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Enhanced Skin Permeability of Ascorbic Acid after CLEAR + BRILLIANT Perméa Laser Treatment

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Introduction

Photoaging results in numerous clinical effects in the skin, including wrinkles, textural changes, dyspigmentation, telengiectasia, and lentigines[1]. Long-term sunlight exposure has been shown to increase the risk of actinic keratoses and nonmelanoma skin cancer[2]. Amongst the approaches undertaken to reduce this risk are laser skin resurfacing as well as topical therapies[1,3,4]. Concerning the latter, ascorbic acid, commonly known as vitamin C, has received much attention for its ability to reduce ultraviolet (UV)-induced photodamage[3]. The mechanism underlying this photoprotective effect is thought to be related to ascorbic acid's antioxidant activity[5]. When human subjects topically applied L-ascorbic acid as a 10% solution for 5 days prior to UVB irradiation, a significant reduction in erythema was observed when compared to vehicle controls[6].

With the advent of nonablative laser technologies, many patients are opting for laser treatment as a method to improve the appearance of photodamaged skin[7]. Recent advances in nonablative laser research and technology have led to the development of a novel approach termed fractional photothermolysis (FP)[8]. This technique, which relies on water as a chromophore, was the first successful demonstration of the delivery of fractional microthermal treatment zones (MTZ), with intentional skin sparing during the delivery of laser energy to the epidermis[9]. Studies have shown that the subsequent wound healing response stimulates exfoliation of both the untreated and laser treated skin[8,10]. Thus, FP offers the distinct advantage of equivalent efficacy with accelerated healing and reduced side effects when compared to other nonablative lasers that do not spare tissue[11,12]. Although previous studies have shown that microdermabrasion and ablative lasers, such as Er:YAG or carbon dioxide, can enhance topical permeation of ascorbic acid, each of these devices achieve this effect only by compromising the stratum corneum barrier[13]. These abrasive or ablative techniques therefore leave the patient at risk for unnecessary complications such as bleeding and skin infections[15,16].

The objective of this study was to determine if topical ascorbic acid permeation would be enhanced by nonablative fractional laser irradiation without breaching the stratum corneum. We utilized a Clear + Brilliant Perméa handpiece (Solta Medical, Inc., Hayward, CA) with an adjusted, low-power 1927 nm wavelength laser, also known for its efficacy in nonablative laser resurfacing (NFR™) treatment on the Fraxel Dual® 1550/1927 Laser System. We hypothesized that fractional treatment of skin with ultrahigh microscopic laser fluences and irradiances facilitated topical permeation of ascorbic acid in the absence of frank stratum corneum barrier disruption and suggested a role for nonablative lasers in the application of hydrophilic small molecule substances.

The study made use of C E Ferulic (SkinCeuticals, Inc., New York, NY) which is an antioxidant serum containing L-ascorbic acid (Vitamin C) as the key active ingredient. C E Ferulic acid helps neutralize free radical damage and is shown to protect against oxidative stress (factors leading to cosmetic skin conditions such as premature aging, loss of elasticity, and hyperpigmentation).

Materials and Methods

All laser treatments were performed using the Clear + Brilliant Perméa handpiece (Solta Medical, Inc., Hayward, CA) on freshly excised human abdominal skin (for *ex vivo* histology and topical permeation testing) and human forearm skin (IRB approved study with biopsies taken at various time points). The topical formulation tested was C E Ferulic (SkinCeuticals, Inc., New York, NY).

A. Laser parameters

The Clear + Brilliant Perméa laser system was used to treat subjects for clinical and histological analysis. Arrays of micro beams of 110 - 180 μm diameter at incidence were delivered to the surface of the skin specimen in each treatment. The laser pulse energy tested for C E Ferulic permeation measurements was 5 mJ. The laser system has three settings; low, medium and high, which deliver 5, 7.5 and 10 % treatment coverage respectively when 8 passes are made over a treatment area. Treatments were carried out at all three settings using a constant handpiece motion velocity. Histologic examination was performed for comparison of lesion dimensions and characteristics with the Clear + Brilliant 1440 nm laser system.

