Precise laser poration to control drug delivery into and through human nail

Simon Vanstone[a,b], Sarah F. Cordery[b], James M. Stone[a], Sergey N. Gordeev[a,c] and Richard H. Guy[b,c,d]

[a]Department of Physics

[b]Department of Pharmacy & Pharmacology

[c]Centre for Nanoscience & Nanotechnology

University of Bath, Claverton Down, Bath, BA2 7AY, U.K.

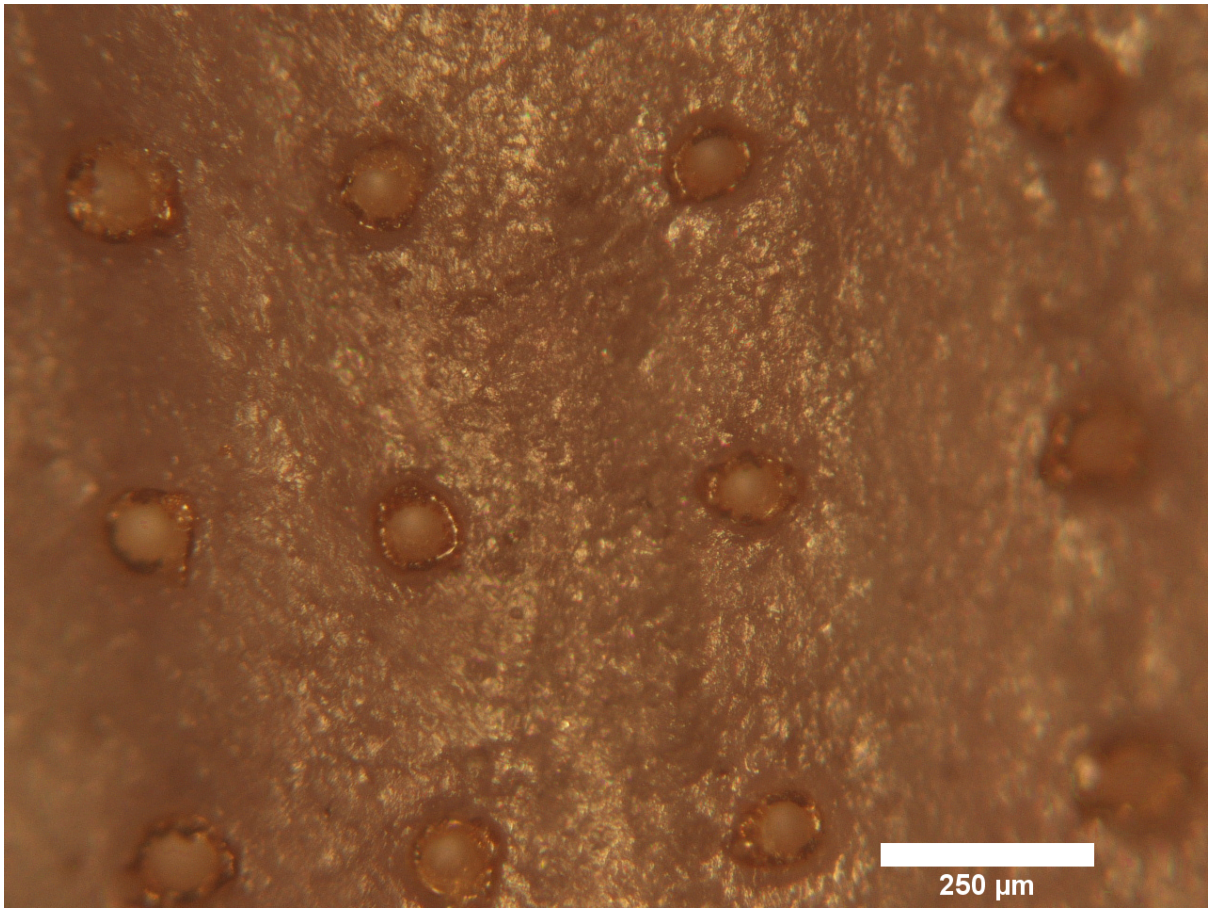
[d]Correspondence: r.h.guy@bath.ac.uk

Abstract

Drug treatment of diseases of the human nail remains a difficult challenge; topical therapy, in particular, is limited by very poor transport of active agents across the nail itself. The objective of this research was to examine the potential of controlled, and fibre-optic delivered, femtosecond laser light pulses to provide new pathways and opportunities for drug access to targets within and beneath the nail plate. Optical, confocal fluorescence and scanning electron microscopies demonstrated partial and complete laser poration of human nail samples, with the energy per pore and the exposure duration being the key modulating parameters that determined the extent of ablation achieved. Parallel measurements of the penetration of a model drug across laser-treated nails showed that complete poration resulted in essentially complete circumvention of the diffusion barrier, an array of 100 pores in 0.2 cm^2 area of nail permitting a 10^3-fold increase in initial drug uptake. Partial ablation of the nail created pores that extended to a range of depths; the nail material adjacent to the ablated area was rendered porous in appearance presumably due to local thermal perturbation of the nail structure. These openings offer, as a result, potential sites in which topical drug formulations might be sequestered post-poration and from which slow, sustained delivery of the active agent into and through the nail may be envisaged.

Keywords: human nail; laser poration; drug delivery; onychomycosis; enhanced nail permeability

Table of contents graphic



A femtosecond pulsed laser has created an array of ~50 µm diameter ‘micro-pores’ in a human nail permitting a substantial increase in topical drug delivery.

1. Introduction

Diseases of the nail, especially fungal infections (onychomycosis) and nail psoriasis, have proven notoriously difficult to treat [1]. Patient populations such as the elderly, diabetic and immunocompromised, are particularly vulnerable to long-term, unresolved nail problems [2]. Neither oral nor topical treatments of nail disease have proved very successful and have been associated with poor patient adherence. Oral drug therapy involves prolonged drug administration (typically over several months) and significant incidences of adverse drug effects and drug-drug interactions have been reported. Topical drug delivery, while in large part avoiding these issues, suffers from very low (or even no) bioavailability primarily due to the extremely limited and very slow permeation of therapeutically useful compounds into and across the nail [3]. Further, in general, topical formulations have failed either to maintain the active agent in a molecularly diffusible form post-application (because of the rapid evaporative loss of solvent from the vehicle) or to ensure a decent period of substantivity on the nail surface and allow drug to partition efficiently into the nail [4].

The attainment of effective drug concentrations in the nail bed, therefore, remains an elusive goal and has stimulated a number of attempts to disrupt nail structure so as to enhance the rate and extent of topical delivery [5]. Chemical enhancers, for example thiols, sulphites and hydrogen peroxide, have been used to destabilise keratin conformation, while physical techniques investigated include iontophoresis [6], low-frequency ultrasound (sonophoresis) [7], acid etching [8] and microporation [9, 10].

