

Lasers and Microdermabrasion Enhance and Control Topical Delivery of Vitamin C

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The objective of this study was to evaluate the ability of lasers and microdermabrasion, both of which are skin resurfacing modalities, to enhance and control the *in vitro* skin permeation and deposition of vitamin C. The topical delivery of magnesium ascorbyl phosphate, the pro-drug of vitamin C, was also examined in this study. All resurfacing techniques evaluated produced significant increases in the topical delivery of vitamin C across and/or into the skin. The erbium:yttrium-aluminum-garnet (Er:YAG) laser showed the greatest enhancement of skin permeation of vitamin C among the modalities tested. The laser fluence and spot size were found to play important parts in controlling drug absorption. An excellent correlation was observed in the Er:YAG laser fluence and transepidermal water loss, which is an estimation of skin disruption. Permeation of magne-

sium ascorbyl phosphate was not enhanced by the Er:YAG laser. The CO₂ laser at a lower fluence promoted vitamin C permeation with no ablation of the stratum corneum or epidermal layers. Further enhancement was observed with the CO₂ laser at higher fluences, which was accompanied by a prominent ablation effect. Microdermabrasion ablated the stratum corneum layers with minimal disruption of the skin barrier properties according to transepidermal water loss levels. The flux and skin deposition of vitamin C across microdermabrasion-treated skin was approximately 20-fold higher than that across intact skin. The techniques used in this study may be useful for basic and clinical investigations of enhancement of topical vitamin C delivery. **Key words:** lasers/skin/topical application/vitamin C. *J Invest Dermatol* 121:1118–1125, 2003

Vitamin C (L-ascorbic acid) has important physiologic effects on skin, including inhibition of melanogenesis, promotion of collagen biosynthesis, prevention of free radical formation, and acceleration of wound healing (Darr *et al*, 1992; Austria *et al*, 1997; Boyce *et al*, 2002). The stratum corneum (SC) provides the principal barrier that limits the percutaneous amount and rate of topical vitamin C application (Lee and Tojo, 1998; Zhang *et al*, 1999). Lasers are physical devices that have been used for medical diagnoses and therapeutic purposes, and have been suggested for the controlled ablation or removal of the SC (Lee *et al*, 2002). The duration of laser procedures on the skin is in the range of nanoseconds to microseconds, indicating that enhancement of drug permeation across laser-pretreated skin occurs in a very short time.

Many types of lasers have been developed and are used in dermatology. The erbium:yttrium-aluminum-garnet (Er:YAG) laser can ablate the SC layer with minimal residual thermal damage to the skin; it is currently used for the resurfacing of rhytides, scars, and photodamage (Manaloto and Alster, 1999). The CO₂

laser also has the ability to resurface skin. The procedure for SC ablation by the CO₂ laser is accompanied by heating (Marcellis and Ellis, 2000). The CO₂ laser may be beneficial in enhancing topical drug delivery because the thermal effect can induce skin permeability to a drug. In addition to lasers, microdermabrasion is a process that uses Al₂O₃ crystals and negative pressure to peel off superficially the outer surface of the skin (Lloyd, 2001). Microdermabrasion has the advantages of less bleeding, fewer complications, and no need for local anesthesia in comparison with other superficial peeling techniques (Tsai *et al*, 1995).

The aim of this study was to evaluate the feasibility of lasers and microdermabrasion in controlling and enhancing the topical delivery of vitamin C, a model hydrophilic molecule, using an *in vitro* model of nude mouse skin. Although the nude mouse skin is more permeable than human skin (Catz and Friend, 1990; Fang *et al*, 1999), it is still a good model for examining the skin permeation of drugs because of the limited variability among individuals and similar hair follicle density to human skin. The use of vitamin C is limited due to its low stability in products. Magnesium ascorbyl phosphate (MAP), a new derivative of vitamin C with a hydrophilic characteristic, is more stable than vitamin C in formulations (Semenzato *et al*, 1995). Hence MAP was also used for permeation into and/or across the skin after SC peeling as a comparison with vitamin C. Histologic examination by light microscopy and transepidermal water loss (TEWL) determination were also used to elucidate changes in the skin structure and the mechanism of drug permeation across laser- and microdermabrasion-treated skin. All resurfacing techniques tested in this study used lower energies or shorter treatment durations than those

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Abbreviations: Er:YAG, erbium:yttrium-aluminum-garnet; MAP, magnesium ascorbyl phosphate; SC, stratum corneum.

utilized in clinical situations. The combination of chemical vitamin C and physical lasers or microdermabrasion may be also applicable in the field of medical cosmetology for rejuvenation.

The animal experiment protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Chang Gung University. The committee deemed the animal experiment follows the guidelines as shown in the Guide for Laboratory Facilities and Care.

MATERIALS AND METHODS

Laser and microdermabrasion assemblies The Er:YAG laser, CO₂ laser, and microdermabrasion were used to pretreat nude mouse skin from the region of the back (BALB/C-nu strain, 6–8 wk old). The Er:YAG laser (Continuum Biomedical, Dublin, California) had a wavelength of 2940 nm and a pulse duration of 250 μ s. An articulated arm was used to deliver the laser beam on to the skin. Output energies of 0.35, 0.45, and 0.55 J per pulse and beam spot diameters of 5 and 7 mm achieved fluences of 0.9 to 2.8 J per cm².

The experimental skin was also treated with one pass of a CO₂ laser (Surgipulse 150XJ, ESC Sharplan, Yokneam, Israel) with a pulse duration of 50 ms in the surgipulse or superpulse mode, depending on individual fluences. This laser delivered fluences of 1.4, 4.0, and 7.0 J per cm². The beam diameter of the CO₂ laser was 3 mm.

The microabrasor (Pepita-C, Mattioli Engineering, Florence, Italy) has a system of aspiration-compression within a flexible tube, which is connected to the machine and the hand-piece. Al₂O₃ crystals are fired from the compression system via a nozzle at a vacuum pressure of 3 bar with the hand-piece in contact with the skin for 2, 3, or 4 s. The used microcrystals are reabsorbed through an aspiration system and shunted to a waste container.