B. Histologic examination

For *ex vivo* tests, prior to laser treatment, each skin sample was trimmed to a size of 10 mm × 60 mm and heated in between saline-soaked gauze on a digital hot plate (Cole-Parmer Instrument Co., Vernon Hills, IL) until the skin surface temperature reached 32 ± 3 °C. The top layer of gauze was removed and the sample was treated at predetermined laser parameters. Immediately post-treatment, each sample was cut into smaller pieces and fixed in 10% v/v neutral buffered formalin (VWR International, West Chester, PA) overnight for paraffin embedding and sectioning. The sectioned samples were stained with H&E and then imaged using a DM LM/P microscope and a DFC320 digital camera (Leica Microsystem, Cambridge, UK). The lesion dimensions were measured using a proprietary Visual Basic computer program (Solta Medical, Inc., Hayward, CA). Mean lesion widths and depths were calculated based on measurements of 10-15 MTZs for each treatment parameter.

For *in vivo* assessments up to 10 subjectrs were enrolled in an IRB approved study. Biopsies were taken from human forearm skin prior to and 1, 3, 7 and 14 days post treatment. The samples were fixed in 10% v/v neutral buffered formalin (VWR International, West Chester, PA) overnight for paraffin embedding and sectioning. The sectioned samples were stained with H&E and Fontana Masson, and then imaged for further analysis.

C. C E Ferulic permeation studies

This study made use of C E Ferulic (SkinCeuticals, Inc., New York, NY) which is an antioxidant serum that helps neutralize free radical damage and is shown to protect against oxidative stress (factors leading to premature aging, loss of elasticity, and hyperpigmentation). These studies were carried out using skin permeation systems (LGA, Inc., Berkeley, CA) and 500 μm thick skin grafts from freshly excised human abdominal skin. Non-laser treated skin was used as a control. Immediately after laser treatment, each skin sample was mounted on a permeation system whose donor compartment was then filled with C E Ferulic serum[13]. The prepared sample was incubated at 32 ± 3 °C to simulate body temperature. Aliquots were drawn at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 24 hours from the diffusion chamber, and quantitatively analyzed for permeated ascorbic acid using high-performance liquid chromatography (HPLC). After 24 hours, each skin sample was washed thoroughly in saline, weighed, homogenized and centrifuged[13]. This was followed by HPLC analysis to determine the amount of ascorbic acid retained within the skin sample. The measured retention was then normalized to the effective area of skin sample through which permeation occurred. Each experiment constituted a total of 3 individual runs (n = 3).

D. Data analysis

The total permeation was taken as the sum of the permeated and retained ascorbic acid over the cross-sectional area of the skin through which permeation occurred. The permeation values were calculated at each time point and plotted as a cumulative value. The permeation enhancement ratio represents the total ascorbic acid permeation for laser treated skin divided by the total permeation for untreated skin at 24 hours.

Results

Gross inspection of the skin after treatment with the Clear + Brilliant Permea demonstrated no visually-detectable structural changes. *Ex vivo* measurement of the Clear + Brilliant Perméa induced lesions were wider and shallower than those produced by the Clear + Brilliant 1440 nm handpiece, regardless of the treatment level used (**Tables 1 and 2**).

Parameter	**CLEAR+BRILLIANT (1440 nm)**	**CLEAR + BRILLIANT (Perméa)**
Treatment Level	**Lesion Width (µm)**	**Lesion Width (µm)**
Low	**120.1**	**220.7**
Medium	**163.1**	**220.7**
High	**201.6**	**220.7**

Table 1 Lesion widths obtained from *ex vivo* measurements comparing the 1440 nm handpiece with the Perméa handpiece.

