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Research Article

Enhanced Topical Uptake of Ascorbic Acid in Fractional Photothermolysis-Treated *ex vivo* Human Skin

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Abstract

Fractional photothermolys (FP) is an innovative approach to non-ablative laser skin resurfacing that does not mechanically breach the stratum corneum barrier. Previous studies demonstrated fractional photothermolysis reduced fine wrinkles on the face. Ascorbic acid is a potent antioxidant capable of reducing ultraviolet-induced skin damage. The goal of this study was to assess the impact of fractional irradiation of *ex vivo* human skin on ascorbic acid permeation as well as the influence of contact versus non-contact laser handpiece tips. Pretreatment of *ex vivo* skin with contact mode FP resulted in a 7-fold increase in ascorbic acid uptake relative to control with permeation enhancement increasing as a function of spot density. The stratum corneum remained intact as evidenced by hematoxylin and eosin staining. A 17-fold increase in ascorbic acid uptake was observed in the non-contact mode. It is hypothesized that suprathreshold temperatures achieved during FP treatment disrupted intercellular bonds in the epidermis and altered stratum corneum ultrastructure while preserving its barrier function. Since healing is rapid post-FP, synergistic active ingredients capable of enhancing clinical outcomes post-laser therapy must be delivered during a defined temporal window. This report demonstrates a significant enhancement of topical ascorbic acid uptake by mechanical disruption of the stratum corneum using an infrared non-ablative laser and may serve as a potential avenue to improve clinical outcomes.

Abbreviations

FP: Fractional Photothermolysis; H&E: Hematoxylin and Eosin: HPLC, High Performance Liquid Chromatography, MTZ: Micro Thermal Zone, UV: Ultraviolet

Introduction

Photoaging is comprised of numerous clinical signs including dyschromia, rhytids, lentigines, textural changes, and telengiectasia [1]. An increased risk of solar keratoses and nonmelanoma skin cancer results from long-term exposure to sunlight [2]. Resurfacing lasers and topical prescriptions have been used to treat and prevent both conditions [1,3]. The anti-oxidant activity of vitamin C (ascorbic acid) can reduce the impact of ultraviolet (UV) induced damage [3,4]. Study participants that received a 5-day pretreatment with topical L-ascorbic acid 10% solution exhibited reduced minimal erythema does following UVB irradiation [1].

Non-ablative lasers, especially those employing fractional photothermolysis (FP) [5], are quickly becoming the preferred modality for skin resurfacing to achieve photorejuvenation [6]. FP couples with water as its chromophore and limits its density to ~10-40% of the target skin surface area [7], but still results in exfoliation of even the untreated skin [5,8]. This combination affords faster healing times and decreased side effects while maintaining equivalent levels of clinical efficacy as other non-tissue sparing non-ablative lasers [9,10]. Treatment with ablative lasers, such carbon dioxide and Erbium: YAG, or microdermabrasion devices enhances ascorbic acid skin uptake but only by breaching the stratum corneum [11] leading to increased risk of bleeding and infection [12,13].

This study aimed to assess the impact of FP treatment on topical ascorbic acid uptake and stratum corneum integrity.

Materials and Methods

Laser parameters

An Institutional Review Board approved the study protocol and each participant was consented prior to study initiation. Using freshly excised human abdominal skin (Fitzpatrick skin type II), *ex vivo* laser treatments were performed via a 1550 nm Fraxel® SR laser system (Reliant Technologies Inc., Mountain View, CA). The 1550 nm Fraxel® SR laser system was operated as described previously [14]. Arrays of single mode Gaussian beams of 60 μm $1/e^2$ diameter at incidence were delivered to the surface of

each specimen in contact and non-contact mode. The contact tip abutted the specimen through a sapphire window through which the laser beam was irradiated. The non-contact tip omitted the sapphire window; thus, the laser incidence occurred at the air-tissue interface. Laser pulse energies tested for ascorbic acid uptake measurements were 10 and 20 mJ. For each treatment, 4 passes at 250 microthermal zones (MTZs) per cm^2 were made at a constant velocity of 1.0 cm per second producing a final spot density of 1000 MTZs per cm^2. Histologic examination was performed for pulse energies ranging from 6 to 40 mJ. The pulse durations ranged from 0.5 to 3.2 ms per pulse.