Histologic examination of skin Histologic changes in nude mouse skin were examined after laser irradiation and microdermabrasion. Immediately after treatment, a specimen of exposed area was taken from a live nude mouse for histologic examination. The adjacent untreated skin area was also assessed as the control group. Each specimen was fixed in 10% buffered formaldehyde solution at pH 7.4 for at least 48 h. The specimen was cut vertically against the skin surface. Each section was dehydrated using ethanol, embedded in paraffin wax, and stained with hematoxylin and eosin. For each skin sample, three different sites were examined and evaluated under light microscopy (Nikon Eclipse 4000, Tokyo, Japan). Photomicrographs of the three randomly selected sites of each skin sample were taken with a digital camera (Coolpix 950, Nikon). The digital photomicrographs were then processed with Adobe PhotoDeluxe (Adobe Systems, California), and the SC and epidermal thicknesses were calculated with ImagePro-plus 4.0 (Media Cybernetics, Silver Spring, Maryland). The final thicknesses were the averages from at least three animals.

Test formulations A 50% glycerin aqueous solution was selected as the test formulation in donors based on stability studies of vitamin C in various solutions (Lee and Tojo, 1998). The experimental results also showed that glycerin in the donor solution did not change the skin permeation properties. Hence 85 mM of vitamin C or MAP in 50% glycerin/pH 3.5 citrate phosphate buffer was used as the donor vehicle. All drug-containing formulations were freshly prepared prior to the experiments.

In vitro topical application of vitamin C and MAP The diffusion cell used in this *in vitro* study was a Franz vertical diffusion assembly. Female nude mice (6–8 wk old) were euthanized by ether and full-thickness skin was excised from the dorsal region. A piece of excised skin was mounted on the receptor compartment with the SC side facing upwards into the donor compartment. To obtain SC-stripped skin, adhesive tape (Four Pillars Co., Sanchung, Taiwan) was applied to nude mouse skin with uniform pressure and then removed. This procedure was repeated 20 times. Each stripped skin was examined by light microscopy to avoid the possible discrepancy among the morphology and thickness of these stripped skins. The laser hand-piece was located approximately 3.7 cm from the surface of the skin. After pretreatment with a laser or microdermabrasion, the skin surface was wiped with a cotton wool swab several times. The receptor compartment (10 mL) was filled with a glycerin/pH 7.4 citrate phosphate buffer (1:1) mixture. The donor compartment was filled with 1 mL of the test formulation. The available area of the Franz cell was 1.54 cm². The receptor compartment was

maintained at 37°C and stirred by a magnetic bar at 600 r.p.m. At appropriate intervals, 200 μ L aliquots of receptor medium were withdrawn and immediately replaced by an equal volume of fresh receptor solution. The samples were analyzed by high-performance liquid chromatography (HPLC) methods. Each experiment included four replicates. The *in vitro* permeation experiments demonstrated that within the limit of detection of 2 ng per mL of the HPLC assay, no measurable amount of MAP was obtained in the receptor during 12 h. It may suggest that MAP was almost converted to vitamin C in the nude mouse skin. Hence the vitamin C amount (converted from MAP) was used to calculate the flux and skin deposition of MAP.

The amount of vitamin C retained in the skin was determined at the end of the *in vitro* experiment (12 h). The application site on the skin was washed 10 times using a cotton cloth immersed in distilled water. A sample of skin was weighed, cut with scissors, positioned in a glass homogenizer containing 1 mL of methanol, and ground for 5 min with an electric stirrer. The resulting solution was centrifuged for 10 min at 9,279 g r.p.m. The supernatant was analyzed by HPLC. The weight of each skin piece was used to calibrate the drug amount in skin for the final unit of μ g per mg.

HPLC analytical methods The vitamin C content of the various samples was analyzed with a HPLC system consisting of a Hitachi L-7110 pump, a Hitachi L-7200 sample processor, and a Hitachi L-7400 ultraviolet detector. A 25 cm long, 4 mm inner diameter stainless steel RP-18-NH₂ column (Merck, Darmstadt, Germany) was used. The mobile phase, consisting of 70:30 methanol/pH 3.5 citrate phosphate buffer was used at a flow rate of 1 mL per min. The mobile phase for MAP was 40:60 acetonitrile/pH 3.5 citrate-phosphate buffer at a flow rate of 1 mL per min. The ultraviolet detector was set at a wavelength of 255 nm for both vitamin C and MAP (Semenzato *et al.*, 1995).

TEWL determination The dorsal skin of a live nude mouse was treated with a laser or microdermabrasion. After 30 min, TEWL was quantitatively measured using an evaporimeter (Tewameter 300, Courage and Khazaka, Cologne, Germany). The TEWL value was automatically calculated and was expressed in g per m² per h. An adjacent untreated site was used as a baseline standard for each determination. The measuring area of the TEWL probe was 0.785 cm². Each experiment included four replicates.

Data analysis In the *in vitro* permeation study, the total amount of drug permeating across a unit diffusion surface and into the receptor was calculated and plotted as a function of time. The flux was calculated by the slope of the linear portion of the cumulative amount-time plots for a zero-order model and are expressed as the mass of drug passing across 1 cm² of skin over time. This study shows that limited ablation-pretreated areas of diffusion of the skin were 12.75% and 24.99% for 5 mm and 7 mm diameter hand-pieces of the Er:YAG laser, respectively, for one pulse, 18.36% for the 3 mm diameter hand-piece of the CO₂ laser for four pulses at different sites, and 48.96% for the 0.754 cm² oval-shaped hand-piece of the microdermabrasion unit of the total permeated skin surface area. The data of cumulative amount, flux, drug reservoir within the skin, and TEWL of the treated area were extrapolated to an area of 100% ablation of the permeated area by the following equation:

$$Q_{100\%} = [Q_{\text{treat}} - (Q_{\text{control}} \times (A_{100\%} - A_{\text{treat}}/A_{100\%}) \times 100\%)] \times A_{100\%}/A_{\text{treat}}$$

where $Q_{100\%}$ represents the permeation data (cumulative amount, flux, drug reservoir within the skin, and TEWL) across 100% treated skin (calibrated data), Q_{treat} represents the data across partially treated skin in the Franz cell or in skin surface (original data), Q_{control} represents the data across nontreated skin, $A_{100\%}$ is the area (cm²) of the total diffusion area, and A_{treat} is the area of the laser-treated site or microdermabrasion-treated site. The ratio of the permeation data across 100% treated skin to the value across nontreated skin (control group) was calculated as the enhancement ratio (ER).

The statistical analysis of differences between different treatments was performed using the unpaired Student's *t* test. A 0.05 level of probability was taken as the level of significance. The ANOVA test was also utilized in this study.