Parameter	**CLEAR + BRILLIANT (1440 nm)**	**CLEAR + BRILLIANT (Perméa)**
Treatment Level	**Lesion Depth (µm)**	**Lesion Depth (µm)**
Low	**281.8**	**167.4**
Medium	**339.3**	**167.4**
High	**384.2**	**167.4**

Table 2 Lesion depths obtained from *ex vivo* measurements comparing the 1440 nm handpiece with the Perméa handpiece.

Furthermore, the Clear + Brilliant Perméa induced lesions appeared to optimize the heat concentration superficially with increased superficial disruption (which was associated with audible acoustic during treatment and visible “popcorning” effect (images not shown)) in comparison with the 1440 nm handpiece (**Figure 1**). It should be noted that the stratum corneum was structurally intact and the epidermis was pushed aside, potentially as a result of the laser tissue interaction at this wavelength.

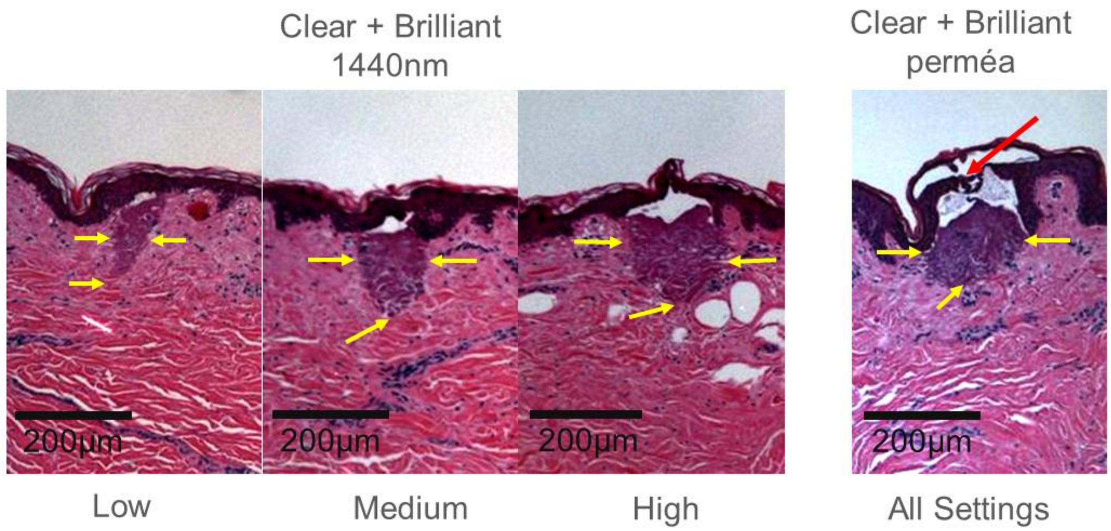

Figure 1 Lesion profile and characteristic comparing the 1440 nm handpiece with the Perméa handpiece. E*x vivo* samples were Clear + Brilliant treated, paraffin embedded, sectioned and, stained with H&E. . The treatment levels of the Clear + Brilliant 1440 nm handpiece delivers three increasing energy levels (4, 7 and 9 mJ) to produce increasing severities of MTZs. The treatment levels of the Perméa handpiece deliver one energy level with increasing coverage which is why only one representative lesion is shown.

Results from previous forearm biopsy studies with the 1927 nm wavelength showed an expected wound healing profile, with MENDs (microscopic epidermal necrotic debris) formation at 1 day post-treatment (**Figure 2B**) as compared to untreated (**Figure 2A**). The epidermis had almost completely regenerated by this time point with slightly visible sub-epidermal clefting and a zone of dermal coagulation underneath, which still appeared to be healing. By 3days post-treatment (**Figure 2C**) the epidermis had completely regenerated and there was a continued dermal remodeling visible. By 7 days post-treatment (**Figure 2D**) the stratum corneum was now underlying the MEND, which is an indication of exfoliation readiness and by 14 days post treatment (**Figure 2E**) the MEND had exfoliated and there was no superficial indication of treatment visible. The dermal remodeling process appeared continual.