Histologic examination

Each specimen was trimmed to a size of 10 mm × 60 mm, warmed in between saline soaked gauze pads on a digital hot plate (Cole-Parmer Instrument Co., Vernon Hills, IL) and upon reaching 98 ± 3 °F surface temperature, exposed to predetermined laser parameters after removing the top layer of gauze, then cut into smaller pieces and fixed in 10% v/v neutral buffered formalin (VWR International, West Chester, PA) overnight in preparation for paraffin embedding. Once sectioned and stained with hematoxylin and eosin (H&E), samples were imaged using a DM LM/P microscope and a DFC320 digital camera (Leica Microsystem, Cambridge, UK). A proprietary Visual Basic computer program (Reliant Technologies, Inc., Mountain View, CA) was used to measure lesion dimensions for at least 20-25 MTZs per treatment parameter.

Ascorbic acid formulation

Topical vitamin C solution contained L-ascorbic acid 15% (VWR International, West Chester, PA), ferulic acid 0.5%, and vitamin E 1% buffered to a pH of 3.2 ± 0.2 with triethanolamine [15]. Ascorbic acid was freshly prepared avoiding light exposure just prior to each experiment.

Ascorbic acid permeation studies

Uptake measurements were conducted using skin permeation systems (LGA, Inc., Berkeley, CA) deployed with freshly excised human abdominal skin of 500 μm thickness. Both laser-treated and untreated skin samples were then mounted on a permeation system which contained ascorbic acid solution in the donor compartment [11]. Aliquots were drawn at 5, 10, 15, 30, 60, and 90 min, and subjected to HPLC analysis. After 90 min, samples were saline rinsed, weighed, homogenized, centrifuged [11], then subjected to HPLC analysis to determine the retained ascorbic acid value. The effective area of skin through which permeation occurred was used to normalize the measured retention component.

Data analysis

Total uptake comprised the sum of permeated and retained ascorbic acid values normalized to tissue weight. Temporal permeation was measured and plotted as a cumulative value. Uptake enhancement ratio was calculated by dividing the total ascorbic acid uptake for laser treated skin by the total uptake for untreated skin at 90 min. A student's t-test (Microsoft Excel, Microsoft, Seattle, WA) was used to assess statistical significance with P-values of ≤ 0.05 taken as significant.

Results

Effect of contact mode laser treatment on ascorbic acid permeation

To assess the effect of the 1550 nm Fraxel® SR laser system on ascorbic acid permeation, a 60 μm incidence microbeam spot size was used to treat *ex vivo* human abdominal skin. Gross inspection of the skin post-laser treatment demonstrated no obvious structural changes.

(Figure 1) demonstrates the HPLC derived standard curve for ascorbic acid values spanning 0 to 19 μg with a strong correlation coefficient. The cumulative permeation of ascorbic acid as a function of time is shown in (Figure 2). Ascorbic acid content of *ex vivo* skin prior to topical application was undetectable by HPLC. Non-irradiated *ex vivo* skin demonstrated no permeation up to 90 min after application as measured by HPLC. However, under these conditions, 0.10 ± 0.01 mg of ascorbic acid was found in the retained fraction (Table 1). Total uptake for non-laser treated skin was 1.17 ± 0.11 mg of ascorbic acid per gram of tissue. In sharp contrast, skin treated at pulse energy of 20 mJ in the contact mode demonstrated permeation within 5 min (Figure 2). By 90 min, 0.24 ±0.02 mg of ascorbic acid permeated the chamber compared to 0 mg for untreated skin. No significant difference was observed ($p > 0.1$) between laser treated (0.11 ±0.02 mg) and untreated skin (0.10 ± 0.01 mg) in terms of retained fraction content.

