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Research paper

Using laser microporation to improve transdermal delivery of diclofenac: Increasing bioavailability and the range of therapeutic applications

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ABSTRACT

The objective of the study was to investigate the effect of laser microporation, using P.L.E.A.S.E.® technology, on diclofenac delivery kinetics. Skin transport of diclofenac was studied from aqueous solution, propylene glycol and marketed formulations across intact and laser-porated porcine and human skins; cumulative permeation and skin deposition were quantified by HPLC. After 24 h, cumulative diclofenac permeation across skins with 150, 300, 450 and 900 shallow pores (50–80 μm) was 3.7-, 7.5-, 9.2- and 13-fold superior to that across untreated skin. It was also found to be linearly dependent on laser fluence; Permeation (μg/cm^2) = 11.35 ∗ Fluence (J/cm^2) + 352.3; r^2 = 0.99. After 24 h, permeation was 539.6 ± 78.1, 934.5 ± 451.5, 1451.9 ± 151.3 and 1858.6 ± 308.5 μg/cm^2, at 22.65, 45.3, 90.6 and 135.9 J/cm^2, respectively. However, there was no statistically significant effect of laser fluence on skin deposition. Diclofenac delivery from marketed gel formulations was also significantly higher across laser-porated skins (e.g. for Solaraze™, cumulative permeation after 24 h across treated (900 pores/135.9 J/cm^2) and untreated skin was 974.9 ± 368.8 and 8.2 ± 3.8 μg/cm^2, respectively. Diclofenac delivery from Solaraze™ across laser-porated porcine and human skins was also shown to be statistically equivalent. The results demonstrated that laser microporation significantly increased diclofenac transport from both simple and semi-solid formulations through porcine and human skin and that pore depth and pore number could modulate delivery kinetics. A similar improvement in topical diclofenac delivery *in vivo* may increase the number of potential therapeutic applications.

1. Introduction

Diclofenac (MW 296.15 Da; log *P* 4.06) is one of the most widely used nonsteroidal anti-inflammatory agents. Although it is almost completely absorbed, an extensive hepatic first-pass effect means that systemic bioavailability following oral administration is ~50%; furthermore, rapid clearance results in a short half-life and the potential need for multiple dosing [1]. Although generally well tolerated, the most common adverse drug reaction is its impact on the gastric mucosa; indeed, a recent study using endoscopic examination of a series of orthopaedic outpatients demonstrated that 83.3% and 73.5% of patients receiving diclofenac and diclofenac SR, respectively, manifested gastric mucosal lesions [2]. Topical administration should decrease the risk of these side effects and such dosage forms are available [3].

Furthermore, clinical studies have shown that topical administration resulted in higher diclofenac levels in the dermis and muscle as compared to oral administration, providing evidence for the efficacy of local administration [4,5]. For example, following application of two tapes containing a total of 30 mg diclofenac, concentrations in the subcutaneous fat and muscle were reported to be 13.46 ± 11.31 and 9.29 ± 8.34 ng/ml, respectively (cf. 3.85 ± 2.28 and 0.66 ± 1.11 ng/ml after oral administration of a 30 mg diclofenac capsule) [5]. However, the converse was true for drug levels measured in the synovial fluid and membrane (topical: 1.96 ± 0.68, 4.99 ± 3.84 ng/ml and oral: 16.76 ± 12.00, 15.07 ± 9.17 ng/ml) [5]. It follows that oral administration is the preferred option for the treatment of rheumatoid and osteoarthritis, whereas topical application is suitable for the relief of pain symptoms in minor strains, sprains and contusions. Pharmacokinetic studies have also shown that diclofenac levels measured in synovial fluid after topical administration from ipsi- and contralateral knee joints were not significantly different; thus, it was likely that drug was principally entering the synovial space from the systemic circulation [6]. Therefore, in order to improve efficacy in the treatment of rheumatoid and osteoarthritis using topical diclofenac dosage forms, it will probably be necessary to increase systemic bioavailability while minimising the risk of adverse drug reactions.

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Several different formulation strategies have been investigated to improve diclofenac transport [7–11]. However, the excellent barrier function of the stratum corneum limits the rate and extent of topical drug delivery [12,13], and focus has moved to the development of new technologies to reversibly impair barrier function and so enhance transport [14]. Several approaches to ablate the stratum corneum have been studied, including mechanical ablation by way of microneedles [15–18] to the use of more sophisticated energy-based strategies to selectively remove the stratum corneum and to facilitate drug entry into the epidermis [19–21].