Laser poration uses a high-energy light source to create well-defined areas of ablation in a biological tissue. Pulsed lasers are typically employed as their power and duration can be modulated to carefully control the selective removal of tissue. Relatively recently, laser microporation of the stratum corneum, skin's external and principal barrier layer, has been successfully applied to the challenge of improving (trans)dermal drug delivery [11-13]. With respect to the nail, however, the literature is more limited. An earlier study demonstrated proof-of-concept with four different lasers [14] and this has been followed up with further work using millisecond-pulsed CO_2 lasers. In one case, nails were imaged post-poration with optical coherence tomography; in another, application of a topical cream to porated nails produced a positive treatment effect in nearly all patients [15, 16]. However, exposure to laser ablation was not without noticeable pain, probably because the long pulse duration caused local heating.

An approach to mitigate this issue is to use recently-developed hollow-core photonic crystal fibres to guide light from a *femto*second pulsed laser in a focused, high energy density beam [17]. When either skin or nail is irradiated in this way, it is possible to create pores of about 100 μm in diameter with minimal damage to the surrounding tissue [18, 19]. Here, therefore, the progressive poration of human nail samples has been achieved in this way, and fully characterised by different microscopic techniques. Subsequently, the degree of poration has been correlated with the permeability of the treated nail to a model, small chemical entity (specifically, caffeine). It is shown that complete poration of the nail enhances permeation by 2 to 3 orders of magnitude relative to an untreated sample, and that control of partial poration

provides a progressive modulation of barrier function that may prove useful for the development of a minimally-invasive drug delivery strategy.

2. Materials and methods

2.1 Nail samples

Nail clippings were obtained (using a protocol approved by the University of Bath Research Ethics Approval Committee for Health (EP 15/16 71)) from 3 healthy adult donors, who had given their informed consent. The samples were cleaned in cold water before being stored until used in zip-lock bags at -20^{o}C. Before laser poration, the nails were immersed for 15 minutes in a 1 mg/mL aqueous solution of Ruthenium Red (Sigma-Aldrich, Dorset UK), a dye that absorbs strongly at the wavelength of the laser used (532nm). This one-application treatment drastically reduces the poration threshold, allowing the nail to be drilled with a lower laser power and, as a result, reducing thermal damage to the surrounding tissue [18, 19]. The nails were then inspected under a light microscope (Conrad Electronic SE, Hirschau, Germany) to verify an even coverage of dye on the surface.

2.2 Laser irradiation of nails

The laser employed for optical poration of the nail (Fianium FP-1060-5-FS laser, Southampton, UK) produced 5W average power, with 200 fs pulses at a repetition rate of 80 MHz. The 1064 nm wavelength beam then passed through a lithium triborate frequency-doubling crystal to yield an output wavelength of 532 nm. A mechanical shutter controlled the exposure time. As the repetition rate of the laser pulses was fixed, an optical chopper (Thorlabs, Newton, USA) was used to provide additional control over the grouping of the pulses. Laser light was delivered to the nail via an accurately positioned hollow-core negative-curvature optical fibre (HC-NCF) [20]. An array of 100 pores was created inside a 0.2 cm^2 area of each nail using a mechanically-driven stage. The ablation regimens used for poration and the geometrical characteristics of the resulting pores produced are summarised in Table 1. The energy per pore was calculated from the average laser power and the exposure time; three nail samples were porated for each regimen.

Table 1*: Ablation regimens and pore characteristics produced (mean ± S.D.; n = 3).*

Poration regimen	**#1**	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**
Power (W)	1.0	1.3	0.5	1.3	1.0	0.5	0.25
Exposure time (s)	0.25	0.063	0.125	0.033	0.033	0.063	0.125
Energy per pore (J)	0.25	0.082	0.063	0.043	0.033	0.031	0.031
Pore diameter (µm)	146 ± 57	97 ± 20	80 ± 38	88 ± 28	70 ± 11	63 ± 27	45 ± 10
Pore depth (µm)	434 ± 52	320 ± 24	170 ± 63	118 ± 63	120 ± 48	115 ± 39	83 ± 20
Nail Thickness (µm)	434 ± 52	320 ± 24	340 ± 14	350 ± 5	365 ±42	335 ± 30	410 ± 65
% nail thickness porated	100	100	50 ± 16	33 ± 2	33 ± 4	34 ± 6	20 ± 4
% nail area porated at the surface	9.8 ± 7.1	3.8 ±0.5	2.7 ± 1.9	3.6 ± 1.2	2.0 ± 0.3	1.8 ± 1.0	0.8 ± 0.2

Optical microscopy (Leitz HM Lux, Wetzler, Germany) was used to confirm that arrays of 100 pores per 0.2 cm^2 of nail had been created, to visualise individual pores, and to measure their dimensions. The depths and diameters of the laser-ablated pores were also evaluated by laser-scanning confocal microscopy (Carl Zeiss LSCM 510 inverted microscope, Jena, Germany). A 10x objective was used and consecutive scanning was performed using 405 nm diode and 488 nm argon lasers. To obtain a 3-dimensional representation of a pore, a series of cross-sectional images acquired at regular intervals of depth into the nail were stitched together using an open-source software platform [21].

2.3 Caffeine permeation across intact and porated nails

Prior to the permeation experiments, nails were soaked in deionised water (MiliQ, Millipore, Billerica, Massachusetts) for 30 minutes to restore flexibility and to limit cracking or splitting when mounted in the diffusion cell. The nail sample separated the two halves of the side-by-side diffusion cells used (PermeGear, Inc., Bethlehem, PA, USA) and was supported by a plastic adapter (PermeGear, Inc., Bethlehem, PA, USA) which was lined with plastic foam (3M Co., St. Paul, MN, USA) to maintain a close seal and prevent any leakage. The donor and receiver chamber volumes were 3.5 mL; the former, facing the nail's outer surface, was filled with a 20 mg/mL aqueous solution of caffeine (Sigma-Aldrich, Dorset UK) the latter with phosphate-buffered saline (PBS) at pH 7.4. Once assembled, the diffusion cells were kept in an incubator at a physiological temperature (37^{o}C) for the entire experiment and the donor and receiver solutions were stirred magnetically. At 0.25, 0.5, 0.75, 1, 2, 3, 6, 24, 48, 72 hours after initiation of the study, 1mL samples of the receiver solution were removed and immediately replaced with fresh PBS. The samples were filtered (0.45 μm nylon filters from SMI- Labhut, Ltd., Maisemore, UK), and the caffeine content therein was measured by high performance liquid chromatography (Dionex P690 HPLC, Camberley, UK) using a reverse-phase column (HiQ sil C18HS, KYA Technologies, Tokyo, Japan) of 4.6 mm internal diameter and 250 mm length, and UV detection at 273 nm. The injection volume was 75 μL and the oven temperature 25^{o}C. The mobile phase (by volume) was 20% acetonitrile, 0.1% diethylamine, and 79.9% deionized water at pH 2.5. The flow rate was set at 1 mL min^{-1} and the caffeine retention time was 5.7 minutes.