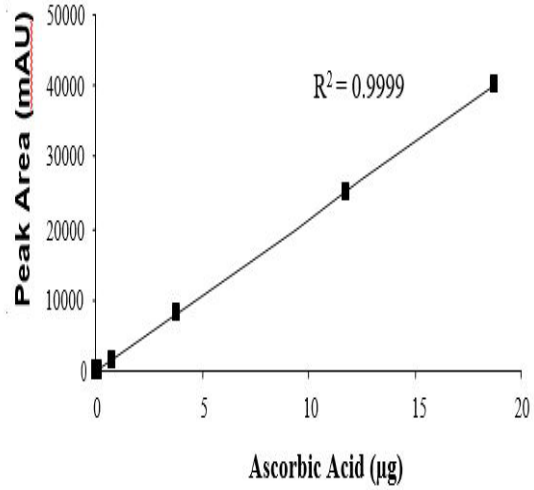

Figure 1: HPLC derived calibration curve for ascorbic acid with quantities tested ranging from 0 to 19 μg.

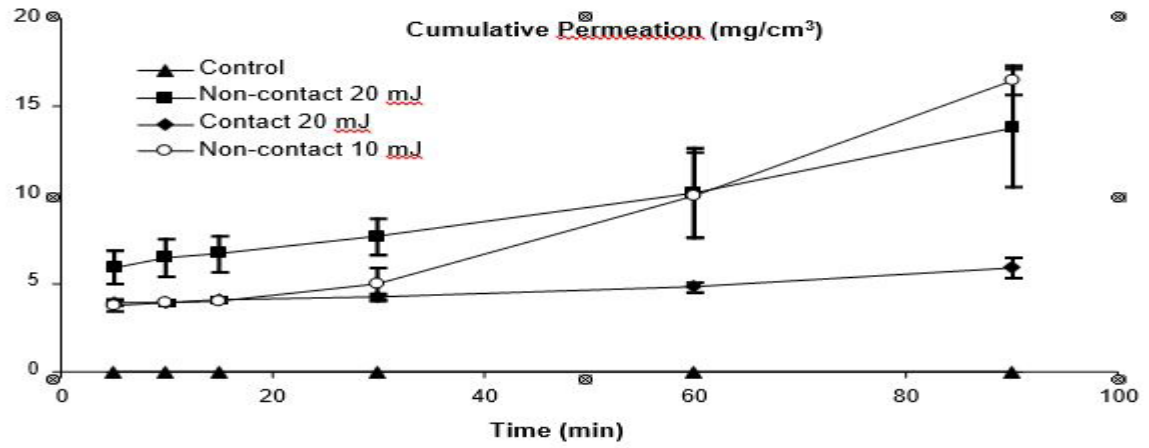

Figure 2: Cumulative ascorbic acid permeation following HPLC measurements of ex vivo skin treated with either 0 mJ (control), 10 mJ in non-contact mode, or 20mJ in contact or non-contact mode. Permeation was measured 5, 10, 15, 30, 60, and 90 minutes after treatment.

Pulse Energy (mJ)	*Treatment mode*	*Cumulative permeation (mg)*	*Retention (mg)*	*Total uptake (mg /g) **	*Enhancement ratio (total uptake by treatment/ total uptake in control*
Control	-	-	0.10 0.01	1.17 0.11	-
10	Non-contact	0.66 0.03	0.18 0.06	20.3 3.4	17
20	Non-contact	0.55 0.14	0.15 0.02	20.2 3.7	17
20	Contact	0.24 0.02	0.11 0.02	7.6 0.6	7

Table 1: Cumulative permeation, retention, and uptake enhancement of ascorbic acid in human ex vivo abdominal skin before and after laser treatment at various pulse energies.

Effect of non-contact mode laser treatment on ascorbic acid permeation

Figure 2 shows at 20 mJ in the non-contact mode, cumulative ascorbic acid permeation was increased relative to both untreated and contact mode (20 mJ) treated skin at each time point tested. A 130% increase in ascorbic acid permeation at 90 min was achieved by switching to the non-contact mode while maintaining the pulse energy at 20 mJ (Table 1). Figure 2 also demonstrates similar permeation kinetics (1st order) between contact and non-contact mode treatments at 20 mJ. The retained fraction for the non-contact mode 20 mJ treatment was approximately 40% greater than the same energy treatment in the contact mode ($p < 0.05$).