RESULTS

Histologic examination of skin Nude mouse skin was exposed to lasers and microdermabrasion using various energies to determine their effects on the integrity of the skin structure. Light microscopy indicated no observable damage to whole skin

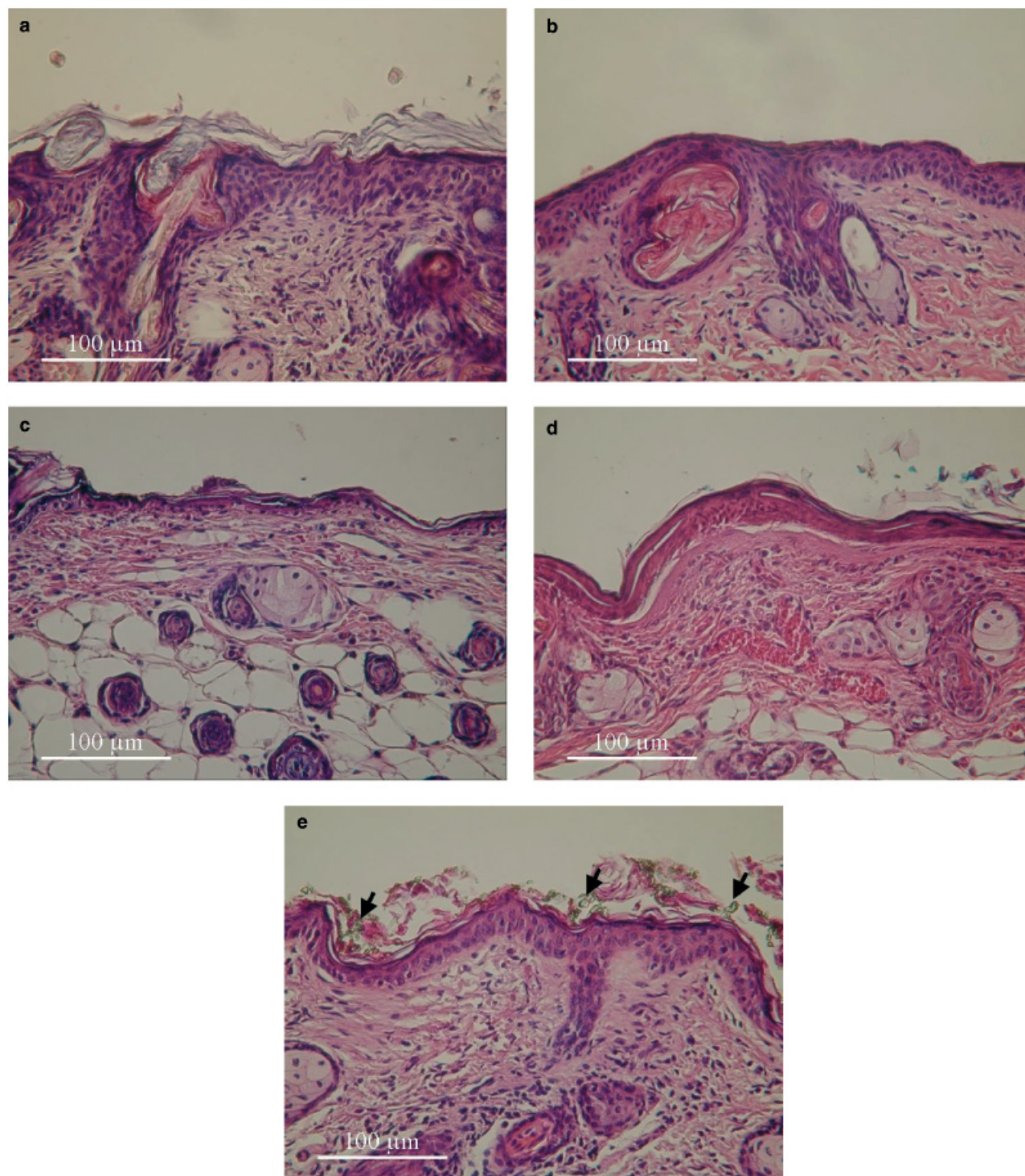


Figure 1. Histologic examination of nude mouse back skin by nontreatment (control group) (a); the tape-stripped group (b); Er:YAG laser at 1.2 J per cm^2 (c); CO₂ laser at 4.0 J per cm^2 (d). microdermabrasion for 3 s (e). Original magnification $\times 200$.

in the nontreated group (**Fig 1a**). The tape-stripped skin showed that almost no SC existed in the stripped skin (**Fig 1b**). Moreover, the thickness of the epidermis was not changed after the strip (**Table I**). Histologic observations demonstrated that Er:YAG laser ablation achieved partial removal of the SC as shown in **Fig 1(c)**. Higher fluences of the Er:YAG laser generally induced deeper SC/epidermal ablation after calculating the thickness by microscopy (**Table I**). The histologic photographs also showed there to be no significant changes in structural features of the epidermis or dermis with treatments at 0.9 and 1.2 J per cm^2 .

As compared with the Er:YAG laser at the same irradiated energies (1.4 J per cm^2), the CO₂ laser induced little tissue ablation or histologic changes to the skin (**Table I**). The higher energies (4.0 and 7.0 J per cm^2) of the CO₂ laser indeed produced significant SC/epidermal ablation; however, thermal necrosis in the epidermis and upper dermis was observed in treated skin as shown in **Fig 1(d)**. Vessel congestion was also shown in the dermis. The higher fluences led to more significant ablation of the SC thickness for the CO₂ laser. There was a linear relationship between the etched depth of the SC and the fluences

Table I. SC and epidermal thickness of nude mouse skin after ablation of Er:YAG laser, CO₂ laser, or microdermabrasion

Ablation type	Fluence (J per cm ² or seconds)	SC thickness (μm)	Viable epidermal thickness (μm)
Nontreatment	0	11.6 ± 2.2	18.5 ± 8.3
SC-stripped	0	1.2 ± 3.2 ^a	16.8 ± 5.9
Er:YAG	0.9 (7 mm, 0.35 J)	6.6 ± 1.7 ^a	17.1 ± 1.2
Er:YAG	1.2 (7 mm, 0.45 J)	5.9 ± 1.2 ^a	16.8 ± 2.8
Er:YAG	1.4 (7 mm, 0.55 J)	1.3 ± 2.3 ^a	5.1 ± 2.0 ^a
CO ₂	1.4 (3 mm, surgipulse)	13.9 ± 1.1	23.3 ± 6.4
CO ₂	4.0 (3 mm, surgipulse)	7.2 ± 2.7 ^a	6.2 ± 1.8 ^a
CO ₂	7.0 (3 mm, powerpulse)	1.9 ± 1.7 ^a	3.0 ± 0.8 ^a
Microdermabrasion	2 s (4 × 6 mm, 3 bar)	6.8 ± 0.9 ^a	18.1 ± 2.3
Microdermabrasion	3 s (4 × 6 mm, 3 bar)	4.9 ± 2.3 ^a	18.1 ± 0.9
Microdermabrasion	4 s (4 × 6 mm, 3 bar)	4.8 ± 0.8 ^a	18.9 ± 2.7