At the end of the permeation experiments, nail samples were microtomed (Reichert-Jung Ultracut E Microtome, Nussloch, Germany) to reveal cross sectional planes through the interior of the pores. The samples were sputter-coated with a 2–5 nm layer of gold (Edwards S150B Sputter-Coater, Burgess Hill, UK) and were examined by scanning electron microscopy (JEOL JSM-6480 SEM, Peabody, USA) to observe the overall structure and, at higher resolution, the finer detail at the pore edges.

3. Results

Laser poration of the nail was achieved to different depths by varying the total energy delivered. In detailed experiments reported elsewhere [19], poration regimens combining high power and short exposure time have been shown to produce pores with the least surrounding thermal damage. For regimens #1 and #2, the exposed nails were fully porated; reducing the power and/or the exposure time in the other regimens resulted in nails partially porated to different extents. The dye (Ruthenium Red) is needed to increase absorption of the laser radiation and thus initiate ablation of the nail tissue at the very beginning of the poration process. All dye molecules rapidly evaporate from the pore and do not play any further role in the poration process which, itself, continues to propagate. This is because the laser radiation is absorbed at the bottom of the pore by thermally damaged proteins which play the role of the dye in the continuing poration process [19].

The pores are barely visible to the naked eye, with only the largest appearing as dots on the surface. Optical microscopy reveals more details (Fig. 1). A full array of pores created with regimen #1 in a nail from one donor is shown in Fig. 1(a); in this case, the nail was fully ablated and the pores were even visible without magnification. In Fig. 1(b), another nail sample from the same donor was treated with regimen #7, the lower power and shorter time used resulting in smaller pores and less damage around them. Fig. 1(c) illustrates the complete poration of a nail from a different donor following exposure to regimen #2; the white areas in the centre of the pores reflect the colour of the background support on which the nail has been placed; note that, due to the curvature of the nails and large aspect ratio of the pores (on the order of 100 μm in diameter and 300-400 μm in depth), it was not possible to see the background support in all cases). Finally, Fig. 1(d) shows a single pore in a nail from a third donor after application of regimen #3.

For a fixed power of the laser beam, the pore diameter decreased with decreasing energy delivered (or, in other words, with decreasing exposure time). At the same time, if the beam power increased (for a fixed exposure time or fixed energy delivered), the pore diameter also increased. The beam power for regimen #4 (1.3 W) is significantly larger than that for regimen #3 (0.5 W). This explains the difference in pore diameter and why that from #4 is larger than #3. Similarly, for #6, the beam power is two times larger than that for #7 and, as a result, the pore diameter is larger.

After laser ablation, the interior of the pores auto-fluoresced strongly (produced by thermally damaged proteins at the pore surface and in the thin layer of nail tissue surrounding the pore) enabling laser scanning confocal microscopy (Fig. 2) to be used to delineate clearly the pore edges and to allow their depth to be accurately measured (as well as the % thickness porated of the nail). Fig. 2(a) is an x-y image of the opening of a complete pore through the nail of one donor post-exposure to regimen #1. In Fig. 2(b), a reconstructed x-z image of a partially porated nail from a different donor after treatment with regimen #3 is illustrated; the pore extends to a depth of 250 μm, corresponding to 71% of the total nail thickness. In general, when nails were fully ablated, the pores were essentially cylindrical in shape; in contrast, when the nail was only partially ‘drilled’ the pores were typically conical (e.g., Fig. 2(b)).