The aim of this study was to use laser-assisted microporation to create transport channels in the skin and so enable controlled enhancement of diclofenac delivery. The laser system employed in these studies (P.L.E.A.S.E.®; Precise Laser Epidermal System) contains an Er:YAG laser that emits light at 2.94 μm [22,23]. This corresponds to a major water absorption peak; the excitation and subsequent evaporation of the water molecules in the epidermis lead to micropore creation without inducing thermal damage to the surrounding tissues [22]. The applied laser energy per unit area (fluence) controls the depth of each micropore, and due to the device's high repetition rate, it is possible to create several hundred micropores with controlled depth in the space of a few seconds. Since the number of pores created in the skin and the depth can be easily varied (in principle, from 1 to nearly 5000), this provides a simple means to control the dose delivered and to individualise therapy; in contrast, other microporation technologies lack this capacity since they create a fixed number of pores dependent on the number of "porating" elements in the device placed in contact with the skin.

The specific objectives of the project were (i) to study the effect of pore number and fluence on diclofenac delivery, (ii) to demonstrate that diclofenac could be delivered rapidly and in significant amounts across P.L.E.A.S.E.® porated porcine skin from both simple and marketed formulations and (iii) to compare transport across P.L.E.A.S.E.® porated porcine and human skins.

2. Materials and methods

2.1. Chemicals

Diclofenac sodium, propylene glycol and sodium dihydrogen phosphate were purchased from Sigma (Buchs, Switzerland). Acetonitrile, methanol (Chromasolv HPLC gradient grade) and nylon membrane filters (0.22 μm) were purchased from VWR (Nyon, Switzerland). Sodium chloride and potassium dihydrogen phosphate were purchased from Acros Organics (Chemie Brunschwig; Basel, Switzerland). Marketed diclofenac formulations: Voltaren Dolo® Emugel® 1% w/w (VDE; Novartis SA), Flector® gel 1.3% w/w (FG) and Flector® EP Tissugel 1.3% w/w (3.0 cm^2–3.4 mg diclofenac epolamine) (FTG; Institut Biochmique SA (IBSA)) were purchased direct from pharmacies. Solaraze™ gel 3% w/w (Almirall Hermal GmbH) was kindly provided by Dr. R. Strohal (Federal Academic Hospital of Feldkirch, Dept. of Dermatology and Venereology, Feldkirch, Austria). Deionised water (resistivity > 18 MΩ cm) was used to prepare all the solutions.

2.2. Skin

Full thickness (1.0–1.5 mm) porcine skin was used to study the effect of P.L.E.A.S.E.® poration on diclofenac delivery. Porcine ears were supplied by a local abattoir (CARRE; Rolle, Switzerland) shortly after sacrifice. After cleaning under cold running water, the whole skin was removed carefully from the outer region of the ear and separated from the underlying cartilage with a scalpel. Full thickness porcine skin samples were wrapped in Parafilm™ and maintained at −20 °C for no longer than a period of 2 months before use.

Human skin samples were collected immediately after surgery from the Department of Plastic, Aesthetic and Reconstructive Surgery, Geneva University Hospital (Geneva, Switzerland); fatty tissue was removed and the skin was wrapped in Parafilm™ before storage at −20 °C for a maximum period of 3 days. The study was approved by the Central Committee for Ethics in Research (CER: 08-150 (NAC08-051); Geneva University Hospital).

2.3. Diclofenac stability in the presence of skin

Solution stability of diclofenac in contact with porcine skin was assessed by preparing (a) 10 mg/ml diclofenac in water and (b) 10 mg/ml diclofenac in pH 7.4 phosphate-buffered saline (PBS, pH 7.4). Solutions (a) and (b) were placed in contact with full-thickness epidermal and dermal skin surfaces, respectively, for 6 h. Samples were diluted in PBS (pH 7.4) and analysed by HPLC. Experiments were performed in triplicate.

2.4. Laser microporation

Skin samples were equilibrated in 0.9% NaCl for 30 min prior to poration using the P.L.E.A.S.E.® device. After removing surface moisture, skin samples mounted in a custom-designed assembly were placed at the focal length of the laser to create the micropores. Laser poration parameters, i.e., the pore number and the fluence (determined by the number of energy pulses applied to create each pore), were fixed by the user.

2.5. Experimental protocol to study diclofenac delivery kinetics

Skin samples (either intact (untreated) or P.L.E.A.S.E.® porated) were mounted in Franz diffusion cells ($A = 3.0 \pm 0.1$ cm^2); silicone grease was applied to ensure a watertight seal. The receptor compartment (~10 ml) was filled with PBS (pH 7.4). After equilibration, the formulations (details given below) were placed in the donor compartment. The receptor phase was stirred at room temperature throughout the experiment; 0.4 ml of the receptor phase was withdrawn hourly for 6 h and then again after 24 h, and each aliquot was replaced with an equivalent volume of fresh PBS buffer.

At the end of the permeation experiment, the diffusion cells were dismantled and the skin surface washed in running water to remove residual donor formulation. The skin samples were then cut into small pieces and soaked in mobile phase (6 ml; see below for composition) for 4 h under constant agitation at ambient temperature so as to extract diclofenac deposited in the membrane during the permeation experiment. After dilution in PBS (pH 7.4), samples were filtered through 0.22 μm nylon membrane filters prior to HPLC analysis.