^ap ≤ 0.05 as compared with the SC and epidermal thickness of non-treatment group.
Each value represents mean ± SD (n = 3).

tested (correlation coefficient, $r = 0.9941$), suggesting that SC removal can be precisely controlled by the CO₂ laser. Light microscopy showed thinning of the SC with focal compaction after treatment. Retention of Al₂O₃ crystals was observed on the surface of the SC (**Fig 1e**). No change in the viable epidermis/dermis of treated skin was noted when compared with the control. The effect of ablation by microdermabrasion was approximately that of levels of the Er:YAG laser at 0.9 and 1.2 J per cm² (**Table I**). Moreover, the depth of SC ablation was comparable ($p > 0.05$) with microdermabrasion treatment for 2, 3, and 4 s.

Topical delivery of vitamin C by Er:YAG laser The cumulative amount of vitamin C (μg per cm²) in the receptor as a function of time is shown in **Fig 2**. As shown in **Table II**, the flux of vitamin C dramatically increased by 350.92-fold after stripping the SC of the skin, suggesting the major barrier presented by the SC to the permeation of vitamin C. Skin deposition of vitamin C also greatly increased with SC stripping, but the ratio of the enhancement was lower than for the data on flux (**Table III**). Results in **Fig 2(a)** demonstrate that the Er:YAG laser was effective in enhancing skin permeation of vitamin C for all intensities tested. Extrapolating the flux data of the laser-irradiated area to an area of 100% exposure resulted in an ER value of about 100 for fluences of 0.9 and 1.2 J per cm² (**Table II**). The comparable changes in skin

structure by fluences of 0.9 and 1.2 J per cm² may have resulted in similar enhancement of vitamin C permeation. The more significant changes in skin structure with the 1.4 J per cm² intensity led to a higher enhancement ratio (142.05) of vitamin C flux. In addition to evaluating the effect of a single pulse, the effect of multiple pulses at the same treated site was determined. The results in **Table II** show that the enhancement of vitamin C permeation by two pulses at 0.9 J per cm² was comparable with that of a single pulse ($p > 0.05$).

The effect of laser spot size on the permeation of vitamin C was additionally investigated as listed in **Table II**. A smaller spot size resulted in greater fluence on the skin based on the same output energy (J). The 5 mm probe produced higher vitamin C flux than did the 7 mm probe at the same outputs (0.35 and 0.55 J). This enhancement was not observed in the skin deposition of vitamin C, however (**Table III**).

Topical delivery of MAP by the Er:YAG laser The *in vitro* topical delivery of MAP across Er:YAG laser-treated skin was studied to compare the permeation data of vitamin C. Preliminary permeation experiments demonstrated that within the limit of detection of 2 ng per mL of the HPLC assay, no measurable amount of MAP was obtained in the receptor during 12 h. The flux of MAP across intact skin was comparable with that of vitamin C as shown in **Table IV** ($p > 0.05$). Tape-stripping only increased the MAP flux by 12.39-fold, which was

Table II. The fluxes and enhancement ratios of vitamin C across nude mouse skin by treatment of lasers and microdermabrasion

Laser type	Fluence (J per cm ² or seconds)	Original flux (μg per cm ² per h) ^a	Normalized flux (μg per cm ² per h) ^b	Enhancement ratio (ER _{flux}) ^c
Nontreatment	0	1.19 ± 0.24	—	—
SC stripping	0	417.59 ± 97.75	417.59 ± 97.75	350.92
Er:YAG	0.9 (7 mm, 0.35 J)	35.99 ± 8.09	140.38 ± 32.37	117.97
Er:YAG	1.2 (7 mm, 0.45 J)	32.40 ± 5.42	126.01 ± 21.69	105.89
Er:YAG	1.4 (7 mm, 0.55 J)	43.15 ± 4.60	169.04 ± 18.41	142.05
Er:YAG	0.9 (7 mm, 0.35 J) × 2	24.49 ± 6.67	102.39 ± 26.66	86.04
Er:YAG	1.8 (5 mm, 0.35 J)	32.10 ± 5.12	243.50 ± 40.16	204.62
Er:YAG	2.8 (5 mm, 0.55 J)	40.63 ± 6.11	310.42 ± 47.86	260.86
CO ₂	1.4 (3 mm, surgipulse)	2.76 ± 0.27	9.75 ± 1.48	8.19
CO ₂	4.0 (3 mm, surgipulse)	11.62 ± 1.06	57.96 ± 5.75	48.71
CO ₂	7.0 (3 mm, powerpulse)	15.28 ± 5.27	77.93 ± 28.68	65.49
Microdermabrasion	2 s (4 × 6 mm, 3 bar)	15.50 ± 4.16	30.41 ± 8.49	25.55
Microdermabrasion	3 s (4 × 6 mm, 3 bar)	11.36 ± 5.39	21.96 ± 11.00	18.45
Microdermabrasion	4 s (4 × 6 mm, 3 bar)	15.60 ± 1.99	30.62 ± 4.06	25.73

^aOriginal flux was calculated directly from the flux across partly laser- or microablation-treated skin.

^bNormalized flux was calculated from the flux across fully laser- or microablation-treated skin (100%) that was calibrated by flux of control group.

^cER was normalized flux of laser- or microabrasion-treated group/flux of control group.

Each value represents mean ± SD (n = 4).