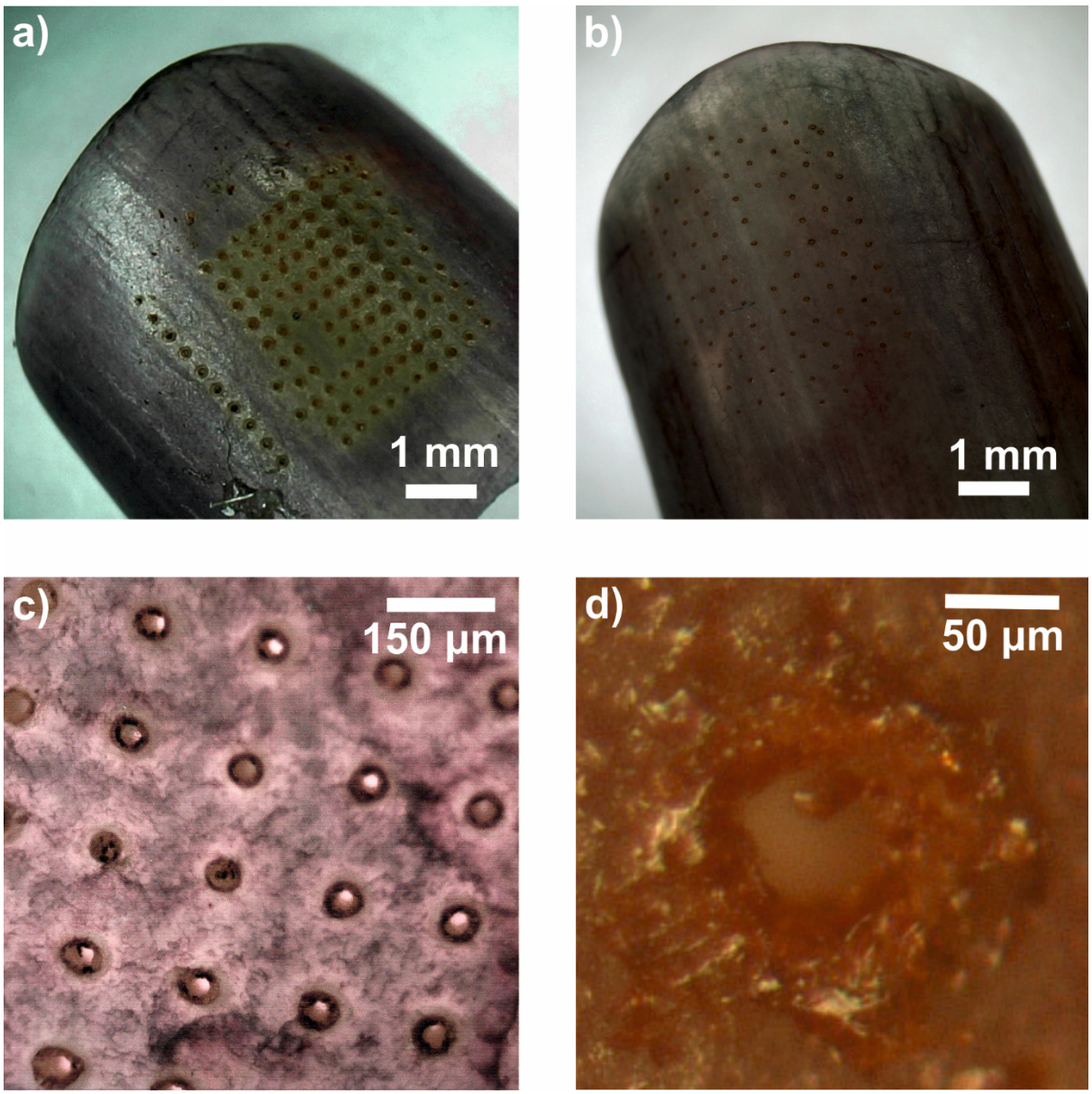


Fig. 1. *(a) Optical microscope image of a fully laser-ablated array of pores on the surface in a nail sample treated with regimen #1 (Table 1). The nails appear dark due to staining with Ruthenium Red. (b) Optical microscope image of a laser-ablated array of pores on the surface in a nail sample treated with regimen #7 (Table 1). (c) Higher magnification image of a fully-porated nail from a different volunteer treated with regimen #2. (d) High magnification image of a single (partial) pore in a nail sample subjected to regimen #3 (Table 1).*

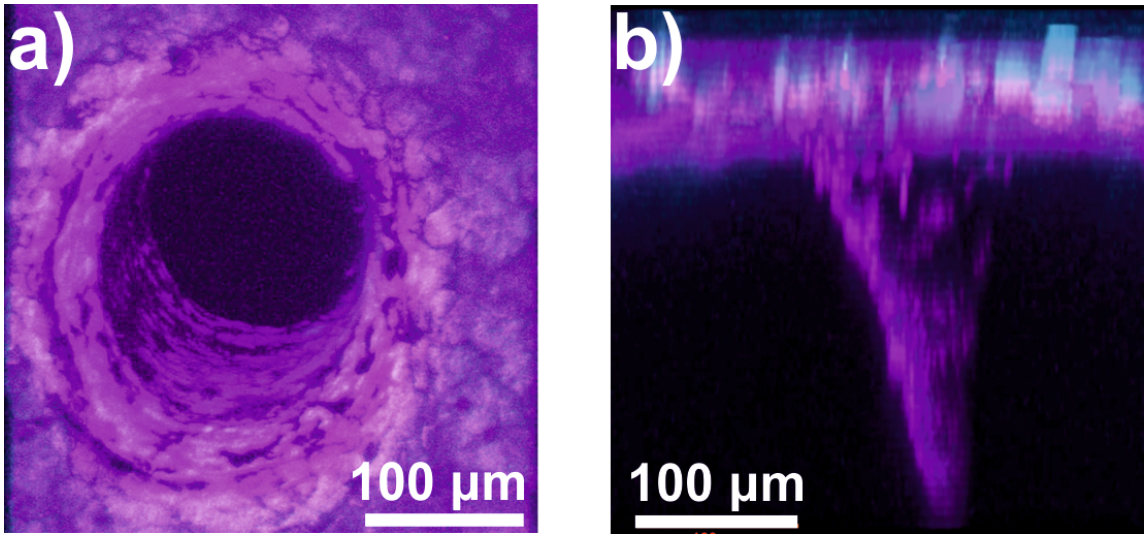


Fig. 2. *Laser scanning confocal microscope images of (a) the pore opening of a fully ablated nail sample after treatment with regimen #1, and (b) a reconstructed cross-section through a partial pore post-exposure to regimen #3.*

Comparison of the permeation of caffeine across intact and laser-porated nails revealed the dramatic potential impact of tissue ablation. Fig. 3 shows the cumulative transport of caffeine as a function of time across nails divided into four broad categories: (i) untreated controls (intact), (ii) porated between 15% and 40% of total thickness, (iii) porated between 40% and 70% of total thickness, and (iv) completely porated. The data are shown on a double-logarithmic axis to emphasise the manner in which complete poration of the nail greatly enhances both the rate and extent of caffeine transport. The cumulative penetration of caffeine across untreated nails amounts to less than 100 µg in 72 hours, consistent with that observed for similar small organic compounds reported in the literature [22]. In contrast, when the nail was fully porated, the initial penetration rate of caffeine was on the order of 1000-fold faster, and total delivery over 3 days was enhanced by more than 100 times. Partial poration to the extent of 40-70% of total nail thickness led to steady-state transport being achieved within 6 hours; the smallest level of poration (15-40% of total nail thickness) resulted in penetration profiles not dissimilar to those of the untreated controls with constant fluxes attained approximately 24 hours after initiation of the experiment (Fig. 4).

Finally, scanning electron microscopy was used to examine the finer structural details of the pores and the nail immediately surrounding the ablated area (Fig. 5). Fig. 5(a) shows an overview of a pore drilled with regimen #1. Fig. 5(b), is a higher magnification capture showing part of the pore edge inside the red box in a). In Fig. 5(c), a partial pore created with regimen #7 is illustrated, revealing the typical conical shape. It can be seen that the nail next to the (smooth grey) ablated region has been rendered porous with apparent openings of several micrometres in diameter extending away from the pore itself. These openings are larger in the samples drilled with the greater energies per pore.

