge about the relative contributions of electro-osmosis and electro-migration to molecular transport during nail iontophoresis suggests that the latter is a more efficient and less variable mechanism for drug delivery.
- Significant and recent advances in the field have elucidated the role of factors such as pH, molar fraction, mobility, competing ions, nail permselectivity and presence of chemical enhancers in nail iontophoresis. This improved knowledge will allow the rational development of nail iontophoretic drug delivery systems.
- There are methodological issues which should be carefully considered prior to data interpretation and comparison.
- There is sufficient evidence to support the feasibility of nail iontophoresis *in vivo*.
- Significant *in vivo* and *in vitro* data support the development of a terbinafine iontophoretic delivery system to treat fungal infections.

This box summarizes key points contained in the article.

1.2 Iontophoresis

Iontophoresis involves application of a small electrical current to a biological membrane to increase molecular transport and has been used primarily in the fields of transdermal and ocular drug delivery [12-17]. As several reviews on the mechanisms of transport underlying iontophoresis and its applications in drug delivery are available [12-16,18-20], only a brief summary is presented here.

Two mechanisms of transport, electro-migration and electro-osmosis (also known as electro-repulsion and convective solvent flow, respectively), may contribute to iontophoretic transport.

Electro-migration results directly from current application [13,16,18,19]: a direct constant current is supplied by a power supply to two Ag/AgCl electrodes (**Figure 1**) and causes electrochemical reactions at the anode (oxidation) and the cathode (reduction). This results in an excess of positive and negative charge in the anodal and cathodal chambers, respectively. This unbalance is corrected by ionic fluxes across the membrane, which restores electro-neutrality. Faraday's law relates the ionic transport across the membrane to the intensity of the electric current applied, the time of current passage and the charge per ion [13,18,19]:

$$M_i = \frac{T.i_i}{F.z_i} \quad (1)$$

where M_i are the moles of the 'i' ion flowing through the membrane during a time T (time of current application), z_i is the valence of the ion 'i', F is Faraday's constant (96 487 $C.mol^{-1}$) and i_i the current carried by the i^{th} species. Ions present on both sides of the membrane compete to transport charge during iontophoresis (**Figure 1**) and so any ion 'i' transports across the membrane just a fraction (i_i) of the total charge applied (I (intensity of current)). The transport number of ion 'i' (t_i or efficiency of transport) is defined as $t_i = i_i/I$. Therefore, Equation 1 may be rewritten:

$$M_i = \frac{t_i.I.T}{F.z_i} \quad (2)$$

Equation 2 can be used to estimate the 'iontophoretic dose' that is the I and T required to deliver a specific amount of a drug whose valence and transport number are known. On the other hand, the current density, or intensity of current applied per unit area, should not exceed certain limits (0.5 $mA.cm^{-2}$ on skin sites) for iontophoresis to be well tolerated [21].

Endogenous and highly mobile and concentrated sodium and chloride ions transport across the membrane a major fraction of the total charge. Therefore, the transport numbers of most drugs are significantly less than one. A drug's maximum transport number is measured in 'single-ion' experiments during which the drug is the only ion available for charge carrying in the donor and competition is reduced to that with endogenous ions beneath the membrane [22-24].

Practical implementation of iontophoresis may require the presence of other ionizable components (buffers, stabilizers, etc.) which may compete for charge carrying across the membrane. In this case, the drug's transport number is directly proportional to its concentration and electrical mobility [22-27]. Drug delivery is optimized by minimizing the presence of competing species and increasing the drug concentration and its ionized fraction [23,26]. Because both ionic mobility and transport numbers are inversely related to molecular size [24,27,] the best candidates to be delivered by electro-migration are small, fully charged and highly water-soluble.

Electro-osmosis is the second mechanism contributing to iontophoretic transport [20,23,28]. Most biological membranes (including the skin and eye) have a net negative charge at physiological pH and are cation-permselective [20,23,28-30]. When an electric field is imposed across a cation-permselective membrane an electro-osmotic, water flow, is induced in the anode-to-cathode direction [20,28-32]. Neutral, zwitterionic and polar solutes are carried along with this solvent flow facilitating their delivery from the anodal chamber. Hence electro-osmosis also reinforces the transport of cations and opposes that of anions [12,15,16].

The electro-osmotic solvent flow is modified by the intensity of current applied and by the pH and ionic composition of the electrode chamber formulations [20,29-31,33,34]. The electro-osmotic flux (J_i (molar flux of a solute 'I')) of a drug 'i' may be expressed as: $J_i = J_{VS} \bullet c_D$ where J_{vs} is the electro-osmotic solvent flow and c_D the concentration of the drug in the vehicle [20,31]. Transdermal transport by electro-osmosis is weakly dependent on molecular size [20,31], hence, while this mechanism of transport is not as efficient as electro-migration, it allows the transport of large cations which have very low transport numbers [14-16,35].

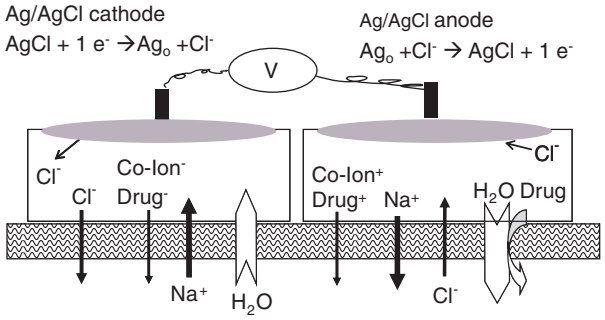

Figure 1. Schematic diagram illustrating drug delivery by iontophoresis. Constant current is delivered to the anode and cathode from a power supply. Cationic and neutral substances are delivered from the anode by electro-migration, electro-osmosis and (to a negligible extent, typically) passive diffusion. Anionic compounds are delivered from the cathode by electro-migration, while convective solvent flow opposes this phenomenon (again, passive diffusion is negligible).