Four separate series of experiments were performed:

2.5.1. Effect of P.L.E.A.S.E.® device parameters on diclofenac delivery

Diclofenac transport (permeation and deposition) from 1 ml of aqueous solution (10 mg/ml) through P.L.E.A.S.E.® porated porcine skin was investigated as a function of (i) pore number (0, 150, 300, 450 and 900 pores; fluence fixed at 22.65 J/cm^2) and (ii) fluence (22.65, 45.3, 90.6 and 135.9 J/cm^2), which determines the pore depth – this was done using 900 pores.

2.5.2. Effect of formulation on diclofenac delivery

Diclofenac transport into and across porcine skin using aqueous and propylene glycol formulations at equivalent concentration (10 mg/ml) was compared in order to investigate the effect of

formulation type. Skin samples were porated to create 900 pores using a fluence of 135.9 J/cm^2.

2.5.3. Studying diclofenac delivery from marketed formulations across P.L.E.A.S.E.® porated porcine skin

Diclofenac delivery kinetics from three gel formulations – Voltaren Dolo® Emugel® (VDE;1% w/w, 300 mg), Flector® (FG; 1.3% w/w, 300 mg) and Solaraze™ (SG; 3% w/w, 300 mg), and a novel diclofenac plaster formulation – Flector® Tissugel, (FTG; 1.3% w/w, 3.0 cm^2) – were compared across untreated (control) and P.L.E.A.S.E.® porated porcine skin with 900 pores and created using a fluence of 135.9 J/cm^2.

2.5.4. Comparison of transport kinetics across porcine and human skin

Cumulative diclofenac permeation from Solaraze™ (300 mg) across full-thickness human skin samples porated with 900 pores at a fluence of 135.9 J/cm^2 was followed over 24 h. The amount of drug retained in the skin during permeation was also quantified.

2.6. HPLC analysis

Diclofenac samples were analysed using a P680A LPG-4 pump equipped with an ASI-100 autosampler and a UVD170U UV/Vis detector (Dionex; Olten, Switzerland) set at 205 nm. The flow rate and injection volume used were 1.5 ml/min and 25 µl, respectively. Column temperature was set at 35 °C. Isocratic separation of diclofenac was performed using a 125 mm × 4.0 mm column packed with 5 µm C18 reversed-phase silica particles (Lichrocart, Merck KGaA, Germany) assembled with prefilter. The mobile phase comprised 0.05 M KH_2PO_4 (pH 7.0)/methanol/acetonitrile (58:21:21 v/v). Under these conditions, diclofenac eluted at ~3.8 min and the method was linear from 0.1 to 100 µg/ml. The limits of detection and quantification were 50 and 150 ng/ml and the RSD of the repeatability was less than 1%. The method was specific for diclofenac, and the peak was clearly separated from solvent peaks and endogenous compounds in the skin.

2.7. Statistics

Statistical analysis was performed using either a paired Student's *t*-test or an ANOVA followed by a Student–Newman–Keuls test; the level of significance was fixed at $\alpha = 0.05$ unless indicated otherwise. Outliers were identified using a Grubbs' test.

3. Results

3.1. Stability of diclofenac in contact with skin

Diclofenac recovery after 6 h contact with epidermis and dermis was 100.5 ± 0.5% and 93.0 ± 0.3%, respectively. The results indicated that diclofenac was stable under the conditions used for the skin transport experiments.

3.2. Effect of laser poration parameters and formulation type on diclofenac transport

3.2.1. Effect of pore number on diclofenac delivery

This was studied by varying the number of pores from 0 (control) to 150, 300, 450 and 900 while fluence was fixed at 22.65 J/cm^2; this ensured the creation of relatively shallow pores with depths typically in the range of 50–80 µm [23].

Diclofenac permeated very poorly across intact skin; cumulative permeation through untreated skin after 6 h was below the limit of detection (Fig. 1a). Laser poration resulted in a statistically significant increase in cumulative skin permeation of diclofenac at