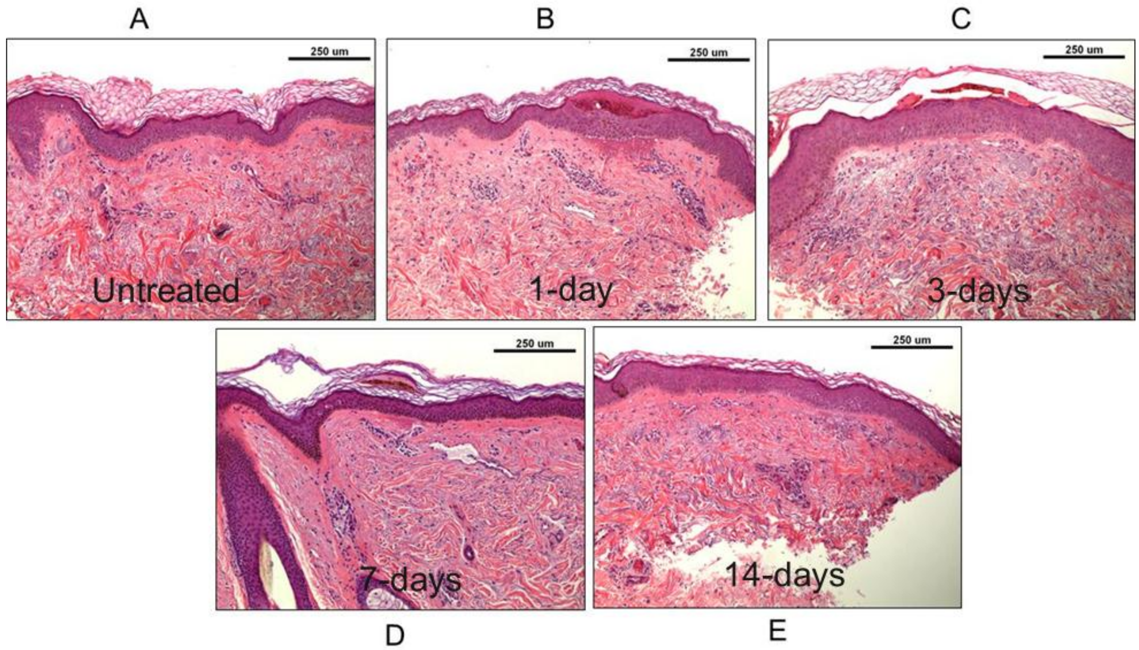

Figure 2 Lesion healing profile post-treatment with the 1927 nm wavelength. Samples were paraffin embedded, sectioned, and stained with H&E. Biopsies were assessed prior to (A), 1 day (B), 3 days (C), 7 days (D) and 14 days (E) post-treatment.

Ex vivo Clear + Brilliant Perméa induced topical permeation of C E Ferulic

Two sets of experiments were run; one was to demonstrate that the Perméa treatments induced greater topical permeation enhancement than the 1440 nm handpiece and the other was to understand dose dependence using C E Ferulic at the three treatment settings.

The first study was carried out using an in-house aqueous based formulation with 10% L-ascorbic acid with typical treatment settings delivered to skin samples with the 1440 nm and Perméa handpieces. Results showed that the Perméa handpiece produced a 5X enhancement over the untreated control and approximately 2X enhancement over the 1440 nm handpiece (**Figure 3**).