Table III. The skin depositions and enhancement ratios of vitamin C within nude mouse skin by treatment of lasers and microdermabrasion

Laser type	Fluence (J per cm ² or seconds)	Original deposition (μg per mg × 10 ²) ^a	Normalized deposition (μg per mg × 10 ²) ^b	Enhancement ratio (ER _{deposition}) ^c
Nontreatment	0	3.68 ± 1.41	—	—
SC stripping	0	471.04 ± 157.46	471.04 ± 157.46	128.00
Er:YAG	0.9 (7 mm, 0.35 J)	76.30 ± 21.43	294.15 ± 85.70	79.93
Er:YAG	1.2 (7 mm, 0.45 J)	109.04 ± 16.08	424.48 ± 64.00	115.35
Er:YAG	1.4 (7 mm, 0.55 J)	71.76 ± 35.87	275.98 ± 143.47	74.99
Er:YAG	0.9 (7 mm, 0.35 J) × 2	68.60 ± 6.46	263.34 ± 25.85	71.56
Er:YAG	1.8 (5 mm, 0.35 J)	45.78 ± 0.78	333.77 ± 6.13	90.70
Er:YAG	2.8 (5 mm, 0.55 J)	61.05 ± 14.55	453.44 ± 114.04	123.22
CO ₂	1.4 (3 mm, surgipulse)	1.44 ± 0.56	—	—
CO ₂	4.0 (3 mm, surgipulse)	10.03 ± 2.79	38.24 ± 15.19	10.39
CO ₂	7.0 (3 mm, powerpulse)	49.93 ± 7.92	255.50 ± 43.11	69.01
Microdermabrasion	2 s (4 × 6 mm, 3 bar)	23.75 ± 5.90	44.65 ± 12.04	12.13
Microdermabrasion	3 s (4 × 6 mm, 3 bar)	45.49 ± 10.65	89.48 ± 21.27	24.32
Microdermabrasion	4 s (4 × 6 mm, 3 bar)	45.11 ± 2.04	87.99 ± 3.45	23.91

^aOriginal deposition was calculated directly from the flux across partly laser- or microablation-treated skin.

^bNormalized deposition was calculated from the flux across fully laser- or microablation-treated skin (100%) which was calibrated by flux of control group.

^cER was normalized deposition of laser- or microabrasion-treated group/deposition of control group.

Each value represents mean ± SD (n = 4).

far less than that of vitamin C. Exposure of skin to the Er:YAG laser did not increase the flux of MAP across the skin at different fluences (**Table IV**). The MAP flux across 0.9 J per cm² treated skin was even significantly lower ($p < 0.05$) than that across intact skin after determining the statistical analysis. The same result was observed for the data of MAP uptake by skin, except that at a fluence of 1.4 J per cm², there was a slight increase in the skin deposition of MAP as shown in **Table V**. It can be inferred that diffusion within the vehicle, but not permeation across the skin, is the rate-determining step for topical delivery of MAP.

Topical delivery of vitamin C by the CO₂ laser An intensity of 1.4 J per cm² was selected to provide identical fluences for both the Er:YAG and CO₂ lasers compared in this study. It is clear that a single pulse of 1.4 J per cm² was sufficient for the CO₂ laser to significantly enhance the transport of vitamin C across skin (**Fig 2b**). As summarized in **Table II**, a comparison of the flux of vitamin C produced by both lasers suggests that the Er:YAG laser exerted a greater enhancement effect on drug permeation. Histologic and structural alterations in the skin may have produced a great increase in flux after exposure to the Er:YAG laser. It is clear that treatment with the CO₂ laser at higher fluences (4.0 and 7.0 J per cm²) significantly enhanced the skin transport of vitamin C by 48.71-fold and 65.49-fold, respectively (**Table II**). The trend of skin deposition of vitamin C after various treatments of the CO₂ laser was the same as that of the flux (**Table III**).

Topical delivery of vitamin C by microdermabrasion The histologic results showed that microdermabrasion could effectively remove the SC layers (**Fig 1d**), resulting in enhanced vitamin C permeation across the skin as shown in **Fig 2(c)**. There was no significant difference ($p > 0.05$) among the flux of vitamin C after microdermabrasion treatment of various durations (**Table II**). On the other hand, microdermabrasion with 2 s treatment produced lower enhancement of skin deposition than did the 3 s and 4 s treatments ($p < 0.05$) (**Table III**).

DISCUSSION

Nude mouse skin was treated by lasers and microdermabrasion to determine their effects on the integrity of the skin structure. There were no statistically significant differences in the SC/epi-

dermis thicknesses determined from treatment using 0.9 and 1.2 J per cm² ($p > 0.05$) for the Er:YAG laser. The Er:YAG laser not only ablated the SC layers, but also removed a part of the epidermal layers at a fluence of 1.4 J per cm². This may suggest that the threshold fluence for ablation of viable epidermis is about 1.4 J per cm² for the Er:YAG laser. The laser-tissue interactions of the CO₂ and Er:YAG lasers significantly differed. A large fraction of the incident energy of the CO₂ laser is invested in tissue heating (Seckel *et al*, 1998; Jacobson *et al*, 2000). According to previous studies, the estimated ablation threshold for the CO₂ laser is 5 J per cm² in human skin (Herd *et al*, 1999). The energy per pulse must be above this level for the CO₂ laser to have a meaningful effect. This threshold approximated the result in our study in that a fluence of 4 J per cm² on nude mouse skin produced a significant zone of ablation in the SC and epidermis (**Table I**).

The mechanism by which microdermabrasion produces abraded skin is based on high-speed compressed Al₂O₃ crystals. The depth of peeling is controlled by the programmed pressure, not by the touch of the operator (Tsai *et al*, 1995). This can theoretically prevent deep tissue damage and allow precise control of ablation of the skin. The depth of SC ablation was similar ($p > 0.05$) with treatment of microdermabrasion for 2, 3, and 4 s. Hence treatment for 2 s may act as a maximum duration to achieve effective peeling as effects of longer durations may be restricted to those at the level of 2 s.

According to light-microscopic photographs (**Fig 1**), lasers and microdermabrasion produced skin ablation and disruption to different levels. The prerequisite of an ideal permeation-enhancing method is that the skin should recover its normal barrier properties following removal of the enhancing method. Previous studies demonstrated that the depth to which the SC and epidermis could completely recover within 3 to 5 d was based on different fluences used (Lee *et al*, 2001, 2002). The recovery of skin after microdermabrasion might not exceed this period, according to histologic observations and the etched thickness of the SC. These results indicate that further investigations on developing these permeation-enhancing methods are warranted.