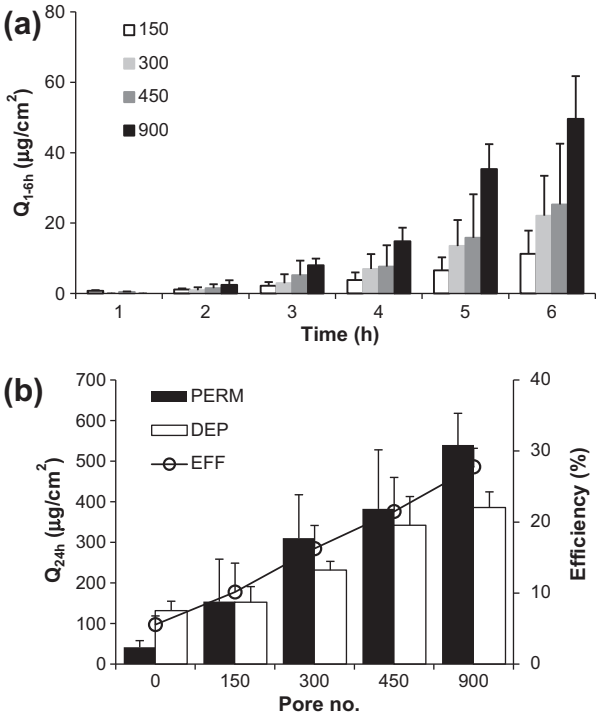

Fig. 1. (a) Cumulative diclofenac permeation across P.L.E.A.S.E.® porated porcine skin during 6 h (Q_{1-6h}) with 0, 150, 300, 450 and 900 pores using a laser fluence of 22.65 J/cm^2. (b) Effect of pore number on total diclofenac delivery (sum of amounts permeated and deposited within the skin) and delivery efficiency (the fraction of the applied dose permeated or deposited) after 24 h (Q_{24h}) (Mean ± SD; $n = 5$–6).

the 6 h time-point (ANOVA; $F(9.41) > F_{crit}(3.20)$, $P = 6.8 \times 10^{-4}$). Post hoc analysis using a Student–Newman–Keuls test ($\alpha = 0.05$) confirmed that delivery using 900 pores (49.6 ± 12.1 µg/cm^2) was statistically superior to that at 450 (25.2 ± 17.2 µg/cm^2), 300 (22.2 ± 11.3 µg/cm^2) and 150 (11.2 ± 6.5 µg/cm^2) pores (Fig. 1a). After 24 h, the dependence of diclofenac permeation on pore number was more pronounced (ANOVA; $F(19.34) > F_{crit}(2.84)$, $P = 8.4 \times 10^{-7}$); at 0 (control), 150, 300, 450 and 900 pores, cumulative permeation was 53.6 ± 33.2, 153.9 ± 104.6, 310.6 ± 106.5, 382.4 ± 145.3 and 539.6 ± 78.1 µg/cm^2, respectively (Fig. 1b). Post hoc analysis using a Student–Newman–Keuls test ($\alpha = 0.05$) confirmed that delivery using 900 pores was superior to that at lower pore numbers; delivery at 450 and 300 pores was superior to 150 and control (0 pores), and finally, transport through skins with 150 pores was also superior to that across control samples.

Increasing pore number also resulted in a statistically significant increase in skin deposition of diclofenac (ANOVA; $F(38.43) > F_{crit}(2.82)$, $P = 1.3 \times 10^{-9}$); the amounts of drug retained within the membrane at 0 (control), 150, 300, 450 and 900 pores were 132.0 ± 22.9, 184.7 ± 86.1, 231.5 ± 21.6, 333.9 ± 66.3 and 386.1 ± 38.1 µg/cm^2, respectively (Fig. 1b). Post hoc analysis using a Student–Newman–Keuls test ($\alpha = 0.05$) confirmed that skin deposition at a given pore number was superior to that at lower pore numbers.

3.2.2. Effect of fluence on diclofenac transport

For these experiments, the pore number was fixed at 900 and the fluence held at four settings – 22.65, 45.3, 90.6 and 135.9 J/cm^2. Histology studies of the P.L.E.A.S.E.® porated skin samples have shown that increasing fluence leads to increase in pore depth [23]. It was shown that a fluence of 22.65 J/cm^2 resulted in consistent penetration into the viable epidermis with pore depths estimated at 50–80 µm. Upon increasing fluence further (45.3, 90.6

and 135.9 J/cm^2), it was possible to reach the epidermal–dermal junction and create deeper pores that extended into the dermis. Significant increases in diclofenac permeation were observed at t = 6 h, upon increasing fluence from 22.65 to 135.9 J/cm^2 (ANOVA; $F(11.15) > F_{crit}(3.20)$, $P = 2.8 \times 10^{-4}$); permeation at 22.65, 45.3, 90.6 and 135.9 J/cm^2 was 49.6 ± 12.1, 120.7 ± 90.9, 232.2 ± 64.7 and 345.2 ± 152.6 μg/cm^2 respectively (Fig. 2a). Post hoc analysis using a Student–Newman–Keuls test ($\alpha = 0.05$) confirmed that delivery at 135.9 and 90.6 J/cm^2 was superior to that at 45.3 and 22.65 J/cm^2. As for the pore number results, the dependence of diclofenac on fluence was more marked at the 24 h time-point (ANOVA; $F(19.71) > F_{crit}(3.16)$, $P = 6.4 \times 10^{-6}$); cumulative permeation at 22.65, 45.3, 90.6 and 135.9 J/cm^2 was 539.6 ± 78.1, 934.5 ± 451.5, 1451.9 ± 151.3 and 1858.6 ± 308.5 μg/cm^2, respectively (Fig. 2b). Post hoc analysis using a Student–Newman–Keuls test ($\alpha = 0.05$) confirmed that delivery at a given fluence was superior to that lower fluences. Cumulative diclofenac permeation was linearly dependent on laser fluence; Permeation (μg/cm^2) = 11.35 * Fluence (J/cm^2) + 352.3; r^2 = 0.99 (Fig. 2c). Given the area of diffusion (3 cm^2), then, at a fluence of 135.9 J/cm^2, ~55% (5.5 ± 0.9 mg) of the applied dose of diclofenac had permeated through the skin in 24 h following P.L.E.A.S.E.® poration. Indeed, approximately 16% of the applied amount of diclofenac was delivered at the lowest fluence (22.65 J/cm^2); thus, selective ablation of the upper epidermal layer was sufficient to enable significant diclofenac delivery while minimising the risk of skin irritation. Increasing fluence did not produce a statistically significant increase in diclofenac deposition.