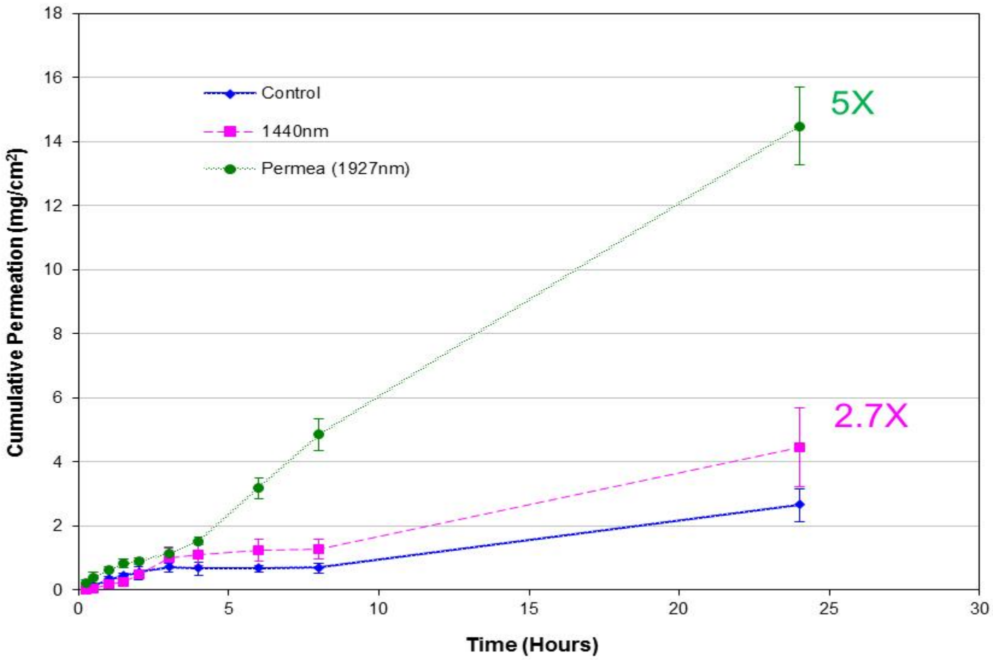

Figure 3 Cumulative permeation as a function of time comparing an untreated control with the 1440 nm handpiece, and the Perméa handpiece.

The second study was carried out using C E Ferulic with all three treatment settings on the Perméa handpiece. Results showed a dose-dependent permeation of C E Ferulic with the amount of permeation increasing as a function of treatment level, with enhancement ratios of 8X, 12X and 17X at the low, medium and high settings respectively, in comparison with untreated control (**Figure 4**). It is important to note that the experimental data displayed below was run on different donor tissue than the one used for the data shown in figure 3.