To assess the effect of pulse energy on the non-contact mode treatment, a similar series of experiments was performed at 10 mJ. A similar magnitude enhancement of ascorbic acid permeation (0.66 ± 0.03 mg) and retention (0.18 ± 0.06 mg) was observed at 20 mJ. At 5 min, no difference in permeation between contact mode at 20 mJ and non-contact mode at 10 mJ was observed. However, by 30 min, a rapid rise in the rate of permeation was observed and this continued through 90 min. Permeation kinetics appeared to be 2nd order in contrast to 1st order kinetics observed for the contact and non-contact treatments at 20 mJ. No significant difference ($p > 0.4$) was found in total uptake between non-contact mode treatments performed at 10 and 20 mJ (Table 1).

Histological analysis of laser irradiated *ex vivo* skin after ascorbic acid treatment

Histologic observations demonstrated that there was no alteration in the structural integrity of untreated skin exposed to

ascorbic acid for 90 min (not shown). Skin irradiated at 10 or 20 mJ in the contact mode demonstrated epidermal disruption without any visible effect on the stratum corneum (Figure 3, A and C, respectively). A region of dermal coagulation was observed to underlie each disrupted epidermal zone. This was also the case when switching to the non-contact mode (Figure 3, B and D). These macroscopic effects seemed to be independent of pulse energy at very high microbeam fluence or irradiance level (Figure 3).

To ensure that an ex vivo skin thickness of 500 μm exceeded the depth of thermal injury induced by the range of experimental treatment parameters, lesion dimensions obtained by H&E staining were characterized. Figures 4A and 4B represent lesion depth and width plots, respectively, and summarize data shown in Figure 3. For both contact and non-contact mode treatments, a linear increase in width (Figure 4B) and non-linear increase in depth (Figure 4A) across the pulse energy range of 5 to 40 mJ was observed. There was no statistical significant difference in lesion dimensions when comparing contact and non-contact treatments ($p > 0.1$). At 10 mJ, both modes resulted in 300 μm deep and 80 μm wide lesions, while lesions at 20 mJ measured 350 μm deep and 110 μm wide (Figure 4, A and B).