3.2.3. Effect of formulation type on diclofenac delivery

Fig. 3 shows the effect of formulation type on diclofenac delivery through the micropores; at equivalent concentration (10 mg/ml), both permeation and deposition were significantly higher from aqueous solution than from propylene glycol formulations (1858.6 ± 308.5 and 435.1 ± 76.1 μg/cm^2 as compared to 87.3 ± 41.0 and 226.2 ± 55.7 μg/cm^2, respectively) (see Fig. 4).

3.3. Diclofenac delivery kinetics across laser-porated skin using marketed formulations

Diclofenac permeation across untreated skin was poor. Cumulative permeation from Solaraze™ (SG) across untreated skin after 6 h was 0.4 ± 0.6 μg/cm^2; no drug was detected in the receiver compartment following the application of Voltaren Dolo® Emugel® (VDE), Flector® (FG) or Flector® Tissugel (FTG). P.L.E.A.S.E.® poration markedly increased diclofenac permeation at 6 h for both FG (37.7 ± 6.5 μg/cm^2) and SG (165.3 ± 122.7 μg/cm^2); changes in delivery from VDE (3.6 ± 3.0 μg/cm^2) and FTG (0.3 ± 0.3 μg/cm^2) were less significant. After 24 h, cumulative permeation of diclofenac across untreated skin from VDE, FG, FTG and SG was 14.6 ± 3.0, 15.0 ± 3.3, 1.1 ± 1.3 and 8.2 ± 3.8 μg/cm^2, respectively. For comparison, diclofenac permeation after 24 h across laser-porated skin was 150.0 ± 28.7, 315.8 ± 55.0, 5.2 ± 5.0 and 974.9 ± 368.8 μg/cm^2 from VDE, FG, FTG and SG, respectively (Table 1). Skin deposition of diclofenac in P.L.E.A.S.E.® porated skin was also greater than that in skin samples without laser treatment (Table 1).

3.4. Comparison of transport kinetics across porcine and human skin

Since Solaraze™ (SG) gave the highest diclofenac permeation with porcine skin, delivery from this formulation was also investigated across laser-treated human skin (900 pores, fluence 135.9 J/cm^2) using samples obtained following abdominoplasty. The results showed that cumulative permeation after 6 and 24 h