2. Iontophoretic drug delivery into and across the nail

The *in vivo* study in 1986 by James *et al.* [36], probably the first publication on nail iontophoresis, reports the plasma levels of prednisolone measured after transungual iontophoresis of the prodrug prednisolone sodium phosphate. Despite the remarkable results presented, little progress was reported in the area until 2007. Since then, a relatively steady stream of work has been performed.

2.1 Methodology considerations

Research on iontophoretic drug delivery to the nail is complicated by several methodological issues. First of all, it is difficult to source nail specimens in large amounts, and so testing numerous formulations and experimental conditions is problematic. Very often, nail tips (nail clippings) from healthy volunteers are used for iontophoretic experiments [37-41], but these must be ~ 8 mm long to fit adequately into a 5 mm diameter diffusion cell. The average nail growth is 3 mm per month [42], meaning that several months are needed for donors to grow nail tips of the right length.

The maximum current density acceptable in nail iontophoresis is unknown, and so the value (0.5 $mA.cm^{-2}$) from transdermal iontophoresis [21] is used instead. However, observance of this limit in nail iontophoresis may result in an insufficient intensity of current, particularly given the small area available for transport. For example, in 'nail clips' experiments, the area is typically 0.2 – 0.25 cm^2 [37,38,40,41] and, as a result, current densities of 0.5 and 1 $mA.cm^{-2}$ correspond to only 0.125 and 0.2 mA, respectively [37,38,40,41]. Alternatively, healthy [39,43-48] and onychomycotic [49] nail plates sourced from cadavers provide larger transport areas up to 0.64 cm^2 in which case passing 0.1 – 0.3 mA results in 0.16 – 0.47 $mA.cm^{-2}$.

The size of a transdermal patch can be increased so that a larger intensity of current can be applied while maintaining an acceptable current density. This strategy is obviously limited in nail iontophoresis by the size of the nails available *in vitro* and *in vivo*. Therefore, establishing the maximum current density acceptable for nail iontophoresis is the key for the future development of the technique.

An *ex vivo* model [50] allows characterizing drug delivery and distribution into the nail plate, nail bed and adjacent soft tissues and consists of healthy intact toes excised from a human cadaver. This model provides valuable information but it is difficult to envisage as a routine model in the future.

Bovine and porcine hooves have also been used [51,52]. Porcine hooves cut to an average 500 µm thickness have been reported to provide results comparable to human nails [51]. However, sufficient data are not available to support the validity of this or any other similar animal model.

Nails are usually kept frozen [39,43-45,47,50] or in desiccators [38,40,41] until use, although some specimens (including onychomycotic nails) have been kept at 4°C [49,51,53-55]. Whether different sourcing and conservation methods modify nail properties, including drug permeation, is unknown and should be taken into account when comparing data. Whether cosmetics (including nail lacquers) change nail properties, and if so, for how long, is not known; however, the possibility must exist as a reduction on transonychial water loss (TOWL), for example, has been observed *in vivo* [56].

Often, nails are hydrated for different lengths of time prior to iontophoresis. This imparts flexibility to the nail plates and/or facilitates current passage by reducing their electrical resistance. Alternatively, iontophoresis may be carried out following a passive control experiment during which the nail plate becomes hydrated. However, the length of the hydration (or passive control) procedure differs significantly between studies in the literature and soaking times ranging from a few minutes to 24 h [38,39,43-45,47-50,52-55,57,58] have been reported. Nevertheless, practical implementation of the technique requires realistic hydration times and, in fact, *in vivo* iontophoresis can be performed successfully after just a few minutes hydration [36,46,59]. Further, because hydration enhances nail permeability and alters nails and bovine hoof microstructure [9,60,61], it is possible that long hydration times could complicate data interpretation.

Variability can be problematic whenever experiments are performed with biological membranes. High inter-nail variability has been typically associated with electro-osmosis whereas transport by electro-repulsion appears to be less sensitive [39-41]. *In vitro* experiments, during which the same nail specimen was submitted sequentially to different experimental conditions, were performed to minimize the impact of inter-nail variability [39-41,43] and elucidate the role of experimental factors.

Most experiments have used constant direct current, [37,38,40,41,48-51,53-55,58,59] with a duration varying between 20 min and 1 h [48-50,55,59], a few hours and

1 day [40,41,43,46,47,51], 2 and 4 days [44,45,] and up to 15 and 70 days [39,57]. Some research has involved constant voltage iontophoresis, allegedly a more cost-effective approach [46,62]. However, ionic transport is determined by the intensity of current and will fluctuate with the circuit resistance when using constant voltage devices; this is acceptable only when a strict dose titration is not necessary. Ag/AgCl electrodes are the most frequent and the best choice for iontophoresis although inert electrodes such as graphite [51] and platinum [47] have been used. Inert electrodes cause electrolysis of water, thus modifying the pH, altering drug ionization and nail permselectivity, and introducing hydronium and hydroxyl species resulting in low and unpredictable drug transport.

A different approach is the device proposed by Power Paper [62]. Thin, low-cost and flexible disposable batteries made of zinc and manganese dioxide are printed on a polymer film and provide current whose intensity will vary with the resistance of the circuit. These devices have been used successfully *in vivo* when an electronic card was incorporated to keep a constant current of 0.1 mA [58].

Laser scanning confocal microscopy has been used to visualize the *in vitro* ungual penetration of sodium fluorescein and Nile blue chloride in passive and iontophoretic conditions [63]. The depth, relative depth uniformity and pathways of penetration of both markers into human nail were estimated via 'transversal' images and 'exposed layer' images. Iontophoresis enhanced moderately the penetration of both markers into the nail plate, and the permeation of sodium fluorescein into the dorsal, ventral and intermediate nail layers was similar.

In vivo, current application for 10 min [36], 2 h [59] and up to 6 – 8 h [46,58] on thumb and toe nails has been reported. Generally, drug delivery takes place from just one electrode and the active delivery area covers completely the nail being treated and some adjacent soft tissue. The second 'inactive or return' electrode is attached to a nearby skin site [36,46,58,59]. Simultaneous delivery of drugs with different polarity to the nail plate would require positioning both electrodes on the plate which is difficult because of space limitations. One solution would be to alternate the polarity of the electrode positioned on the nail so that drugs of different charge are delivered sequentially.