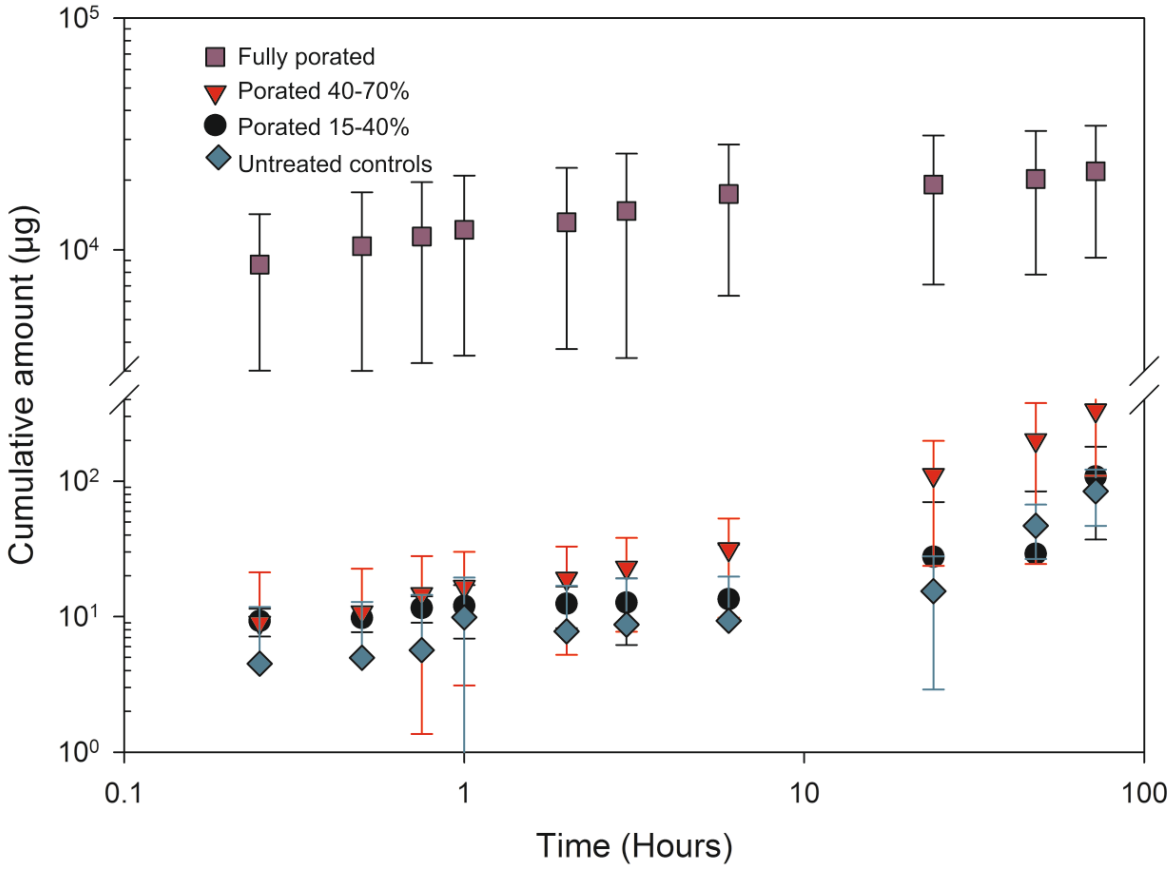


Fig. 3. *Cumulative caffeine penetration as a function of time across human nail samples. The results have been grouped by the degree of laser ablation achieved, specifically, (i) fully porated (n = 5), (ii) porated to 40-70% of the total nail thickness (n = 3), (iii) porated to 15-40% of the total nail thickness (n = 10), and (iv) untreated controls (n = 4).*

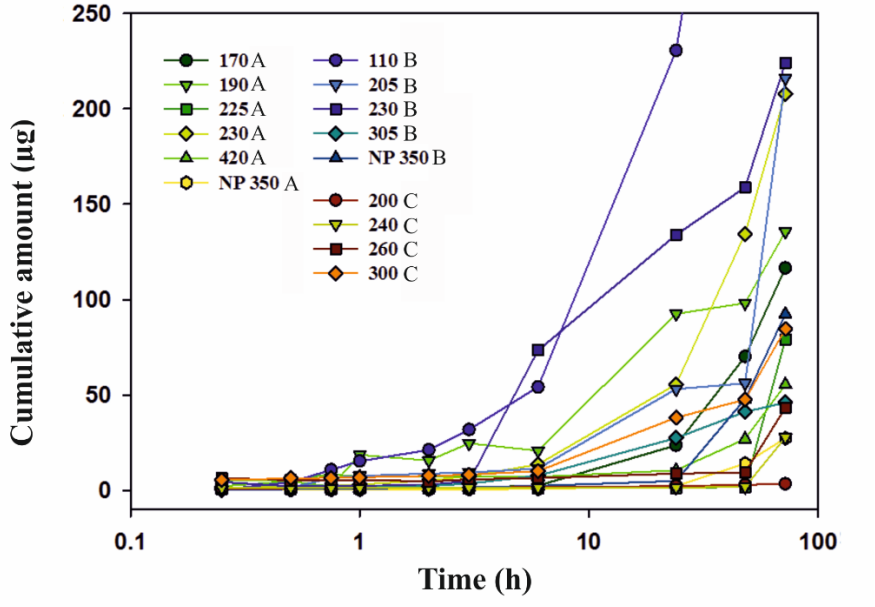


Fig. 4. *Cumulative permeation of caffeine as a function of time across partially porated nails. The legend identifies the thickness of the nail (in µm) remaining from the base of the pore and the nail donor (A, B, C).*