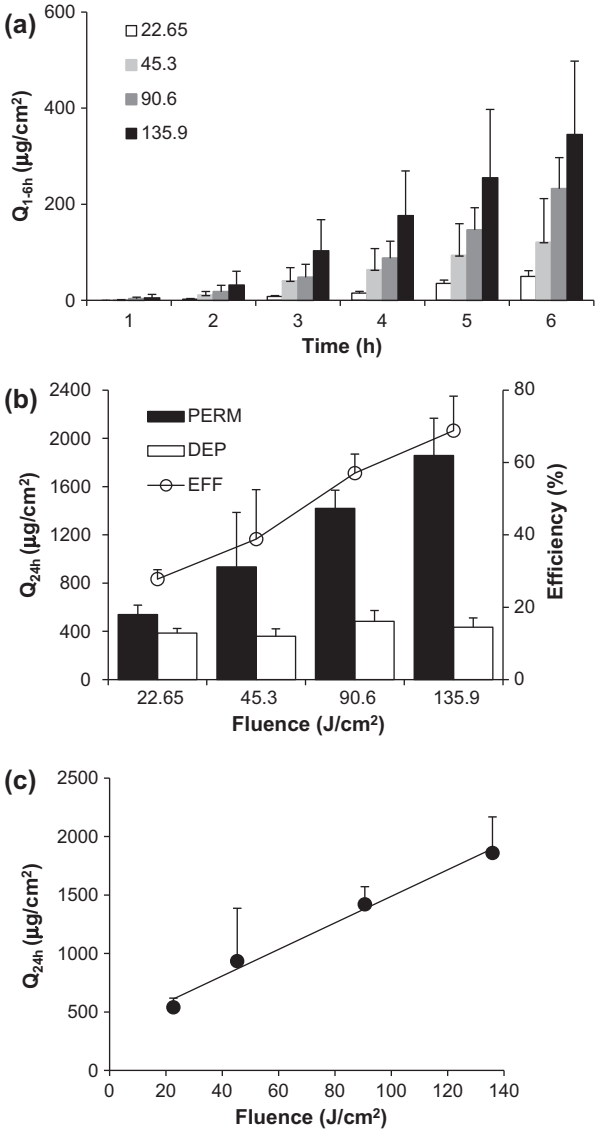

Fig. 2. (a) Cumulative diclofenac permeation across P.L.E.A.S.E.® porated porcine skin with 900 pores during 6 h (Q_{1-6h}) and using laser fluences of 22.65, 45.3, 90.6 and 135.9 J/cm^2. (b) Effect of fluence on total diclofenac delivery (sum of amounts permeated and deposited within the skin) and delivery efficiency (the fraction of the applied dose permeated or deposited) after 24 h (Q_{24h}) and (c) correlation between cumulative diclofenac permeation and fluence (Mean ± SD; n = 5–6).

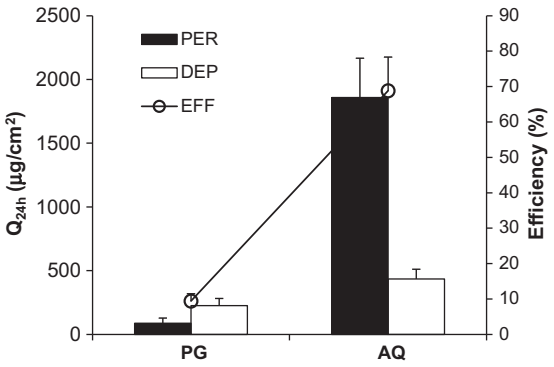

Fig. 3. Comparing diclofenac delivery from aqueous solution (AQ) and from propylene glycol (PG) after 24 h (Q_{24h}) using equivalent formulation (10 mg/ml) and poration conditions (900 pores created using a fluence of 135.9 J/cm^2) (Mean ± SD; n = 5–6).

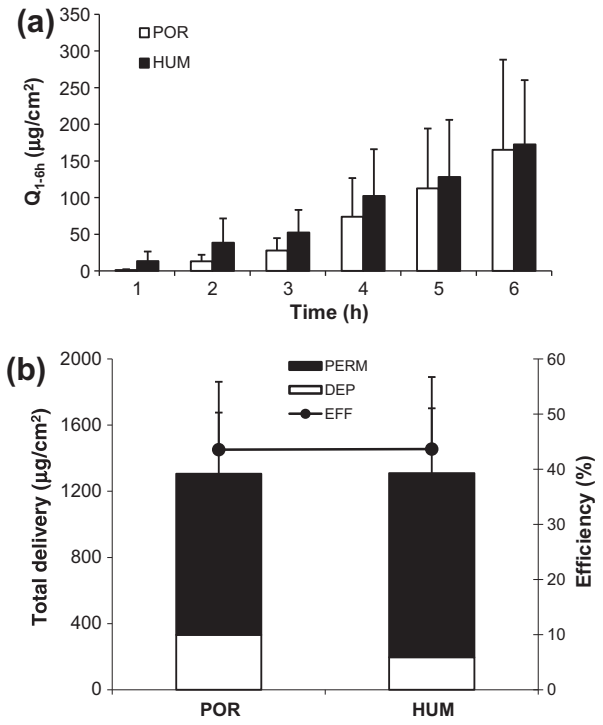

Fig. 4. (a) Comparative diclofenac permeation profile through P.L.E.A.S.E.® porated porcine and human skin during 6 h (Q_{1-6h}) (900 pores created using a fluence of 135.9 J/cm^2) (Mean ± SD; n = 6). (b) Effect of membrane type on total diclofenac delivery (sum of amounts permeated and deposited within the skin) and delivery efficiency (the fraction of the applied dose permeated or deposited) after 24 h (Mean ± SD; n = 6).

(172.6 ± 87.5 and 1112.6 ± 384.5 µg/cm^2, respectively) was statistically equivalent to that observed using porcine skin (cf. 165.3 ± 122.7 and 974.9 ± 368.8 µg/cm^2) using Student's t-test (α = 0.05). The results provide further support for the use of porcine ear as a model for transport in human tissue [24,25].