2.2 Mechanisms and pathways of ungual iontophoretic transport

Transdermal iontophoresis occurs by two mechanisms. Research has recently been undertaken to establish the relative contributions of electro-repulsion (electro-migration) and electro-osmosis (convective solvent flow) [14-16,18,19,25,35,64] to nail iontophoresis.

Murthy *et al.* [37] studied the iontophoretic transport of the uncharged glucose and griseofulvine (pK_a = 0.15) across nail tips in the pH range 3 – 7. Electro-osmosis was higher from the anode at pH 7 (**Figure 2**), but greater from the cathode at pH 3. At pH 5, the fluxes were similar, suggesting that the nail's isoelectric point is around 5. Like skin, therefore, the nail is cation-permselective at physiological pH and has a similar PI [29,30], the value of which is consistent with those of nail keratins [65]. The anodal solvent flow at pH 7, estimated from the glucose data reported, was 0.31 $\mu l.h^{-1}$ or ~ 2.4 $\mu l.h^{-1}.mA^{-1}$.

Nevertheless, an essentially minor role for electro-osmosis has been deduced from mannitol and urea data [39]. The threefold or less enhancement measured for these neutral markers contrasted with the nearly 30-times increase in transport observed for the cationic tetraethyl ammonium (TEA). Subsequent work [43] has found further evidence for nail permselectivity. The anodal and cathodal delivery of mannitol and urea were similar at pH 5 – 6, but increased at the anode when the pH was increased to 7.4 (and, likewise, increased for the cathode when the pH was lowered below 5).

Additionally, the sodium ion transport number of ion 'i', cation t_{Na+} (t_{Na+}) was 0.35 ± 0.03, 0.54 ± 0.05 and 0.88 ± 0.05 at pH 4, 5 and 7, respectively, in symmetric pH experiments [41], a clear demonstration of cation permselectivity at physiological pH. Moreover, t_{Na+} increased from 0.25 to 0.6 in the pH range 3 – 11 in asymmetric pH experiments (i.e., pH 7.4 receptor) [57].

Further information on this issue was provided by Dutet and Delgado-Charro [40]. While mannitol delivery showed high inter-nail variability, the anodal transport of mannitol across individual nails was always higher at pH 7.4 than 4 (**Figure 2**). The cumulative amount delivered from the anode was as high as 19.4 nmol (pH 4) and 82.5 nmol (pH 7.4) for some nails, and as low as 0.48 nmol (pH 4) and 0.89 nmol (pH 7.4) for others. In comparison, 1.5 nmol (pH 4) and 7.7 nmol (pH 7.4), on average, were delivered passively. Cathodal delivery increased from 1.6 to 2.6 nmol when the pH was lowered from 7.4 to 4. The average anodal solvent flow at pH 7.4 was 0.4 ± 0.5 $\mu l.h^{-1}.mA^{-1}$ (range 0.06 – 0.95 $\mu l.h^{-1}.mA^{-1}$), which is lower than that (2.4 ± 0.3 $\mu l.h^{-1}.mA^{-1}$) estimated previously [37]. In sum, the inter-nail variability associated with mannitol iontophoresis overwhelmed factors such as current application, polarity and pH. It is unknown whether these findings would be duplicated *in vivo* or if they would be applicable to other neutral or zwitterionic compounds.

In contrast, the contribution of electro-migration to molecular transport in transungual iontophoresis appears more significant and efficient. A series of *in vitro* experiments performed with binary combinations of sodium and lithium ions enlightened how transport numbers are determined during transungual iontophoresis (**Figure 3**) [41]. First, t_{Na+} and t_{Li+} (transport number of ion 'i', cation Li^+) at pH 4, 5 and 7 were directly related to their respective molar fractions (X_{Na+} and X_{Li+}) in the donor solutions. The gradient ('a') of the linear regression ($t_{C+} = a \bullet X_{C+} + b$; where t_{C+} is transport number of ion 'i', cation t_{C+} and X_{C+} is molar fraction of cation C^+) increased with pH, reflecting changes in nail permselectivity and allowing prediction of the transport number in the 'single ion' situation (X_{C+} = 1). A linear relationship was found between the ratio of the cations' transport number

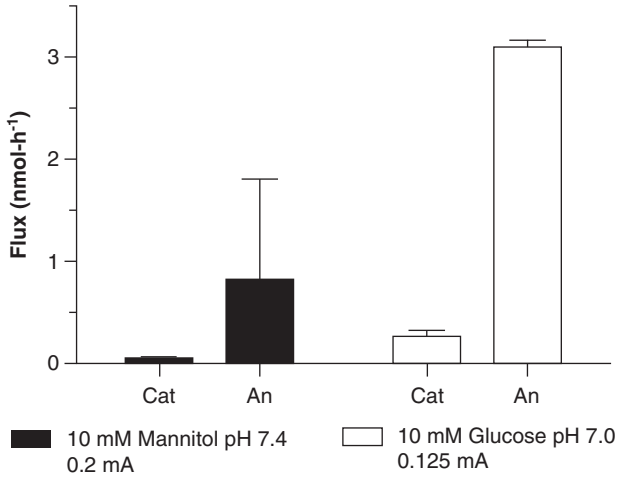

Figure 2. Electro-osmotic transport of mannitol and glucose across human nail tips. 'Cat' and 'An' bars indicate delivery from the cathodal and anodal compartments, respectively.
Data taken from [37,40].

(t_{Li+}:t_{Na+}) and their concentration ratio (C_{Li+}:C_{Na+}) in the vehicle; the gradient or mobility ratio (μ_{Li+}:μ_{Na+}) was very similar in the 4 – 7 pH range (0.65 – 0.71) and close to the value determined using skin (0.79) [26,41] and to the ratio of their aqueous mobilities (0.77) [66]. These results indicate that molar fraction and mobility are the key determinants of cationic transport numbers during nail iontophoresis, as has been observed for transdermal iontophoresis.