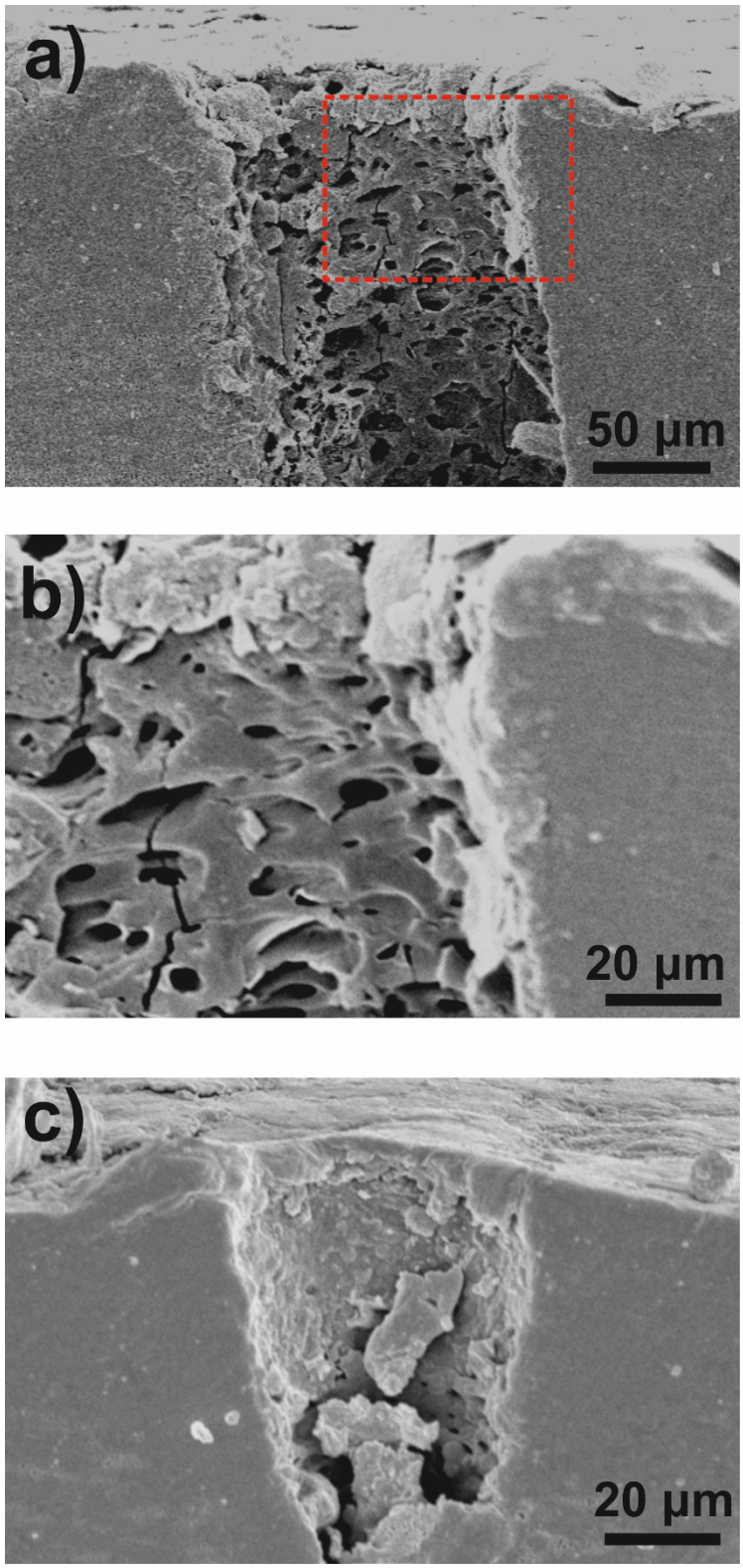


Fig. 5. *Scanning electron micrographs of microtomed nail sections after poration showing the porous appearance of nail structure adjacent to the ablation regime. (a) Pore drilled with*

regimen #1; (b) higher magnification micrograph captured inside the red box in (a); and (c) pore drilled with regimen #7.

4. Discussion

Most obviously, the results of this study demonstrate that full poration of nails with an array of new, laser-drilled pathways completely circumvents the barrier provided by an intact, untreated sample. Consequently, a very rapid permeation of caffeine is observed (Table 2). As the pores created in the nail can be reasonably anticipated to instantaneously fill with water, the classic lag-time (T_{lag}) for caffeine diffusion across the treated nail is given by:

$$T_{lag} = L^2/6D_{aq} \quad (1)$$

where D_{aq} is the diffusion coefficient of caffeine in water and L is the nail thickness. If it is assumed that, relative to the transport of the permeant via the pores, diffusion across the nail itself can, to a first approximation, be ignored, then, given that the aqueous diffusivity of caffeine is on the order of 10^{-5} cm^2s^{-1} [23], it follows that, for a nail with L = 400 μm (= 0.04 cm):

$T_{lag} = (0.04\ cm)^2/\{6 \times 10^{-5}\ cm^2s^{-1}\}\ s \approx 30\ s = 0.5\ minute$

In other words, across fully porated nails, a very short lag-time would be anticipated, and attainment of steady-state transport (which requires approximately $2.7 \times T_{lag}$) across the porated nail would be achieved within less than two minutes [24].

Table 2: *Penetration of caffeine (mean ± S.D.; n = 5) across fully porated nails as a function of time.*

Time (h)	**0.25**	**0.50**	**0.75**	**1**	**2**	**3**	**6**	**24**	**48**	**72**
Cumulative caffeine penetration (mg)	8.6 ± 5.6	10 ± 7.4	11 ± 8.2	12 ± 8.7	13 ± 9.4	14 ± 10	15 ± 11	17 ± 12	18 ± 12	20 ± 13
% permeation of caffeine dose applied	12 ± 8.0	14 ± 11	16 ± 12	17 ± 12	19 ± 13	20 ± 15	22 ± 15	24 ± 17	26 ± 17	28 ± 18
Caffeine permeation normalised by porated nail area and inverse thickness (μg $μm^{-1}$)	2.4 ± 0.46	2.8± 0.42	3.1 ± 0.43	3.3 ± 0.42	3.6 ± 0.43	3.9 ± 0.44	4.2 ± 0.43	4.7 ± 0.41	5.0 ± 0.39	5.6 ± 0.35

Because, steady-state transport is likely to occur so rapidly, Fick's 1st law of diffusion (i.e., a constant rate of transport assuming an effectively infinite applied 'dose' and sink conditions on the 'downside' of the nail) can be used to predict the cumulative penetration of caffeine (Q_T) over the experimental duration (T); that is:

$$Q_T = (a \times A) \times (D/L) \times C_0 \times T \quad (2)$$

In the case of the nails subjected to the fully-porating regimen #1 (Table 1), the average fraction (a) of the total nail surface area (A = 0.2 cm^2) porated was 0.098; caffeine diffusivity (D) is

given above [23] and the mean value of L was 0.0432 cm (= 432 μm); the 'driving' caffeine concentration (C_0) on the nail surface was 20 mg cm^{-3}, and T was 72 hr (= 259,200 s). Hence,

$$Q_{72h} = (0.098 \times 0.2) \times (10^{-5}/0.0432) \times 20 \times (72 \times 3600) \text{ mg} = 23.5 \text{ mg}$$

a value completely consistent with the experimentally observed result (20 ± 13 mg) reported in Table 2. Nevertheless, it should be noted that the initial penetration of caffeine is particularly rapid, suggesting that there is an element of a somewhat classic 'burst' effect occurring early on, presumably because the pores are filled so quickly with the drug solution from the donor.

The data in Fig. 4 re-plots the majority of the data in Fig. 3 for caffeine penetration across the partially porated nails; in this case, however, the cumulative transport is presented on a linear (as opposed to logarithmic) scale. It is clearly apparent that the corresponding lag-times are on the order of 10 hours, i.e., about 1000-fold greater than that across fully-porated nails, and reflecting the fact that diffusion through the remaining nail, which is evidently orders of magnitude slower than that in water, is controlling caffeine permeation.

The relatively high variability in the amount of caffeine delivered is attributed in part to the variation in thickness of the nail samples examined (Table 1). There was also variation in the total porated area of each nail (Table 1), reflecting the effect of the curvature of the samples; that is, not all pores in the array had the same diameter because the distance between the optical fibre tip and the surface of the nail was not constant [19] . The beam diameter increases with the distance from the fibre tip to the nail surface, meaning that a highly curved nail had a larger percentage of its area porated on exposure to the laser beam. The important contributions of nail thickness and porated area variability may be appreciated by the manner in which normalisation of cumulative caffeine penetration by these factors reduces considerably the coefficient of variation observed in the results (Table 2).