4. Discussion

4.1. Effect of poration parameters on diclofenac transport kinetics

Diclofenac transport into and across intact skin from aqueous solution was relatively poor, and significant improvements in diclofenac delivery were observed upon using laser-porated skins (Figs. 1 and 2). P.L.E.A.S.E.® poration leads to the formation of well-defined approximately cylindrical pores with a diameter of ~150 µm. The "height" of the cylinder – in other words the pore depth – is determined by the applied fluence. In addition to the cross-sectional area of the ablated stratum corneum, the cylinder walls provide permeants with facile access to the viable epidermis, and it can be envisaged that vertical diffusion in the pore is complemented by lateral entry into surrounding tissue followed by passage into the deeper skin layers. Thus, increasing the number of pores results in a corresponding increase in diclofenac transport; indeed, a linear correlation was observed between cumulative permeation in 24 h and pore number (Permeation (µg/cm^2) = 0.63 × pore no. + 41; r^2 = 0.90). Even at a relatively low fluence (22.65 J/cm^2) and hence shallow pores (50–80 µm), total delivery (sum of the amounts permeated and deposited) with 900 pores was 925.8 ± 87.0 µg/cm^2 and given the application area, the absolute amount of diclofenac delivered was 2.78 ± 0.61 mg, which corresponds to ~27.8% of the applied dose (Fig. 1b).

For the majority of molecules, transit across the stratum corneum is the rate-limiting step. Since the viable epidermis is a more favourable environment for hydrophilic molecules and presents a much smaller diffusional resistance, it might be assumed that removal of the stratum corneum alone would be sufficient to enable their rapid delivery. However, the linear dependence of cumulative permeation on fluence (and hence pore depth, Fig. 2c) implied that for diclofenac, decreasing the diffusional pathlength in the skin facilitated delivery (Fig. 2c). These results were in contrast to those seen with lidocaine, where there was no statistically significant correlation between permeation and fluence [23]. Lidocaine and diclofenac are relatively small molecules (MW 234.34 and 296.15 Da, respectively), and they have similar lipophilicities (estimated log $D_{7.4}$ of 1.2 and 0.84, respectively); thus, the causes for their different behaviours remain unclear. Since diclofenac shows greater permeation through deeper pores, where more tissue has been removed, the results suggest that diclofenac has a greater tendency to interact with structures in the epidermis or encounters greater diffusional resistance than lidocaine.

4.2. Effect of formulation conditions on diclofenac delivery

Although microporation creates new transport channels that can facilitate delivery, the formulation needs to have the appropriate rheological properties to enable efficient transfer of the drug from the vehicle to the micropore. Increased viscosity or other properties that prevent the formulation from creating an interface with the interstitial fluid released from the bulk skin into the micropore will hinder delivery. The data obtained using the 10 mg/ml diclofenac formulations in water and propylene glycol support this hypothesis. Although the thermodynamic activity of diclofenac in both formulations was similar (solubility in each case of ~20–25 mg/ml), cumulative permeation from the aqueous system was >21.3-fold higher than that from propylene glycol, which was considerably more viscous. The difference was quantified by rheological measurement, which showed that the viscosities of the propylene glycol and aqueous formulations were significantly different – 42.51 and 2.67 mPa, respectively; similar results were

Table 1
Comparison of diclofenac transport kinetics from marketed formulations into and across untreated and P.L.E.A.S.E.® porated porcine skin in 24 h (Mean ± SD; n = 5–6).

Formulation	Permeation (µg/cm^2)			Deposition (µg/cm^2)			Total delivery[e] (µg/cm^2)		
	Control[b]	Porated[c]	ER[d]	Control	Porated	ER	Control	Porated	ER
VDE[a]	14.6 ± 3	150.0 ± 28.7	10.3	28.5 ± 3.2	93.3 ± 23.8	3.3	43.1 ± 4.4	243.3 ± 37.3	5.6
FG	15.0 ± 3.3	315.8 ± 55.0	21.1	52.1 ± 21.9	181.4 ± 60.4	3.5	67.1 ± 22.1	497.2 ± 81.7	7.4
FTG	1.1 ± 1.3	5.2 ± 5.0	4.7	11.8 ± 8.8	35.8 ± 15.7	3.0	12.9 ± 8.9	41.2 ± 16.5	3.2
SG	8.2 ± 3.8	975.0 ± 368.8	118.9	8.9 ± 4.2	331.7 ± 19.8	37.3	17.1 ± 5.7	1306.7 ± 369.3	76.4

[a] VDE, Voltaren Dolo® Emugel®; FG, Flector®; FTG, Flector® Tissugel; SG, Solaraze™.
[b] Permeation or deposition in 24 h across untreated porcine skin.
[c] Permeation or deposition in 24 h across laser-porated porcine skin.
[d] Enhancement of permeation or deposition in 24 h across laser-porated porcine skin with respect to delivery across intact (untreated) skin.
[e] Total delivery represents the sum of the amounts permeated or deposited in 24 h across either laser-porated or untreated porcine skin.

recently reported concerning the effect of vehicle composition on the transdermal delivery of naltrexone hydrochloride across microneedle-treated Yucatan minipig skin *in vitro* [26].