The effect of pH on t_{Cl-} (transport number of ion 'i', cation t_{Cl-}) and t_{Na+} was nicely demonstrated in the range 1 – 11 [57]. t_{Na+} Increased and t_{Cl-} decreased as the pH of the donor solution was raised (the receptor was always at pH 7.4). t_{Na+} And t_{Cl}- were very low at pH 1 due to charge carrying competition with the highly mobile hydronium ions.

Little work has been done to elucidate pathways of iontophoretic transport across the nail. In the case of transdermal iontophoresis, molecular transport occurs through both the appendegeal and intercellular pathways [16,67-70] but only the transcellular and paracellular pathways are possible for transungual permeation. The routes of penetration of two fluorescent markers during passive and iontophoretic experiments have been investigated using laser scanning confocal microscopy [63] and the images obtained suggest that both the transcellular and paracellular pathways are implicated in both passive and iontophoretic permeation across the nail.

2.3 Influential factors in nail iontophoresis

A first consideration is drug selection. Candidates have been typically chosen from drugs used to treat nail psoriasis and onychomycosis [37,47-52,54,55,58]. Ideally, the drug should be ionized in an acceptable pH range, and preferably cationic to benefit from the nail cation-permselectivity at neutral pH. *A priori*, low molecular mass drugs will have higher electrical mobility and transport numbers [24]. Highly soluble drugs allow their formulation at a high molar fraction, thus increasing their transport number. A chlorhydrate salt of the drug is preferred to provide chloride ions for the Ag/AgCl anode electrochemistry such that additional chloride salts, introducing competing counter-ions, are not required. Unfortunately, few of the currently used antifungals meet all these requirements (**Table 1**). Of course, drug potency and target site concentration must also be considered when selecting an appropriate candidate.

The next consideration is whether current application increases drug transport into and across the nail relative to passive delivery. While available data suggest that this is generally the case, the efficiency varies for different chemicals. Iontophoresis was not advantageous for urea and mannitol [39,40,43], while providing a 5- to 10-fold enhancement for glucose, griseofulvine, salicylic acid (SA) and ciclopirox and terbinafine [37,38,47,51], and a 10- to 600-fold increase for inorganic cations [41]. *In vivo*, iontophoresis increased the reverse iontophoretic extraction of chloride and sodium across the nail 8- and 27-fold, respectively [59]. Finally, the superiority of iontophoresis to better deliver terbinafine *in vitro* and *in vivo* has been demonstrated (**Figure 4**) [48-51,55,58].

The intensity of current is used to control iontophoretic transport accordingly to Faraday's law. The cathodal delivery of SA was proportional to the intensity of current in the range 0.04 – 0.125 mA [38]. The permeation of terbinafine was also proportional to the iontophoretic dose (mA.min) and to the intensity of current applied [48,51,55] although a very modest effect of current density was reported [52]. Interestingly, while terbinafine trans-nail transport was linearly dependent on current density, the magnitude of the drug reservoir formed was less sensitive to current application and intensity [48,51,55].

Formulation variables to optimize are the drug ionization and concentration in the vehicle as these factors determine the transport number, which is expected to increase with concentration (relative to any competing ions) in the vehicle. The presence of competing co-ions in the donor will decrease the drug's transport number. As discussed above, therefore, it is the molar fraction of the drug (i.e., its relative concentration to that of competing ions), rather than its nominal concentration, which determines its transport number [26,27,41]. Thus, SA cathodal fluxes increased linearly with drug concentration [38], but decreased as the concentration of competing ions was increased. The detrimental effect of counter-ion competition on the transport number of TEA and on the delivery of terbinafine [45,51] has been reported.

Consequently, additional formulation components and excipients should preferably be neutral; whenever salts are required, priority should be given to those providing the largest and least mobile co-ions. In fact, extremely high fluxes have been reported for terbinafine hydrochloride (TH) formulated as a 'single ion' [48,55] although other formulation and experimental factors might have contributed to these results. TH flux was 0.444 $\mu g.cm^{-2}.min^{-1}$ (or 16 $nmol.h^{-1}$) for a 0.2 mA current

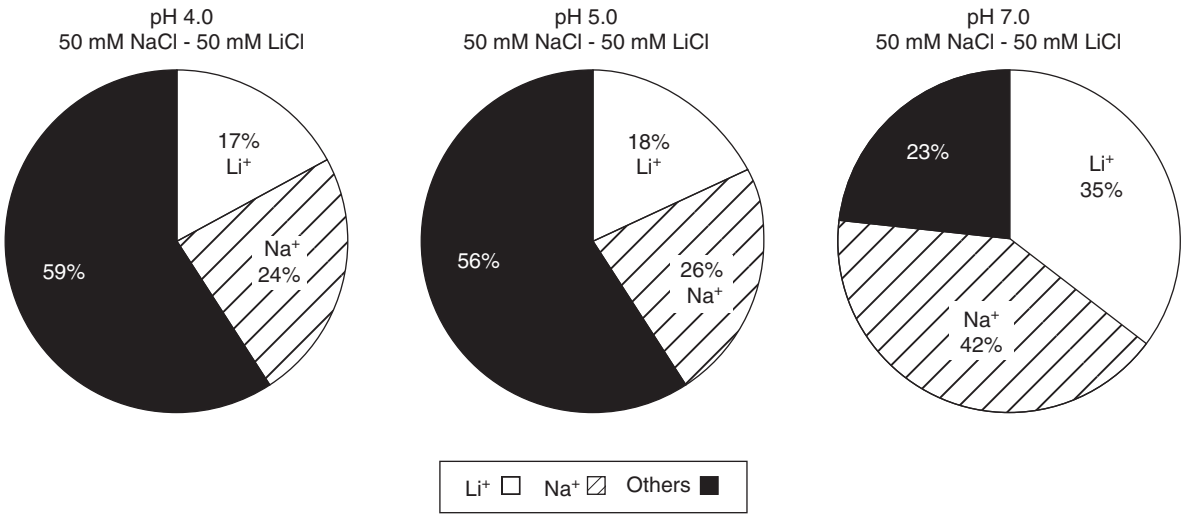

Figure 3. Distribution of charge-carrying during iontophoresis across human nail tips as a function of pH. Only lithium and sodium transport numbers were directly measured; the remaining charge is carried by unidentified ionic species, most probably chloride counter-ions, and is grouped as 'others'.
Data taken from [41].