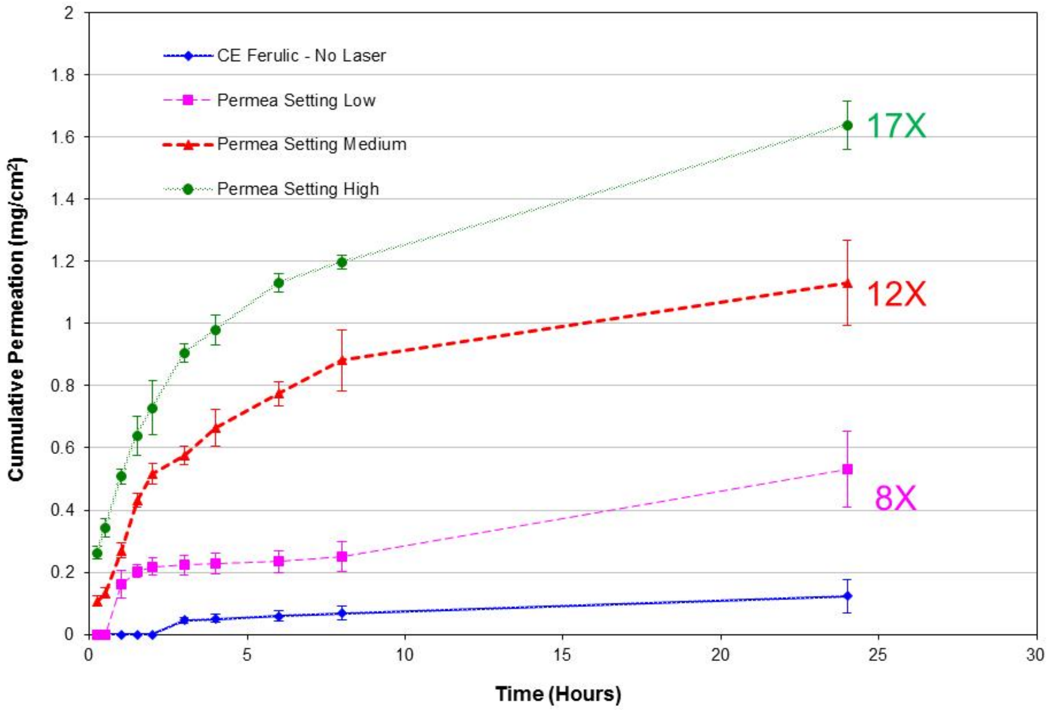

Figure 4 Cumulative permeation as a function of time demonstrated a dose-dependent permeation using the Perméa handpiece.

Kinetics of permeation

Permeation results clearly illustrated that the kinetics for permeation with the Perméa handpiece were superior, as compared to the use of topical alone (**Figure 4**). This was even more significant for the first 90 minutes, where there was no diffusion past the tissue skin graft in the untreated control (topical alone), while all three laser treatment settings enabled the diffusion of the topical into and past the skin graft. Also noted was the dose-dependent rate of kinetics, with the higher treatment settings producing greater amount of permeation and increased rate of diffusion (**Figure 4**). Finally, we also noted that treated samples did not appear to be saturating at 24 hours post-application, while this was clearly visible in the untreated control.

Efficacy with removal of pigment

Results from forearm biopsy studies showed increased pigment removal with 1927 nm laser treatment and the use of C E Ferulic in comparison with the untreated control, laser only, and topical only. The pigmented cells were seen migrating upward from the basement membrane and shuttling into the MEND via the known process of transepidermal exfoliation, wherein the MENDs are expected to flake further off in time, thereby creating the effect of pigment removal. There were a greater number of pigmented cells migrating upward and in the MENDs when using the laser plus topical treatment (**Figure 5**). This is an indication of increased efficiency of pigment removal with the laser plus topical treatment .

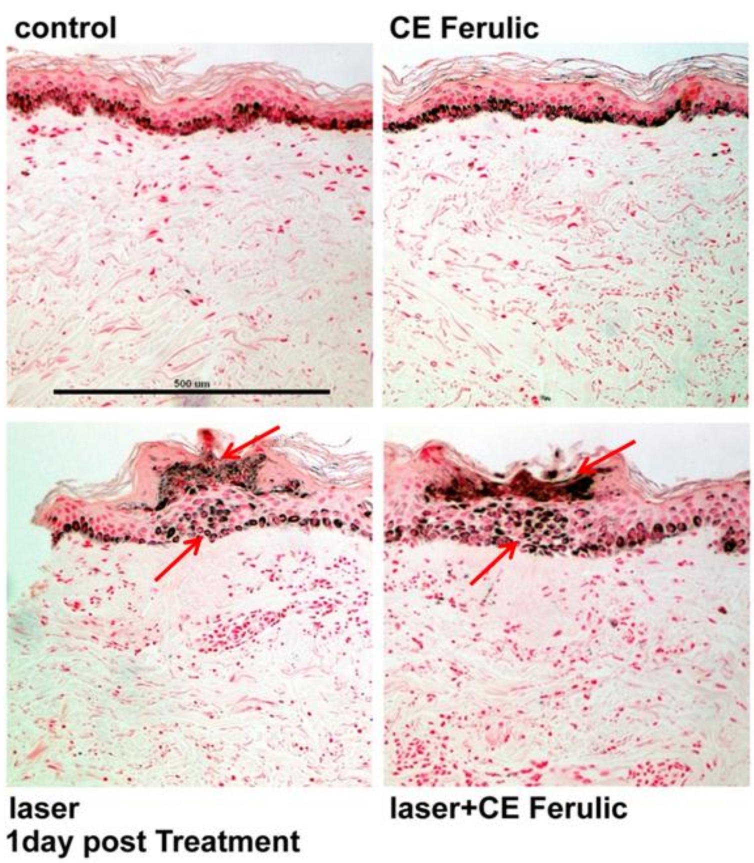

Figure 5 Fontana Masson stained images of control, C E Ferulic only, laser only and laser + C E Ferulic at 1 day post-treatment. Red markers indicate the migration of pigmented cells into the MEND.