Vitamin C possessed a very low passive permeability in the control group. Owing to the lipophilic nature and the ordered matrix of intercellular lipids of the SC, a hydrophilic molecule such as vitamin C has difficulty partitioning and permeating into the SC. When stripping the SC by tape, vitamin C could easily traverse across the viable epidermis/dermis because of the disappearance of this predominant barrier, which is why the

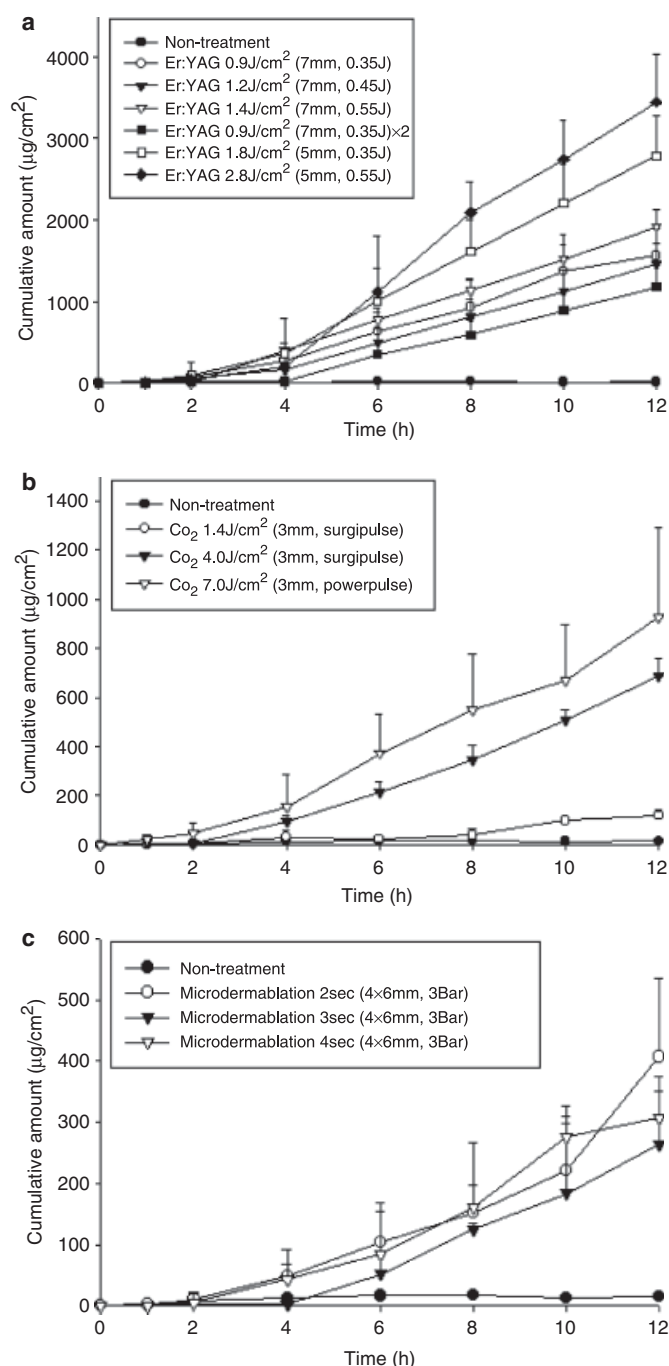


Figure 2. Transdermal permeation of vitamin C by Er:YAG laser (a), CO₂ laser (b), and microdermabrasion (c) at various fluences or treatment durations. Each value represents the mean $\pm$ SD ($n = 4$).

tape-stripping technique possessed the highest ER_{flux} value among all methods examined in this study. Although the skin deposition of vitamin C was also greatly increased by SC stripping, the ratio of enhancement was lower than the data for flux. This may have been due to a lack of the SC drug reservoir within the skin, resulting in insufficient space for the deposition of vitamin C. The Er:YAG laser greatly enhanced skin permeation of vitamin C for all intensities tested. Histologic observations and ablation levels in the skin may have produced this great increase. An observation was that the Er:YAG laser at 1.4 J per cm² not only removed SC layers, but also ablated a part of viable epidermis (**Table I**); however, the vitamin C flux across Er:YAG laser-treated skin was greatly lower than that across SC-stripped skin. Besides the ablation effect on skin, improvement of drug partitioning into the skin may be also important for the enhancement in vitamin C permeation. Although removal of the epidermis can reduce the inherent barrier properties of skin, the partitioning of vitamin C into the viable skin should be decreased. As a result, increased permeation of the skin due to ablation may be partly offset by a decrease in the partition coefficient between epidermis and drug vehicle.

Two pulses of irradiation would theoretically produce a greater disruption of the skin than would one pulse. TEWL indicates the rate at which water migrates from viable tissues through the layer of the SC to the external environment. This method demonstrates a good correlation between skin barrier disruption or thickness and increase in TEWL (Fang *et al*, 1997; Alberti *et al*, 2001). **Figure 3** shows TEWL values of skin after treatment with lasers. Baseline values of nontreated sites were subtracted from the achieved measurements to give actual changes in TEWL (Δ TEWL). The results show that multiple pulses indeed caused more significant Δ TEWL values than that did single pulses ($p < 0.05$). Although removal of a part of the SC and epidermis can reduce the inherent barrier properties of the skin and thus increase skin permeation, the partitioning of drugs into the SC and/or epidermis should be decreased after this removal by two-pulse treatment.

Discrepancies in skin deposition among various modes of the Er:YAG laser were not large (**Table III**). A low correlation coefficient ($r = 0.6088$) was calculated between the fluence of the Er:YAG laser and its $ER_{deposition}$ value. The offset effect by the reduced skin volume for vitamin C to be deposited at higher fluences may be responsible for this low correlation. On the other hand, a good relationship ($r = 0.9588$) between Er:YAG fluence and vitamin C flux was found (**Table II**), indicating that the Er:YAG laser with various modes can precisely control the skin permeation of vitamin C. There was also an excellent relationship ($r = 0.9952$) between the fluence and Δ TEWL value.