Table 1. Physicochemical properties of selected antifungal drugs.

Drug	MM	pK_a	LogP/logD	Water solubility	Ref.
Ciclopirox	207.25	8.07 ± 0.05	1.4-2.3	8.6 mg/ml	[86,87]
Ciclopirox olamine	268.35			32.8 ± 0.6 mg/ml	
Amorolfine HCl	353.51	6.6	-1.45 (pH 4) 1.24 (pH 7.4)	9.2 ± 0.06 mg/ml	[86]
Terbinafine HCl	327.89	6.7 ± 0.3	5.5 (pH 6.8)	0.02 mg/ml (pH 6.8)	[83,84]
Itraconazole	705.63	3.7	5.66	Sparingly insoluble	[88,89]
Fluconazole	306.27	1.76 ± 0.1	0.5	8 mg/ml	[90]
Tioconazole	387.71	6.5-6.42	4.5	Sparingly soluble	[91,92]
Triamcinolone acetonide	434.50	12.9	2.54	17.5 μg/ml	[93,94]

MM: Molecular mass.

(1 mA.cm^{-2}, 0.2 cm^2) which corresponds to a transport number of 0.2% [55]. Apparently, the amount of TH loaded into the nail after 1 h of iontophoresis was approximately three orders of magnitude greater than that observed after 7 days of oral (250 mg/day) therapy; an astonishingly short lag time of only 15 min for TH was reported as well [55].

The formulation pH determines iontophoretic efficiency via changes in drug ionization and solubility, modifications of nail permselectivity, and charge carrying competition with the very mobile hydronium and hydroxyl ions at extreme pH values. This is nicely illustrated by the iontophoretic delivery of SA (pK_a = 3.1) whose ionization state was altered by varying the pH between 1.2 and 7 [38]. Cathodal SA fluxes were low in the 1.2 – 3 pH range, where the drug is non- or partially ionized and the nail is positively-charged driving any electro-osmotic transport in the cathode-to-anode direction. At pH = 5, the drug is ionized and is efficiently transported by electro-repulsion from the cathode; however, little contribution of the convective solvent flow occurs at this pH. Similar cathodal fluxes were measured at pH 5 and 7 consistent with a weak effect of electro-osmosis which would be in the anode-to-cathode direction at this pH.

The pH of a formulation can be altered if inert electrodes are used; for example, the donor pH increased from 8.7 to 10 – 11 when platinum cathodes were used [47]. Reversible electrodes such as Ag/AgCl do not modify pH, but sufficient chloride ions must be available at the Ag/AgCl anode to avoid silver precipitate staining of the nail [46], elevated voltages and unpredictable electrochemistry including pH change.

Iontophoresis has been combined with chemical and other enhancement techniques. Thioglycolic acid (but not urea) improved the iontophoretic transport of mannitol and TEA; glycolic acid had no effect on mannitol transport but decreased that of TEA [44]. SA and sodium sulfite have been delivered iontophoretically in an attempt to increase TH delivery [54]. The effect of PEGs on the passive and iontophoretic transport

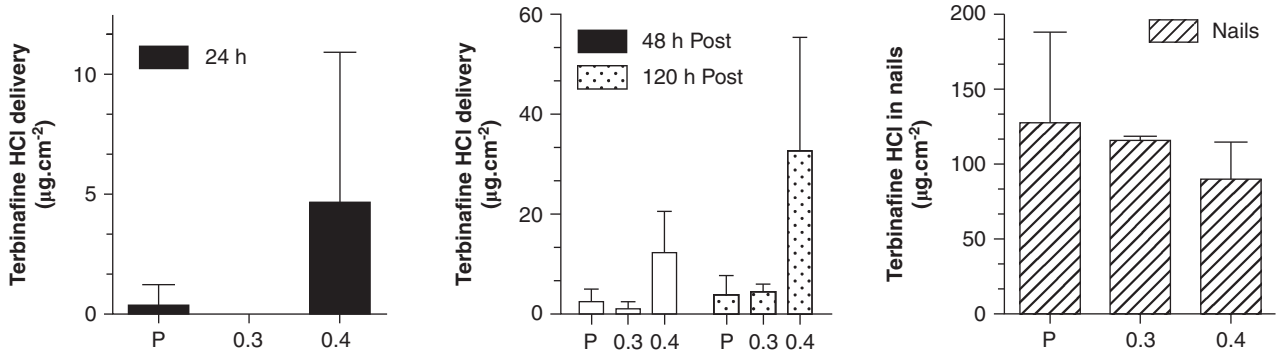

Figure 4. Passive and iontophoretic delivery of terbinafine HCl across human cadaver fingernail plates. The left panel shows terbinafine delivery into the receptor after 24 h of passive (P), 0.3 mA.cm^{-2} (0.3) and 0.4 mA.cm^{-2} (0.4) iontophoresis. The formulation was removed at 24 h and further delivery of terbinafine to the receiver was measured 48 and 120 h post-treatment (middle panel). The amount of terbinafine HCl in the nails at the end of the experiments (24 h treatment + 120 h post-treatment) is shown in the right panel.
Data taken from [51].

of TH has been investigated [53]. The formulation 'TPI-DF-507' containing 4% TH, 21% ethanol 95%; 5% polysorbate 80; 40% glycerin; 0.3% ethyl cellulose, 0.2% benzoic acid, 0.01 % hydroxyl toluene and 0.01 % disodium EDTA in water at pH 3.2 was used. To this formulation was added one of several different PEGs. PEG 400 and 200 enhanced the iontophoretic delivery of the drug but PEG 1000 and 3350 had no effect. The authors claimed t_{TH} to be as high as 0.17 for the PEG 400 iontophoretic experiments. In other words, TH would transport 17% of the charge, which seems surprisingly high for this drug. The data are difficult to interpret because several factors, such as low pH, ethanol, glycerol (a nail softener [71]), surfactants, PEG and current, could all play a role. In a different study the addition of 3% DMSO to a formulation (37% ethoxydiglycol and 1.5% hydroxyethyl cellulose) enhanced delivery of TH into and across porcine hooves [51]. Finally, abrasion of the nail dorsal layer increased the iontophoretic delivery of terbinafine but abrasion of the ventral layer and extraction of the nail lipids had little effect [54].