The variability is also apparent in the evolution of the cumulative caffeine permeation as a function of time (t) (see Fig. 3). Fig. 4 reinforces this point and plots the cumulative penetration of the chemical (Q) across partially porated nail samples from three volunteers. The profiles are consistent with diffusion across a plane sheet from an infinite source [24] and reveal clear lag-times that shorten as the thickness of the nail remaining post-poration (L) decreases. Mathematically, the profiles can be modelled with Eq. (3):

$$Q = L \cdot C_0 \left[\frac{D \cdot t}{L^2} - \frac{1}{6} - \frac{2}{\pi^2} \sum_{n=1}^{\infty} \frac{(-1)^n}{n^2} exp\left(\frac{-n^2 \cdot \pi^2 \cdot D \cdot t}{L^2}\right)\right] \quad (3)$$

As before, C_0 is the concentration of caffeine applied to the nail surface and D is its diffusivity through the nail. The variability of the dependency of Q on time (a) between nails from different donors (of different total thicknesses), and (b) between different remaining partial thicknesses post-poration, is self-evident. However, from Eq. (3), it is clear that dividing time by the remaining nail thickness squared can normalise, at least in part, the variability observed, as shown in the main panel of Fig. 6. Indeed, when the normalisation is performed on partially porated nails from a single donor (B), the resulting profiles effectively 'collapse' onto a single curve (inset of Fig. 6). The inability of the normalisation approach to completely eliminate variability is undoubtedly due to a number of factors, not the least of which are (i) differences in caffeine diffusivity across nails from different donors (or even different digits of the same

donor), and (ii) the fact that the partial pores are conical in shape (Figure 2(b)), not rectangular as we have assumed in using the simplified Eq. (3).

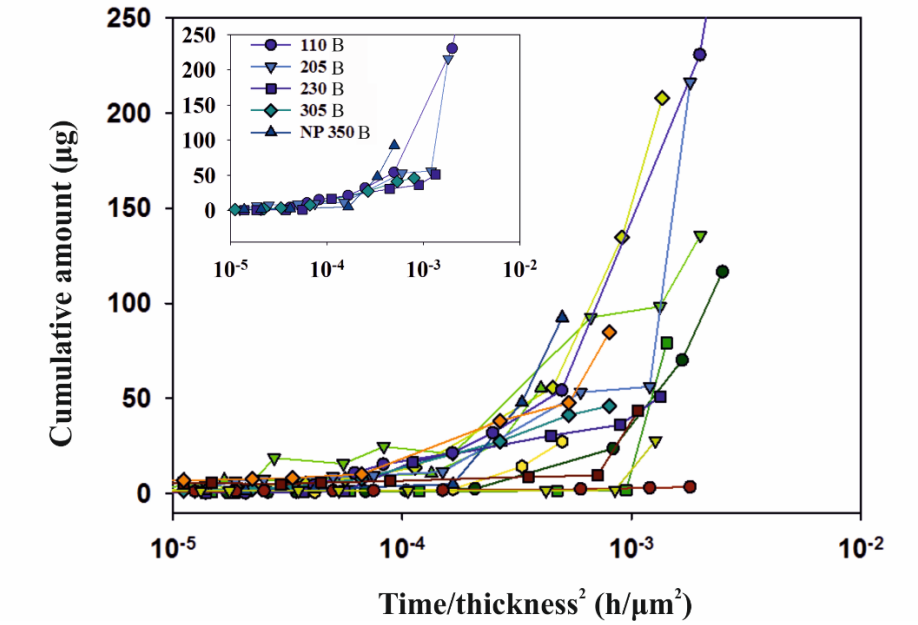


Fig. 6. *The data in Fig. 4 normalised by the squared nail thickness remaining; inset: the results obtained specifically from the porated nails of a single volunteer (B).*

The partially porated nails shorten the time required for caffeine permeation to reach an apparent steady-state but they retain a decent level of barrier function. It seems that poration to a depth of at least 40% of the total nail thickness is necessary to significantly improve caffeine penetration over that across an intact, untreated nail (Fig. 3). However, from a sustained drug delivery standpoint, an array of partial pores offers an opportunity for sequestration of a drug formulation, from which slow, continuous release might be envisaged [25] (perhaps using a varnish to seal off the pore openings at the surface once the drug formulation has been applied). The scanning electron microscopy images in Fig. 5 also indicate that drug release from formulation 'reservoirs' in the partial pores may be enhanced by the evidently open, porous nature of the nail structure surrounding the ablated areas. It follows that lateral diffusion of drug into the nail may be anticipated (as has been observed in other experiments not involving laser poration [25]), a useful feature, perhaps, for dealing with infecting organisms present within the nail plate itself.

With respect to potential, future therapeutic applications, it is reason able to ask why caffeine was used in this work rather than (for example) a drug used to treat nail disease, such as onychomycosis? The simple answer is that, for the purpose of this research, caffeine represents a convenient 'model' compound, for which a sensitive and straightforward assay method was available. Further, as there was no intention in this study to demonstrate therapeutic efficacy associated with improved drug delivery, there was no *a priori* reason to select a compound that is used clinically. In addition, and perhaps most importantly, it is evident from the results obtained in this investigation that the poration technology creates, across the nail, new

pathways, the size of which, relative to the physical dimensions of any small molecular weight drug, are many orders of magnitude greater. There is no risk, therefore, in extrapolating the very substantial increases in caffeine permeation observed to any drug used currently in treating nail disease topically.

The fibre laser system described provides a low-cost, easy-to-use, and compact light source when compared to others reported in the literature [14-16]. Although the frequency doubling optics used in this work were external to the laser system, a simple turnkey system incorporating doubling optics is a commercially available option that allows the set-up to be further simplified. The use of an optical fibre for pulse delivery allows easy, flexible alignment with the nail target that could be incorporated into a small, ergonomic laser delivery head separate from the laser unit for use with patients.

The ablation wavelength of 532 nm may have the additional advantage over other reported nail poration systems of not being tuned to the strong OH absorption peak (primarily from water). The water content of the nail plate (7-25%) is low compared to that of the nail bed (which is the same as that of the viable epidermis (70%)) [26, 27]. Using lasers for nail poration based on the strong water absorption band around 3 μm is therefore likely to cause significant damage to the nail bed once the pore has been drilled through the nail. This is likely to be a serious practical consideration given the varying thickness of nails from patient to patient. However, such a concern would be circumvented with poration via the 532 nm ablation wavelength used in the work described here and additional damage to the nail bed should therefore be minimal.

5. Conclusion

The optical fibre delivery of femtosecond laser radiation pulses described here offers a highly controllable approach to the poration of human nail for enhanced drug treatment. Complete poration of the nail enables orders-of-magnitude increases in the transport of a model drug to the underside of the nail plate (to the nail bed, in other words). Partial poration, on the other hand, while not provoking such substantial effects on drug delivery, offers a strategy by which prolonged and controlled drug input to the nail itself, as well as the nail bed, may be achieved from sequestered formulations confined to the pores after their creation. Further work is now required to improve the reliability and repeatability of pore formation, ensuring that the variability of pore depths across an array is better controlled (for example, by design of a system to keep the distance between the tip of the optical fibre and the outer nail surface as constant as possible). In addition, the degree to which complete laser poration of the nail elicits local sensation – and how this might be mitigated – require additional investigation.