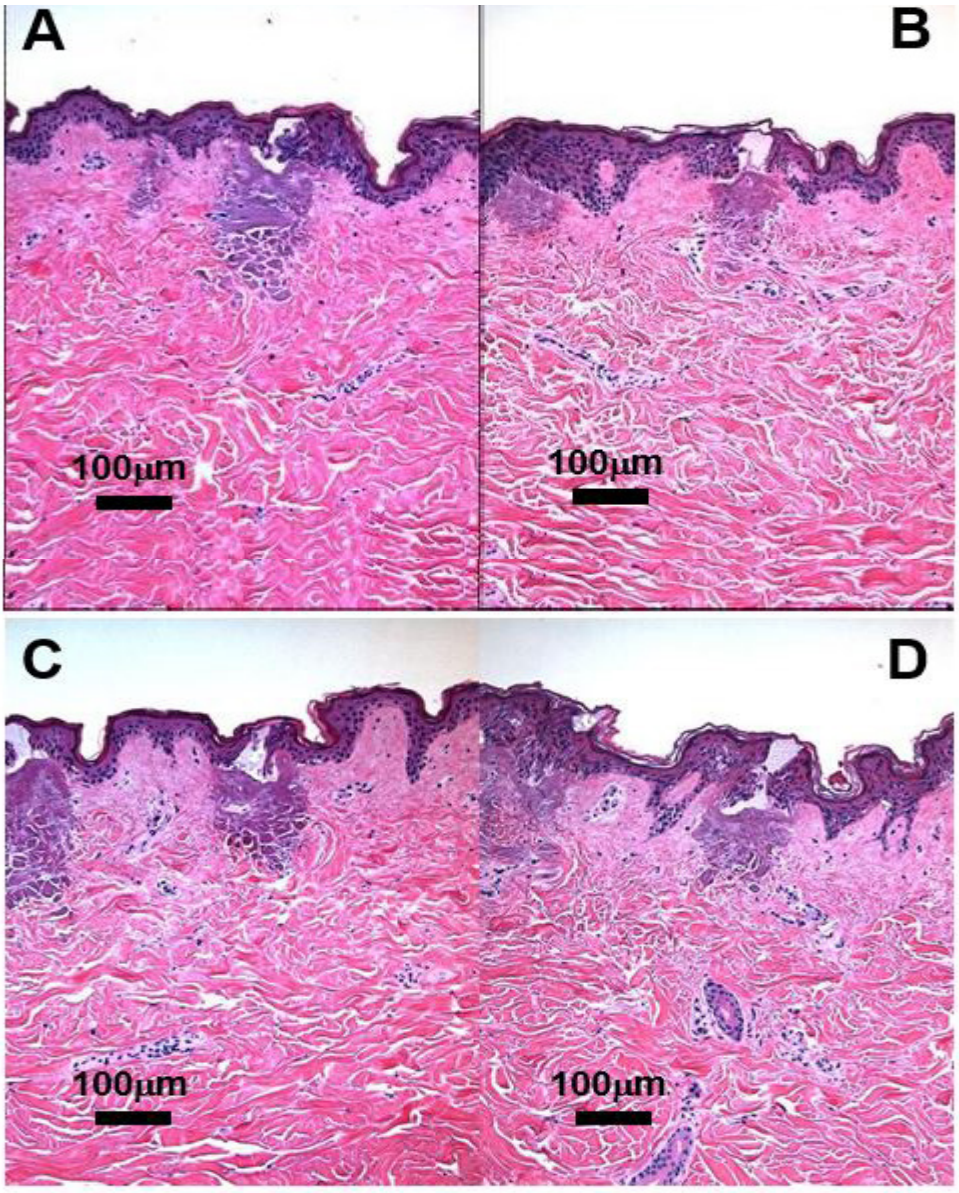

Figure 3: Ex vivo human abdominal tissue treated with the 1550 nm Fraxel® SR laser system at 10 mJ using a contact tip (A) or non-contact tip (B), and at 20 mJ using a contact tip (C) or non-contact tip (D). Paraffin embedded, H&E stained sections show epidermal disruption at 60 μm spot size. Note, in all figures the stratum corneum is not breached.