2.4 *In vivo* nail iontophoresis

The *in vivo* iontophoresis of prodrug prednisolone sodium phosphate across the nail was one of the first reports [36]. A 0.63 mA.cm^{-2} current density was applied for 10 min; the active electrode (surprisingly described as the anode) was a metal electrode in contact with a gauze soaked in an aqueous solution of the drug. The homemade device was held against the thumbnail which had been hydrated with soapy water for 5 min. The indifferent electrode was attached to the arm. The amount of prednisolone sodium phosphate delivered in this experiment is difficult to estimate for several reasons. The plasma levels of the metabolite prednisolone, measured in the iontophoresis treated arm, are difficult to relate to the delivery of the parent compound due to complex prodrug–drug inter-conversion and nonlinear kinetics [72,73].

One case report on the iontophoretic delivery of dexamethasone presents an interesting alternative treatment to treat nail psoriasis [74]. The procedure involved only one patient whose fingers were immersed in the drug solution through which a low electrical current was passed. The disadvantage of this method is that most of the current is expected to flow through the water or pathway of least resistance instead of through the skin or nail.

Hao *et al.* [46] investigated the effect of hydration on nail conductivity *in vivo*. Nail plates were kept hydrated for 6 h; a 0.1 mA current was applied for 1 min at different times and the voltage measured. Nail resistance decreased on hydration, reaching an approximately constant value after 2 h, a time consistent with the kinetics of water uptake investigated in parallel experiments [46]. The nail resistance was lower when more concentrated buffers were used. In another series of experiments, the nail resistance dropped relatively quickly during constant voltage experiments (1.5 and 9 V) reaching a constant value again after 2 h [46]. A concomitant increase in the intensity of current passing through the nail was observed until a plateau was reached [46]. No adverse effects were reported during the 1.5 V experiments. A shock-like sensation when the current was switched on–off during the 9 V experiments and some nail soreness on pressure following the treatment was reported. The effects were reversible in 2 – 3 days. Some stains were also observed on the nail surface probably due to an insufficient amount of chloride [46]. During this 6-h constant voltage experiment, the current density increased from 0.01 to 0.8 mA.cm^{-2} (or 6.4 µA to 0.5 mA for a 0.64 cm^2 application area), possibly the highest current administered so far *in vivo*. It is not clear, however, whether these results provide unequivocal information about the maximum current acceptable during nail iontophoresis as water hydrolysis may have occurred at the anode and contributed to the effects reported. The authors concluded that their

data suggested that constant current iontophoresis at an efficient level (e.g., 0.3 mA.cm^{-2}) would require excessive voltages to be practical.

However, it was later shown that direct, constant current iontophoresis (0.2 mA, 0.5 mA.cm^{-2}) was feasible and well-tolerated by healthy volunteers (as self-reported by the participants) and by measurements of TOWL [59]. TOWL was elevated after iontophoretic and passive experiments but returned to baseline values in ~ 1 h. These preliminary data suggest the TOWL changes are primarily due to hydration (similarly to transdermal iontophoresis [75-78]) and in agreement with preliminary observations done via FT-IR and impedance spectroscopy experiments [79].

Surprisingly, nail resistance appears to behave differently under *in vitro* and *in vivo* experiments. Hao *et al.* [46] observed an initially higher resistance *in vivo* than *in vitro*; however, the resistance of the nail during hydration declined faster *in vivo*. The *in vitro* work used cadaver fingernail plates, alterations of which during excision were suggested as a possible explanation for the differences observed. In contrast, in constant current iontophoretic experiments, lower voltages *in vivo* [59] were reported than *in vitro* [40,41]. Here, 0.2 mA was passed across 0.2 cm^2 nail tips *in vitro* and across 0.4 cm^2 of the thumbnail *in vivo*. These data indicate, therefore, that voltages required *in vitro* cannot be directly extrapolated to the *in vivo* situation and should not be used to make a decision concerning feasibility of an approach. For reasons not completely understood, current passage across the nail *in vivo* appears easier than *in vitro*.

A current application of 0.5 mA for 30 min [80] required a higher voltage (100 – 200 V) when the applicator made contact only with the nail. When contact was also made with adjacent soft tissues the voltage needed was much smaller (~ 25 V) as current then flowed through a more conductive pathway. The initial values from this study are consistent with those reported by Dutet and Delgado-Charro [59], who measured voltages in the 30 – 80 V range when a 0.2 mA current was initially applied across the nail plate.

Terbinafine has been administered via iontophoresis *in vivo*. Patients with toenail onychomycosis were treated with a 1% TH patch applied either passively or with iontophoresis [58]. The 0.1 mA iontophoretic treatment was applied for 6 – 8 h overnight, 5 days a week for 4 weeks and was well-tolerated.

Dutet and Delgado-Charro [59] were first to describe reverse iontophoresis across the nail; the outward fluxes of sodium and chloride ions across the nail plate were significantly enhanced. The transport number determined for sodium was t_{Na+} = 0.51 ± 0.11 which is close to values reported in transdermal reverse iontophoresis [81,82].