Authors' Contributions

SV performed the poration and microscopy experiments, which were set up by JS. Measurements of drug delivery across intact and porated nails were made by SV and SC. All co-authors participated in the analysis and interpretation of the data, and in the preparation of the paper. SG and RG were primarily responsible for the study concept and design.

Acknowledgements

We thank Fei Yu for fabrication of the negative curvature hollow core fibre, Begoña Delgado-Charro for helpful discussion of the results, Fianium for loan of the femtosecond pulsed laser, and EPSRC for financial support in the form of a PhD studentship. James Stone is supported by the EPSRC under the Proteus IRC (EP/K03197X/1)

References

[1] M. Lawry, P. Rich, The nail apparatus: A guide for basic and clinical science, Current Problems in Dermatology, 11 (1999) 161-208.

[2] J. Thomas, G.A. Jacobson, C.K. Narkowicz, G.M. Peterson, H. Burnet, C. Sharpe, Toenail onychomycosis: an important global disease burden, J. Clin. Pharm. Ther., 35 (2010) 497-519.

[3] M.V. Saner, A.D. Kulkarni, C.V. Pardeshi, Insights into drug delivery across the nail plate barrier, J. Drug Target., 22 (2014) 769-789.

[4] J.C.T. Wang, Y. Sun, Human nail and its topical treatment: Brief review of current research and development of topical antifungal drug delivery for onychomycosis treatment, J. Cosmet. Sci., 50 (1999) 71-76.

[5] S. Murdan, Enhancing the nail permeability of topically applied drugs, Expert Opin. Drug Deliv., 5 (2008) 1267-1282.

[6] M.B. Delgado-Charro, Iontophoretic drug delivery across the nail, Expert Opin. Drug Deliv., 9 (2012) 91-103.

[7] D. Abadi, V. Zderic, Ultrasound-Mediated Nail Drug Delivery System, J. Ultrasound Med., 30 (2011) 1723-1730.

[8] M.A. Repka, P.K. Mididoddi, S.P. Stodghill, Influence of human nail etching for the assessment of topical onychomycosis therapies, Int. J. Pharm., 282 (2004) 95-106.

[9] D. Ciocon, T. Gowrishankar, T. Herndon, A.B. Kimball, How low should you go: Novel device for nail trephination, Dermatol. Surg., 32 (2006) 828-833.

[10] A. Boker, D. Cioccon, A. Kimball, A randomized, double-blind, placebo-controlled, pilot study of 1% terbinafine cream applied twice daily and delivered via nail plate microporation for the treatment of subungual toenail onychomycosis, J. Am. Acad. Dermatol., 56 (2007) Ab114-Ab114.

[11] Y.G. Bachhav, A. Heinrich, Y.N. Kalia, Controlled intra- and transdermal protein delivery using a minimally invasive Erbium:YAG fractional laser ablation technology, Eur. J. Pharm. Biopharm., 84 (2013) 355-364.

[12] J.Y. Fang, W.R. Lee, S.C. Shen, H.Y. Wang, C.L. Fang, C.H. Hu, Transdermal delivery of macromolecules by erbium : YAG laser, J. Control. Release, 100 (2004) 75-85.

[13] S.L. Jacques, D.J. McAuliffe, I.H. Blank, J.A. Parrish, Controlled removal of human stratum-corneum by pulsed laser, J. Invest. Dermatol., 88 (1987) 88-93.

[14] J. Neev, J.S. Nelson, M. Critelli, J.L. McCullough, E. Cheung, W.A. Carrasco, A.M. Rubenchik, L.B. DaSilva, M.D. Perry, B.C. Stuart, Ablation of human nail by pulsed lasers, Lasers Surg. Med., 21 (1997) 186-192.

[15] C.H. Yang, M.T. Tsai, S.C. Shen, C.Y. Ng, S.M. Jung, Feasibility of ablative fractional laser-assisted drug delivery with optical coherence tomography, Biomed. Opt. Express, 5 (2014) 3949-3959.

[16] E.H. Lim, H.R. Kim, Y.O. Park, Y. Lee, Y.J. Seo, C.D. Kim, J.H. Lee, M. Im, Toenail onychomycosis treated with a fractional carbon-dioxide laser and topical antifungal cream, J. Am. Acad. Dermatol., 70 (2014) 918-923.

[17] F. Yu, J.C. Knight, Negative Curvature Hollow-Core Optical Fiber, IEEE J. Sel. Top. Quantum Electron., 22 (2016) 10.

[18] H. Garvie-Cook, J.M. Stone, F. Yu, R.H. Guy, S.N. Gordeev, Femtosecond pulsed laser ablation to enhance drug delivery across the skin, J. Biophotonics, 9 (2016) 144-154.

[19] S.J. Vanstone, J.M. Stone, R.H. Guy, S.N. Gordeev, Thermal mapping of the human nail by Raman microspectroscopy following poration by femtosecond pulsed laser ablation, Phys. Rev. Applied, (Submitted for publication).

[20] P. Jaworski, F. Yu, R.R.J. Maier, W.J. Wadsworth, J.C. Knight, J.D. Shephard, D.P. Hand, Picosecond and nanosecond pulse delivery through a hollow-core Negative Curvature Fiber for micro-machining applications, Opt. Express, 21 (2013) 22742-22753.

[21] J. Schindelin, I. Arganda-Carreras, E. Frise, V. Kaynig, M. Longair, T. Pietzsch, S. Preibisch, C. Rueden, S. Saalfeld, B. Schmid, Fiji: an open-source platform for biological-image analysis, Nature methods, 9 (2012) 676-682.

[22] Y. Kobayashi, T. Komatsu, M. Sumi, S. Numajiri, M. Miyamoto, D. Kobayashi, K. Sugibayashi, Y. Morimoto, In vitro permeation of several drugs through the human nail plate: relationship between physicochemical properties and nail permeability of drugs, Eur. J. Pharm. Sci., 21 (2004) 471-477.

[23] W.E. Price, Tracer caffeine diffusion in aqueous solutions at 298K, J. Chem. Soc. Faraday Trans. I, 85 (1989) 415-419.

[24] J. Crank, The mathematics of diffusion, Clarendon Press, 1975.

[25] W.S. Chiu, N.A. Belsey, N.L. Garrett, J. Moger, G.J. Price, M.B. Delgado-Charro, R.H. Guy, Drug delivery into microneedle-porated nails from nanoparticle reservoirs, J. Control. Release, 220 (2015) 98-106.

[26] T.M. Cortazar, M. Guzman-Alonso, H. Novoa, M. Riano, Comparative study of temporary effect on the water content at different depths of the skin by hot and cold moisturizing formulations, Skin Res. Technol., 21 (2015) 265-271.

[27] M. Egawa, Y. Ozaki, M. Takahashi, In vivo measurement of water content of the fingernail and its seasonal change, Skin Res. Technol., 12 (2006) 126-132.