Discussion

In this report we studied the effect of the Clear + Brilliant Perméa handpiece on the permeation of ascorbic acid (C E Ferulic) through *ex vivo* skin. We discovered that ascorbic acid permeation could be significantly enhanced by Perméa treatment. Our studies included qualitative histologic and quantitative HPLC data analyses.

Ascorbic acid is a small molecule (MW 176 Da) when compared to pathogens, bacteria, or proteins. Due to the lipophilic nature of the stratum corneum, hydrophilic molecules such as ascorbic acid are unable to penetrate the stratum corneum due to steric hindrance and opposing polarity. Infrared lasers with 1927 nm wavelength possess a high absorption coefficient in human skin ($\mu_a \sim 90\ cm^{-1}$) resulting in relatively low microbeam threshold for collagen denaturation[17]. In addition, the high absorption coefficient at this wavelength also appeared to cause intense vaporization of the water which is present in the stratum corneum, thereby creating the formation of micropores that run through the stratum corneum in tortuous paths (**Figure 6**).

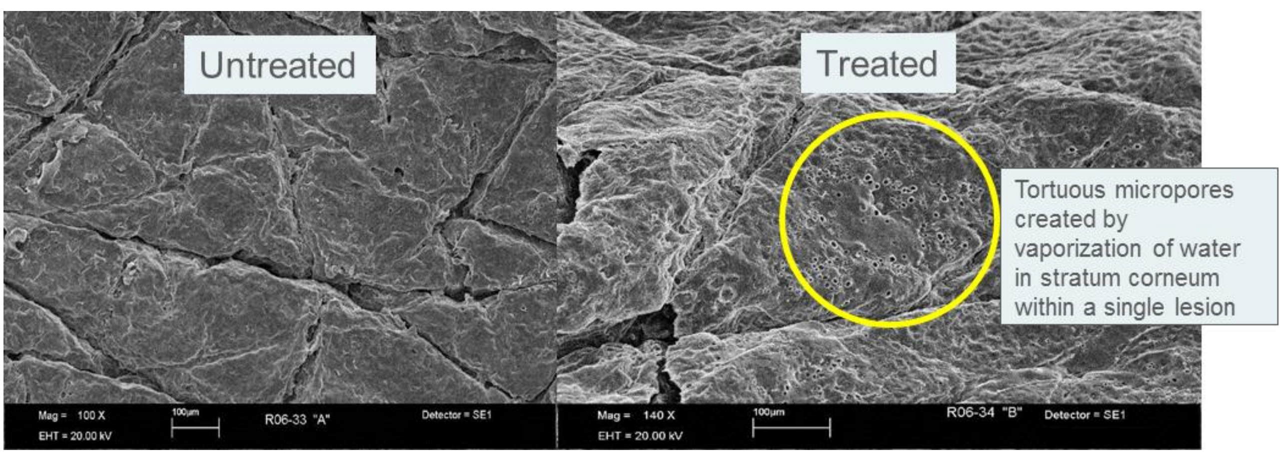

Figure 6 Scanning electron microscopic images of the stratum corneum in an untreated control and in laser treated tissue. Note the formation of micropores within the confines of a single lesion..

Despite the formation of these micropores, the stratum corneum remained structurally intact (**Figures 1 and 2**). Given the small microbeam spot size (110-180 μm) of this laser system, the microfluence levels were relatively high, which further added to the effect of superficial disruption by vaporization of epidermal cells which created the visual effect of the epidermis being pushed aside (**Figure 1**). It was also evident that because of the higher absorption coefficient and microbeam fluence levels, the Clear + Brilliant Perméa treatment was optimal for the superficial heat concentration and distribution (Tables 1 and 2 showed that the lesions were wider and shallower) in comparison with the 1440 nm handpiece (Figure 1). Furthermore, it explained why the Perméa handpiece treatment produced greater permeation than the 1440 nm handpiece (**Figure 3**).