2.5 Iontophoretic delivery of terbinafine to the nail

The antifungal terbinafine is taken orally to treat onychomycosis; while this treatment is regarded as efficient, some disadvantages, such as side effects and toxicity, are observed making topical therapy an interesting alternative [2,4]. Terbinafine is poorly soluble in water. Hendriksen *et al.* [83] estimated TH solubility and logD at pH 6.8 to be 0.02 mg.ml^{-1} and 5.5, respectively. TH solubility is pH-dependent, decreasing from 0.01 M at pH 3 to 4.5.10^{-6} M at pH 9 (pK_a = 6.7 ± 0.3) [84]. Surfactants and co-solvents such as Tween 80, Pluronic F127, ethanol, propylene glycol and ethoxydiglycol are frequently added to the vehicle to increase TH solubility and the pH is usually acidified to 3 – 4.5 [48-51,57,53-55,58]. The receptor solution has also been acidified to pH 3 or supplemented with surfactants to ensure adequate solubility of the drug in this compartment during *in vitro* experiments [48,49,55].

Iontophoresis increased terbinafine transport across and into the nail plate in very short applications of 0.1 mA for 1 h [48]. The donor contained 10 mg.ml^{-1} (~ 30 mM) of the drug and 5% Tween 80 in water adjusted to pH 3. TH was delivered principally by electro-repulsion in these experiments, against the electro-osmotic flow which occurs in the cathode-to-anode direction at pH 3. The resulting transport number was ~ 0.2% [48]. According to this research, 0.76 ± 0.07 and 0.13 ± 0.02 µg.mg^{-1} of TH were loaded into the nail after only 1 h of iontophoretic and passive transport, respectively [48]. An extraordinarily short lag time (< 15 min) was reported for iontophoresis performed with the 'TPI-DF-507' formulation (pH 3.2, see composition above) [55]. TH permeation increased 0.27 – 0.45 µg/unit increase in the electrical dose (mA.min) depending on the donor formulation used [48,55].

An *ex vivo* model [50] was used to study TH distribution into the nail plate, nail bed and adjacent tissues after 20 min of passive and iontophoretic (0.5 mA) application of the TPI-DF-507 formulation. The applicators covered either the soaked (1 h in saline) nail plate or the soaked nail plate plus surrounding skin, resulting in different patterns of TH distribution. For example, the 'nail-only' applicator delivered more TH into the active diffusion area of the nail plate, while the 'skin-nail' treatment transported TH efficiently to the nail proximal fold. Surprisingly, drug transport could be measured after only 20 min of passive and iontophoretic delivery. In fact, it was claimed that a 0.5 mA treatment for 20 min was sufficient to provide TH nail levels comparable to those measured after 28 days of continuous oral Lamisil treatment [50]. It is unclear whether formulation (see composition above) or other experimental factors could have contributed to these remarkable results.

Iontophoresis and passive diffusion were combined with other enhancing techniques to further increase TH transport [53,54]. The enhancers were loaded into the nail via iontophoresis to shorten the duration of the enhancer pretreatment [54]. Control (untreated) nails were loaded with 0.2 ± 0.08 µg.mg^{-1} of TH, whereas 11.0 ± 2.6 µg.cm^{-2} of the drug had permeated after 24 h of iontophoresis at 0.5 mA.cm^{-2}. These values are quite similar to the amount (0.76 ± 0.07 and the 0.14 ± 0.02 µg.mg^{-1}) loaded into the nail plate after iontophoresis at 0.1 mA for 1 h (0.5 mA.cm^{-2}–0.2 cm^{-2}) [48] and after 20 min at 0.5 mA, respectively [50], and to the permeation of 12.3 ± 3.0 µg.cm^{-2} after the former current profile [48]. It is unclear why short- [48,49,55] and long-duration periods of iontophoresis [54] result in comparable

drug delivery. The authors suggest that TH loading into the nail plateaus at 2 – 6 $\mu g.mg^{-1}$ and is not increased by further current application, while drug permeation into the receptor increases linearly with the iontophoretic dose applied [48,55]. Further enhancement of TH transport was observed when PEG 200 and 400 were added to the 'TPI-DF-507', pH 3.2 formulation [53].

The iontophoresis of terbinafine on onychomycotic nails [49] resulted in very similar findings. The formulation (pH 3.2, 4% TH, 21% ethanol 95%; 5% polysorbate 80; 40% glycerin; 0.3% hydroxyethylcellulose; 0.2% benzoic acid; 0.01% butylated hydroxyl toluene; and disodium EDTA) delivered more TH to and across onychomychotic fingernails than toenails under both passive and iontophoretic conditions. The amounts of TH loaded into finger (~ 1.5 $\mu g.mg^{-1}$) and toe nails (0.75 $\mu g.mg^{-1}$) after 1 h of 0.5 $mA.cm^{-2}$ (0.2 cm^2) iontophoresis was claimed to be two to three orders of magnitude higher than that measured in the nail (0.5 $\mu g.g^{-1}$) after weeks of oral therapy (daily dose = 250 mg).

The nail can be loaded with TH in relatively short iontophoretic applications providing a reservoir from which the drug is slowly released to agar plates and inhibits the growth of *Trichophyton rubrum* [48,49,53,55]. This outcome could provide therapeutic advantages.

An *in vivo* study compared the 'masked drape' applicator, which restricts delivery to the nail plate, with the 'full drape' applicator which also delivers the drug to the surrounding soft tissue [80]. The volunteers received doses ranging from 3 to 15 mA.min, with 0.5 mA for 30 min being the largest. Nail clippings were taken 24 h post-treatment and analyzed for TH content; the average values for the 'masked' and 'full draped' treatments at the 8 mA.min dose were 423 and 842 $\mu g.g^{-1}$, respectively. It is unclear why nail levels were higher when the treatment involved the surrounding tissues because, as the authors stated, 70% of the current was expected to pass through the skin and not through the nail.