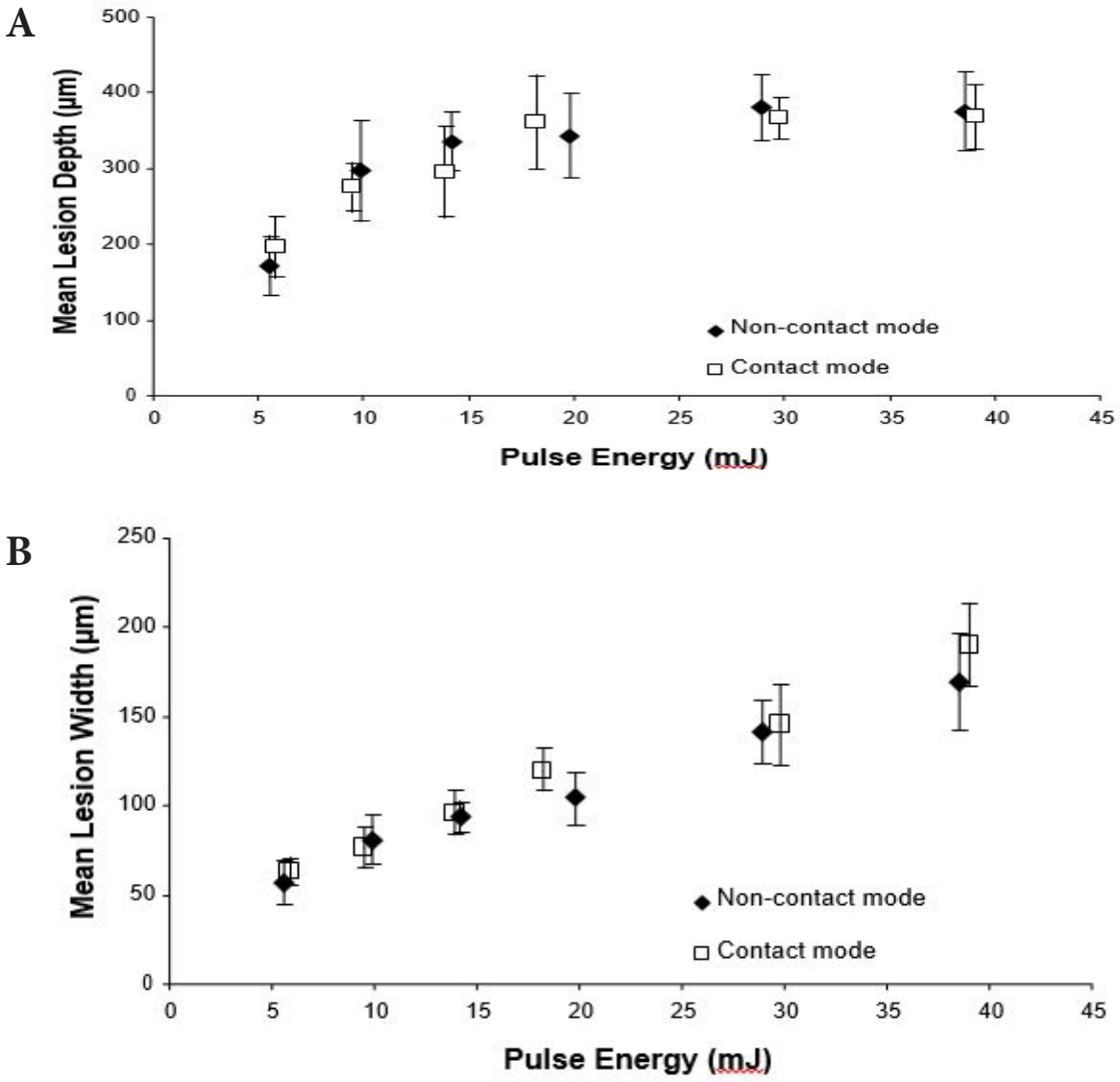

Figure 4: Mean lesion depth (A), and width (B) following treatment of human ex vivo abdominal skin at varying pulse energies using the 1550 nm Fraxel® SR laser system. Plots represent an average of over 250 sample measurements obtained from experiments exemplified in Fig. 3. Note the lack of statistical significance in lesion dimension measurements between the contact and non-contact tips.

Discussion

In this report, the effect of a 1550 nm Fraxel® SR laser on the permeation of ascorbic acid through ex vivo skin was evaluated. Ascorbic acid total uptake was significantly enhanced by FP treatment as shown by qualitative histologic and quantitative HPLC data analyses.

In comparison to pathogens, bacteria, or proteins, ascorbic acid is a small molecule (MW 176 Da). Stratum corneum lipophilicity prevents transcutaneous penetration of hydrophilic molecules such as ascorbic acid, an effect attributed to steric hindrance and opposing polarity. This point was supported by results (Figure 2 and Table 1) that showed no ascorbic acid uptake in untreated skin. Under these conditions, no ultrastructural changes to either the stratum corneum or the epidermis by H&E staining were observed. However, in a previous report, treatment with the 1550nm Fraxel® SR laser system at 60 µm spot size caused epidermal disruption [16]. The 1550 nm laser wavelength exhibits a low absorption coefficient in human skin (µa ~ 7 – 8 cm-1) resulting in a relatively high threshold for collagen denaturation [17]. However, this served as an advantage since the low absorption coefficient helped to prevent stratum corneum ablation at fluence levels in excess of 1 kJ/cm2. Given the short pulse dura-

tions (0.5 – 3.2 ms) and the small microbeam spot size (60 μm) of this laser system, the microfluence and irradiance levels were extremely high at 10 mJ (350 J per cm2 and 440 kW per cm2 respectively) and at 20 mJ (700 J per cm2 and 880 kW per cm2 respectively). High microfluence may have resulted in rapid vaporization of a small epidermal fraction leading to the observed disruptive effect (Figure 3, A and C). Also, at these high levels of irradiance the thermoacoustic effects could account for a significant amount of epidermal disruption via stratum corneum ultrastructural modifications that create higher permeability nanochannels. Once the principal skin barrier (stratum corneum) is overcome, ascorbic acid could easily diffuse through the epidermis into the water rich reservoir of the dermis. Indeed, under all conditions of laser treatment tested, ascorbic acid was observed to permeate into the recipient chamber (Table 1). Since the extracellular matrix of the dermis is a hydrophilic compartment, a significant amount of ascorbic acid was also found in the retained fraction (Table 1).