4.3. Comparing diclofenac delivery across laser-porated skins from marketed semi-solid dosage forms

Diclofenac transport across porated skin was also significantly improved for the marketed, "off-the-shelf", semi-solid dosage forms tested (Table 1). Cumulative diclofenac permeation after 24 h across laser-porated skin from Voltaren Dolo® Emugel® (VDE; 1% w/w), Flector® (FG; 1.3% w/w) and Solaraze™ (SG; 3% w/w) was 10.3-, 21.1- and 118.9-fold superior to that seen across intact (untreated) skin. Diclofenac deposition in the skin was also increased but to a lesser extent – 3.3-, 3.5- and 37.3-fold for VDE, FG and SG, respectively, as compared to that seen with intact skin (Table 1). The concentration gradient across the skin is a key determinant of transport kinetics [27–29]. Its effect in controlling diclofenac permeation from the marketed formulations across laser-porated skin was evident (Table 1); cumulative diclofenac permeation across P.L.E.A.S.E.® porated skins after 24 h from Solaraze™ (SG; 3% w/w) was ~3-fold higher than that seen with Flector® (FG; 1.3% w/w), 975.0 ± 368.8 and 315.8 ± 55.0 μg/cm^2, respectively. Delivery efficiency from both the formulations was also high – 38 and 43%, respectively, for FG and SG. The least significant enhancing effect of laser microporation was observed for Flector® Tissugel (FTG; 1.3% w/w); in this case, cumulative permeation and deposition across porated skins after 24 h were 4.7- and 3.5-fold higher than those with untreated skin. This can be attributed to the fact that although laser poration created transport conduits in the skin, poor contact between the FTG plaster and the micropore limited drug transfer into the pore. Transport across the formulation/micropore interface will be a key issue for all "two-step" (i.e., poration followed by formulation application) microporation systems including microneedles.

4.4. Therapeutic applications and considerations

As described earlier, topical diclofenac delivery produces higher drug levels in the muscle and subcutaneous tissue than oral administration [4,5]; however, levels in synovial fluid and membrane are lower [5,6]. Moreover, it has also been reported that there is considerable inter-individual variability in the local distribution of topically delivered diclofenac in the different skin layers [30]. The results of this study show that laser microporation enables order of magnitude increases in diclofenac delivery; in addition, removal of the stratum corneum may help to reduce inter-individual variability observed in conventional passive delivery across intact skin. Extrapolation of the results reported here to *in vivo* conditions would suggest that it should be possible to achieve significantly higher drug levels in the blood and hence in the synovial fluid. This could enable the effective use of topically administered diclofenac for the treatment of arthritic conditions. The objective is to validate this hypothesis in preliminary Phase I clinical trials. Diclofenac is also used to treat actinic keratosis [31,32]; efficacy is contingent upon local bioavailability, and again, laser microporation may substantially increase drug levels in the epidermis and so improve the likelihood of successful treatment.

As with other microporation-based technologies, several issues need to be investigated and addressed in order to develop therapeutic products using the P.L.E.A.S.E.® laser system. These include patient compliance – which will probably be strongly dependent upon the risk of irritation, infection and recovery time – and the demands of the regulatory authorities. The successful completion of preliminary clinical studies into the laser-assisted delivery of triptorelin and follicle-stimulating hormone points to the potential of the technology and its acceptability [33,34]. Tolerability studies in human volunteers showed that laser microporation was well accepted by the subjects. They rated the laser poration process as causing none to slight discomfort (0–1 on a scale from 0 to 4); it induced slight to moderate local erythema and oedema, which persisted for a maximum of 4–5 days. The effect on skin barrier function and the rate of recovery was followed by transepidermal water loss measurements – 4.5- to 6.3-fold increases were observed 30 min after microporation, and they returned to baseline within 4 days. This raises the question of the frequency of patch application and whether pores can be "reused" – although the pores may indeed remain open for longer than 24 h, in terms of practicality we would probably favour a "once-a-day" patch application regimen. Another key point will be the effect of repeated use especially for the treatment of chronic conditions – in terms of both patient compliance and practicality; these will obviously have to be investigated in further clinical studies.

5. Conclusions

Laser microporation was shown to significantly increase diclofenac delivery across porcine skin; these results were subsequently confirmed using human skin. The linear dependence of diclofenac permeation on both pore number and fluence means that drug delivery can be easily modulated and so provide the clinician with a means to individualise treatment. The significant increase in delivery could not only improve existing treatments where local bioavailability following topical administration is a limiting factor but also might open the door to new therapeutic applications. Furthermore, given that NSAIDs are among the most widely used therapeutics and that mortality and morbidity associated with their use is a major public health issue, this approach offers a means to achieve significant levels of NSAID in the body while at the same time sparing the gastrointestinal tract. These aspects will be explored in future clinical studies using the P.L.E.A.S.E.® technology.

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