Amichai *et al.* in collaboration with Power Paper Ltd. have also explored TH iontophoresis [58]. Thirty-eight patients with toe onychomycosis participated in a clinical study in which the primary measure of efficacy was outgrowth of at least 1.5 mm of new clinically unaffected nail plate, and mycological improvement of the nail (reduced number of fungal elements and decreased rate of fungal culture growth during and after treatment) of the nail. The 1 cm^2 patch contained 1% terbinafine HCl, 25% Pluronic F127, 2% absolute ethanol, 5% propylene glycol, 0.8% Sharonmix 824 (a blend containing pethylparaben, ethylparaben, propylparaben and phenoxyethanol used as preservative against fungi and bacteria) and 0.12% triethanolamine in water at a pH 4.6. The patches were applied to the infected toenail for 6 – 8 h overnight, 5 days a week for 4 weeks and a new patch was used for each application. The iontophoretic device applied a 0.1 mA constant current via a graphite active electrode and an Ag/AgCl return cathode. Follow-up visits took place at weeks 1, 3, 5, 8 and 12. The active (iontophoresis) group showed significant growth of healthy nails and reduction of fungal elements compared to the passive group. Overall the patch was well-tolerated; local irritation and discomfort were reported only by two patients at the beginning of the treatment and a tingling sensation was reported by those wearing the iontophoretic patch. The amounts of terbinafine (mean ± s.e.m.) in the nails measured in the fourth visit were 5.69 ± 2.15 and 1.34 ± 0.54 $\mu g.cm^{-2}$ for the active and passive groups, respectively. The levels declined to 2.41 ± 0.85 $\mu g.cm^{-2}$ (active) and 0.15 ± 0.51 $\mu g.cm^{-2}$ (passive) by the end of the follow-up period. According to the authors, the MIC of terbinafine corresponds to 0.1 – 1 $\mu g.cm^{-2}$, and hence the improved efficacy in the active group was claimed to be due to the deeper penetration of the drug. While there has been some controversy [85] about the nail growth measurement, this study provides the first clear indication of the potential of iontophoresis to treat onychomychosis. The same group investigated the *in vitro* delivery of TH across human nail and porcine hoof (**Figure 4**) [51]. The pH 4.5 formulation consisted of 1% TH, 37 – 40% ethoxydiglycol as solubilizer, 1.5% hydroxyethylcellulose as gelling agent, 0 – 3% DMSO and 1% NaCl or KCl. The results suggested a minimum time and intensity of current application for effectiveness. After 24 h of passive, 0.3 $mA.cm^{-2}$ and 0.4 $mA.cm^{-2}$ treatment, the amounts (mean ± s.e.) of terbinafine in the receptor compartment were 0.3 ± 0.9, 0, and 4.6 ± 6.3 $\mu g.cm^{-2}$, respectively. The formulation was removed at 24 h and further release of TH was measured (**Figure 4**) with the 0.4 $mA.cm^{-2}$ treatment providing better results. However, the amount of TH remaining in the nails after 5 days was quite similar for all treatments (90 – 128 $\mu g.cm^{-2}$).

3. Conclusions

Nail iontophoresis has received increasing attention in recent years but remains a niche area; ~ 30 research articles on nail iontophoresis have been published since 2007. Nail iontophoresis, including one clinical study with terbinafine, has been successfully implemented *in vivo*, which dissipates initial doubts about its feasibility. The nail plate permselective properties have been clearly demonstrated; even so, it is unclear whether electro-osmosis provides a significant contribution to drug transport. Transport by electro-migration is, on the contrary, efficient and less variable. The factors determining transport numbers during nail iontophoresis are in line with those expected from theory and those observed in transdermal applications. The few data available suggest nail iontophoresis to be efficient and well-tolerated but more work is required to establish the safety of the technique on repeated application. The clinical potential of iontophoresis to treat nail psoriasis and onychomycosis and its advantages with respect to other drug delivery methods (including cost effectiveness) have not been fully established.

4. Expert opinion

Recent work on optimization of topical nail therapies responds to unmet clinical needs and is expected to continue until more satisfactory treatments are made available. At the moment, nail iontophoresis is being tested along with other enhancing techniques and it is too early to know whether it will provide an efficient and cost effective method to treat nail psoriasis and onychomycosis. The field of nail iontophoresis was largely unexplored until 2007, yet since then impressive advances have established the necessary knowledge base from which rationale development of the technique can proceed. This rapid progress is due in large part to concepts and methodology 'imported' from transdermal iontophoresis.

Key findings include a better understanding of nail permselectivity, the relative contributions of electro-osmosis and electro-repulsion to iontophoretic transport, and the factors (mobility and molar fraction) which determine the magnitude of transport numbers during nail iontophoresis. Another decisive milestone was the successful application of iontophoresis *in vivo* by independent research teams.

However, there are still some gray areas: the unclear role of electro-osmosis questions the usefulness of the technique for neutral, zwitterionic and large molecules having low transport numbers. Electro-transport and the transport numbers of small ions are well documented, but not much is known about structure–transport relationships. That is, how transport numbers are modified as a function of drug physicochemical properties, most importantly molecular weight and lipophilicity. This is the key to the selection of the most suitable drug candidates to be delivered by iontophoresis.

The different methodologies reported in the literature make data comparison and interpretation difficult, and caution should be exerted when *in vitro* results are extrapolated to *in vivo* situations. Some strikingly high transport data have been published which contrast with other, more expected, results for the same drug. These extreme discrepancies hinder the development of the field and challenge the competence of nail iontophoresis as an efficient and reliable controlled drug delivery technique. It is crucial, therefore, that methods and results are well-detailed so that independent and critical analysis of the data is possible.

Hopefully, the future will soon witness more *in vivo* studies establishing the safety and efficiency of nail iontophoresis. The pioneering clinical study by Amichai *et al.* [58] has provided an exciting illustration of how nail iontophoresis may be implemented in clinical practice. Ultimately, the future of nail iontophoresis will depend on its clinical efficacy and cost relative to that of alternative enhancing techniques being investigated in parallel.

Declaration of interest

The authors declare no conflict of interest and have received no payment in preparation of this manuscript. MB Delgado-Charro is a co-author of several patents in the field of Iontophoresis.

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