MAP, as the prodrug of vitamin C, has been introduced commercially as a skin whitener and radical scavenger (Semenzato *et al*, 1995). No measurable amount of MAP was obtained in the receptor during 12 h, indicating the complete conversion of the prodrug to vitamin C during the permeation process. Evidence suggests that MAP is transported into the dermis, where it is converted into vitamin C as a result of dephosphorylation, then it

Table IV. The fluxes and enhancement ratios of MAP across nude mouse skin by treatment of Er:YAG laser^a

Laser type	Fluence (J per cm ² or seconds)	Original flux (μ g per cm ² per h) ^b	Enhancement ratio (ER_{flux}) ^c
Nontreatment	0	1.02 $\pm$ 0.16	—
SC stripping	0	12.64 $\pm$ 1.37	12.39
Er:YAG	0.9 (7 mm, 0.35 J)	0.76 $\pm$ 0.10	0.75
Er:YAG	1.2 (7 mm, 0.45 J)	0.86 $\pm$ 0.15	0.84
Er:YAG	1.4 (7 mm, 0.55 J)	1.02 $\pm$ 0.03	1.00

^aThe vitamin C but not MAP was detected in the receptor because of the conversion in skin.

^bOriginal flux was calculated directly from the flux across partly laser- or microablation-treated skin.

^cER was original flux of laser- or microablation-treated group/flux of control group.

Each value represents mean $\pm$ SD ($n = 4$).

Table V. The skin depositions of MAP within nude mouse skin by treatment of Er:YAG lasers^a

Laser type	Fluence (J per cm ² or seconds)	Original deposition (μg per mg × 10 ³) ^b	Normalized deposition (μg per mg × 10 ³) ^c
Nontreatment	0	0	—
SC stripping	0	20.08 ± 2.85	20.08 ± 2.85
Er:YAG	0.9 (7 mm, 0.35 J)	0	0
Er:YAG	1.2 (7 mm, 0.45 J)	0	0
Er:YAG	1.4 (7 mm, 0.55 J)	1.28 ± 0.28	4.93 ± 1.08

^aThe vitamin C but not MAP was detected in the skin because of the conversion in skin.

^bOriginal deposition was calculated directly from the flux across partly laser- or microablation-treated skin.

^cNormalized deposition was calculated from the flux across fully laser- or microablation-treated skin (100%) which was calibrated by flux of control group.

Each value represents mean ± SD (n = 4).

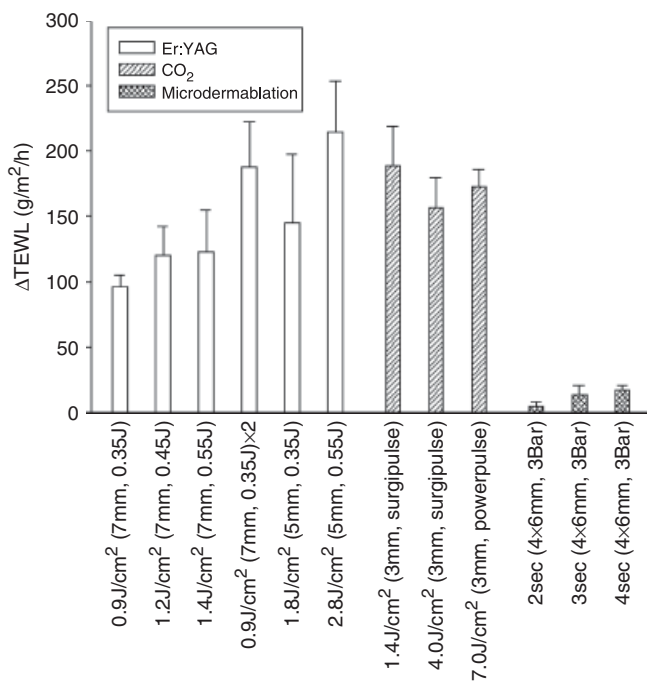


Figure 3. TEWL of nude mouse back skin by Er:YAG laser, CO₂ laser, and microdermabrasion at various fluences or treatment durations. Each value represents the mean ± SD (n = 4).

permeates across the epidermis (Lupo, 2001). The low enhancement of flux after tape-stripping may indicate that not only the SC, but also the epidermis/dermis, presents resistance to MAP permeation. Another possible reason is the affinity of MAP for the vehicle. Hydrophilic MAP may have been better incorporated into the test formulation and thus produced a stronger affinity with this vehicle. This may have contributed to the difficulty of MAP in escaping from the donor vehicle to the skin; that is, the release of MAP from the formulation was very low. Essentially, no detectable amount being observed within the intact skin may indicate the constraint of MAP permeating from the vehicle to the skin. Neither the flux nor the skin deposition of MAP application increased after exposure of skin to the Er:YAG laser. The MAP flux across 0.9 J per cm² treated skin was even significantly lower ($p < 0.05$) than that across intact skin. This may be due to the decrease of the partition coefficient of MAP, which was similar to the effect of the two-pulse treatment for vitamin C permeation. Although a partial removal of SC can reduce the inherent barrier property of skin, the partitioning of drug into the SC and the drug reservoir within the skin should be limited after this removal. In this case of MAP, partitioning may play an important part governing skin permeation, thus showed no or negligible effect after treatment with the Er:YAG laser.

With respect to drug permeation across the skin from the aqueous vehicle, a drug should first diffuse out from the vehicle to the skin surface. To clarify the mechanism of the skin absorption of vitamin C and MAP, the release rate of drug from the test formulation across a cellulose membrane (Spectra-Por, molecular weight cut-off of 12,000–14,000) was studied. The mediums in the donor and receptor were the same with the experiments of nude mouse skin. The release rate of vitamin C and MAP after examination was 353.77 ± 18.16 μg per cm² per h (2.01 ± 0.10 μmol per cm² per h) and 20.83 ± 4.45 μg per cm² per h (0.08 ± 0.02 μmol per cm² per h), respectively. Hence it is concluded that the difficulty of releasing MAP from the vehicle and partitioning it across the skin contributed to the low permeation of MAP following laser treatment.

It was noticed that the CO₂ laser at 1.4 J per cm² caused no ablation of either the SC or the epidermis (Table I). The 8.19-fold increase of vitamin C flux by the CO₂ laser at 1.4 J per cm² may have been due to the slight thermal effect induced at lower fluences. The pulse duration of 50 ms used in this study may be higher than the thermal relaxation time of the skin (1 ms), resulting in thermal injury to the skin without ablation (Herd *et al*, 1999). Two major observations constituting structural changes in CO₂ laser-treated skin (4.0 and 7.0 J per cm²) include thermal injury and skin ablation. As observed in Table I, the CO₂ laser at 7.0 J per cm² showed a similar ablation to the Er:YAG laser at 1.4 J per cm². The enhancement of vitamin C flux, however, was greater for the Er:YAG laser than for the CO₂ laser. This may indicate that mechanisms other than the ablation effect dominate the permeation of vitamin C. The large wavelength of the CO₂ laser (10,600 nm) allows deep penetration of skin tissues. The heat diffusion by the CO₂ laser causes dermal collagen shrinkage, which is believed to be responsible for tightening of the dermis (Collawn *et al*, 1998; Jacobson *et al*, 2000), resulting in loss of the extracellular gel matrix surrounding the collagen fibers. This effect may cause a negative result for vitamin C transport across the skin.