Although no statistically significant difference in lesion dimensions was observed between treatments in the contact and non-contact mode (Figure 4), the latter consistently resulted in slightly more epidermal disruption. Neither mode of treatment caused ablation of the stratum corneum effectively maintaining barrier function against pathogen entry (Figure 3). In contact mode, the sapphire window abutted the stratum corneum acting as an acoustic impedance matching material, dampening or eliminating any thermoacoustic perturbation on the stratum corneum as a result of laser irradiation. It is hypothesized that enhanced ascorbic acid uptake was achieved in non-contact mode due to greater thermoacoustic alteration of the stratum corneum, a result of an acoustic impedance mismatch at the stratum corneum–air interface during laser irradiation [18]. Indeed, results demonstrated a 17-fold enhancement of ascorbic acid uptake in the non-contact mode, representing nearly a 150% improvement over contact mode treatments at identical pulse energy (Figure 2, Table 1).

It was thus evident that ascorbic acid uptake across laser-treated skin was less dependent on pulse energy (10 or 20 mJ) when operating in non-contact mode (Table 1). Both treatments were carried out at spot densities of 1000 MTZs per cm2, suggesting that final treatment density may play a more important role than absolute pulse energy or microbeam fluence. Previous reports established that lower energy settings at any final spot density [9] and adjunctive use of handheld forced cold air device [10] result in less pain. Thus, FP-enhanced ascorbic acid uptake may be achieved clinically in the absence of significant pain.

Although no statistical difference was detected in total uptake after 90 min for non-contact mode treatments at 10 mJ versus 20 mJ, their kinetics were noticeably different as early as 30 min (Figure 2). As noted above, both treatments maintained identical final spot densities, and only the microbeam fluence differed. Under these conditions, the permeation values measured for the 10 mJ treatment were initially lower (0-30 min) than those at 20 mJ (Figure 2). A shift to 2nd order kinetics took place between 30 and 60 min allowing the 10 mJ treatment to catch up to the 20 mJ treatment which continued to demonstrate 1st order kinetics. From 60 to 90 min, the kinetic order of both treatments remained unchanged, which allowed the 10 mJ treatment to surpass the 20 mJ treatment by 90 min. It would be interesting to explore the kinetic behavior of both treatments for extended periods of time (for example, through 24 hours). The exact reasons for these differences remain unclear but are under current investigation through high resolution ultrastructural studies.

It is important to point out that the 17-fold enhancement reported here was observed after only a 90 min ascorbic acid exposure (Figure 2). Fang and colleagues showed a 20-fold enhancement of ascorbic acid was achieved after microdermabrasion treatment [11]. It is unclear if this enhancement is clinically relevant since measurements were made on nude mouse skin which has an 11.6 μm and 18.5 μm thick stratum corneum and epidermis, respectively, for a total of 30.1 μm. Human skin thickness ranges from from 40 μm (eyelid) to 1.5 mm (palms and soles) with a minimum 10 μm stratum corneum [19]. Furthermore, the enhancement required removal of 41-59% of the stratum corneum and a 12-hr exposure [11]. In the present study, only a 90 min exposure was necessary to generate a 17-fold enhancement in ascorbic acid uptake. Furthermore, unlike ablative lasers and microdermabrasion devices, efficacy of the Fraxel® laser system did not depend on removal of the stratum corneum [11].

In conclusion, this study demonstrated a significant increase of ascorbic acid uptake after treatment of human skin with the Fraxel® laser system without ablating or removing any stratum corneum, unlike devices such as microdermabrasion and ablative lasers where this is a prerequisite for efficacy. It also did not involve the use of any exogenous chromophore, whether superficial or delivered subcutaneously, to achieve ultrastructure disruption. Moreover, the ultrastructure disruption is temporary and normal wound healing would restore skin integrity. This report shows that non-ablative infrared FP laser treatment can enhance ascorbic acid topical uptake and suggests a novel mechanism for transcutaneous topical delivery of potentially numerous active ingredients that could result in breakthrough treatments for difficult to treat conditions such as skin aging, melasma, and scarring. Future studies will focus on uncovering the ultrastructural alterations that are responsible for the observed increase in skin permeability, assessment of additional active ingredients of therapeutic interest based on molecular weight, structure, and charge, as well as on understanding the 1st and 2nd order time-resolved permeation observed in this study.

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