At these levels of absorption, thermoacoustic effects triggered by rapid vaporization accounted for a significant amount of epidermal disruption, while allowing the stratum corneum to maintain its overall structural integrity. The mechanism underlying this effect, however, may be secondary to the thermomechanical alterations of the stratum corneum structure, resulting in the formation of the aforementioned microchannels for increased permeability to smaller molecules. Once the principal barrier, the stratum corneum, is overcome, there is diffusion of ascorbic acid through the epidermis into the water rich reservoir of the disrupted epidermis and the dermis (**Figures 3 and 4**).

Because the microchannels formed in the stratum corneum are nonlinear and take torturous paths, the allowance of a certain sized of molecules is possible. We have carried out studies which showed that there is a size exclusion criterion which is smaller than the sizes of bacteria and viruses (data not shown). Fractional non-ablative lasertreatments with the Clear + Brilliant Permea allow topical permeation yet retain the protective nature of the stratum corneum, unlike in microdermabrasion or ablative resurfacing procedures where the stratum corneum is no longer intact.

CLEAR + BRILLIANT laser technology delivers treatment without requiring direct surface contact with the skin. In addition to the high absorption coefficient and microbeam fluence, we hypothesized that this non-contact mode of treatment imparted a greater thermoacoustic alteration to the stratum corneum due to an acoustic impedance mismatch at the stratum corneum–air interface during laser irradiation. Ultimately, this allowed greater permeation of ascorbic acid[18], while retaining the protective nature of the stratum corneum, true to the nature of nonablative fractional resurfacing treatments. Further studies investigating the role of spot density and pulse energy are warranted. Since lower energy setting treatments have been reported to be less painful for any final spot density[12], the enhancement of ascorbic acid permeation may be clinically achieved in the absence of significant pain, as is true for the Perméa handpiece[19]. This is further supported by a recent study reporting diminished pain in patients undergoing nonablative fractional laser resurfacing (NFR) treatment with concurrent use of a handheld forced cold air device[12].

In the study by Fang and colleagues, a 20X enhancement of ascorbic acid was achieved after microdermabrasion treatment[13]. Although interesting, the relevance of this study remains unclear for several reasons. First, the measurements were made on nude mouse skin not human skin. According to their study, the stratum corneum of nude mouse skin measured approximately 11.6 µm and epidermis 18.5 µm in thickness for a combined 30.1 µm. Human skin varies in thickness from 40 µm (eyelid) to 1.5 mm (palms and soles) with a minimum stratum corneum thickness of 10 µm[20]. Thus, it is not possible to extrapolate data obtained using nude mouse skin to human subjects due to the variability in both stratum corneum and epidermal layer thickness. Other studies have determined that microdermabrasion removed between 41-59% of the stratum corneum without affecting the epidermis[13]. In the present study, not only did we generate 17X enhancement in ascorbic acid permeation, but, the efficacy did not depend on removal of the stratum corneum, a critical distinction over ablative lasers and microdermabrasion devices.

Conclusion

In conclusion, we have demonstrated a statistically significant ($p < 0.01$) enhancement of ascorbic acid permeation after treatment of human skin with the Clear + Brilliant Perméa handpiece. This occurred in the absence of any stratum corneum ablation or removal, unlike modalities such as microdermabrasion and ablative lasers where this is a prerequisite for efficacy. It also did not involve the use of any exogenous chromophore in conjunction with laser irradiation to disrupt or alter the ultrastructure of the skin[21]. In addition, the importance of treatment level (spot density) needs to be emphasized given the apparent higher total permeation as a dose-dependent response. This also led to increased pigment removal as shown in this report via histological evidence. To our knowledge, this is the first report to demonstrate enhancement of ascorbic acid permeation using a nonablative infrared laser and suggests a unique mechanism by which topical small molecule substances may be delivered through the skin without compromising the barrier function of the stratum corneum.

Acknowledments

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