The trend for skin deposition of vitamin C after treatment with the CO₂ laser was the same as that for the flux. Moreover, the CO₂ laser at 1.4 J per cm² showed an inhibitory effect of skin partitioning ($p < 0.05$), which may have been due to dermal shrinkage. Further studies are needed to elucidate these mechanisms. Unlike the Er:YAG laser various fluences of the CO₂ laser produced comparable ΔTEWL values (Fig 3), although a linear relationship was determined between the ablated SC thickness and the fluence. This may suggest that the SC thickness is not a strict factor predominating the ΔTEWL value. Structural changes other than skin ablation are possibly other mechanisms that can modulate ΔTEWL.

Microdermabrasion has become an extremely popular form of superficial skin resurfacing. The nonsignificant difference in the flux of vitamin C after treatment with microdermabrasion at various durations was due to the comparable ablation effects of various treatment durations as shown in Table I. Microdermabrasion with 2 s treatment caused lower enhancement of skin deposition

than for 3 s and 4 s treatments (Table III). The same trend was observed for Δ TEWL by microdermabrasion (Fig 3). A linear correlation ($r = 0.9538$) was found to exist between skin deposition of vitamin C and Δ TEWL exposed by microdermabrasion at various durations. A good relationship ($r = 0.9695$) was also observed between treatment duration and Δ TEWL.

The Δ TEWL caused by microdermabrasion was much lower than those caused by the lasers (Fig 3). It was perceived that the Er:YAG laser at 0.9 and 1.2 J per cm^2 and microdermabrasion ablated the SC and epidermis to similar thicknesses ($p > 0.05$) (Table I). The results of flux and skin deposition enhancement as well as Δ TEWL, however, showed a great discrepancy between these two techniques.

This may suggest that factors other than skin ablation contribute to this difference. Histologic observations of skin treated by microdermabrasion showed that it may simply remove the SC layers while reserving intact epidermis/dermis (Fig 1d). Previous studies also demonstrated the same results (Lloyd, 2001; Tan *et al*, 2001). The Er:YAG laser may also affect skin layers with ultrastructural alterations not visible in photomicrographs from light microscopy (Lee *et al*, 2001). One explanation for this inference may be the photomechanical acoustic effect generated by the laser. Local propagation of the acoustic shock wave may cause "cracking" of the epidermis (Nelson *et al*, 1991). The scanning electric micrograph also showed intervening spaces between the keratinocyte aggregates after irradiation with the Er:YAG laser (Lee *et al*, 2002). These effects on the skin could lead to greater drug permeation across and within the skin by the Er:YAG laser.

In conclusion, all resurfacing techniques tested in this study show promise for promoting vitamin C topical delivery. Permeation of the prodrug of vitamin C (MAP), however, was not enhanced by the Er:YAG laser because of the affinity between the vehicle and the drug. SC and epidermal removal with these resurfacing techniques can be fast and precisely controlled and may offer a distinct advantage over tape-stripping, which is both macroscopic and unpredictable. The results of this study indicate that each resurfacing technique had its own characteristics in modulating skin histology and topical delivery of vitamin C. Hence lasers and microdermabrasion exhibit their own abilities to enhance drug transport into and/or across the skin to fit different needs.

1 The Er:YAG laser greatly enhanced vitamin C permeation with a 86.04-fold to 260.86-fold increase. Skin deposition also showed 71.56-fold to 123.22-fold enhancement. A good correlation was determined between the laser fluence and vitamin C flux; however, there was relationship between fluence and skin deposition of vitamin C. Δ TEWL could be a factor reflecting the magnitude of Er:YAG laser fluence because of an excellent correlation between them.

2 The CO_2 laser at a lower fluence (1.4 J per cm^2) induced a 8.19-fold increase in vitamin C flux without ablating any of the SC or epidermal layers. Skin deposition, however, was somewhat lower at this fluence. The CO_2 laser at higher fluences (4.0 and 7.0 J per cm^2) was sufficient to ablate the SC and epidermal thickness, resulting in enhanced vitamin C flux and skin deposition. The effect of enhancement was lower than that of the Er:YAG laser, which may have been due to skin tightening by the CO_2 laser.

3 Microdermabrasion moderately increased skin permeation and deposition of vitamin C by simply ablating a portion of the SC layers. Both treatment duration and skin deposition showed good correlations with the value of Δ TEWL.

Further studies on the feasibility of *in vivo* and clinical uses of these techniques are in progress to explore their practicality.

REFERENCES

- Alberti I, Kalia YN, Naik A, Bonny J, Guy RH: In vitro assessment of enhanced topical delivery of terbinafine to human stratum corneum. *J Control Release* 71:319–327, 2001
- Austria R, Semenzato A, Bettero A: Stability of vitamin C derivatives in solution and topical formulations. *J Pharm Biomed Anal* 15:795–801, 1997
- Boyce ST, Supp AP, Swope VB, Warden GD: Vitamin C regulates keratinocyte viability, epidermal barrier, and basement membrane in vitro, and reduces wound contraction after grafting of cultured skin substitutes. *J Invest Dermatol* 118: 565–572, 2002
- Catz P, Friend D: Transdermal delivery of levonorgestrel VIII. Effect of enhancers on rat skin, hairless mouse skin, hairless guinea pig skin, and human skin. *Int J Pharm* 58:93–102, 1990
- Collawn SS, Boissy RE, Vasconez LO: Skin ultrastructure after CO_2 laser resurfacing. *Plast Reconstr Surg* 102:509–515, 1998
- Darr D, Combs S, Dunston S, Manning T, Pinnell S: Topical vitamin C protects porcine skin from ultraviolet radiation-induced damage. *Br J Dermatol* 127:247–253, 1992
- Fang JY, Tsai MJ, Huang YB, Wu PC, Tsai YH: Percutaneous absorption and skin erythema: Quantification of capsaicin and its synthetic derivatives from gels incorporated with benzalkonium chloride by using non-invasive bioengineering methods. *Drug Dev Res* 40:56–67, 1997
- Fang JY, Fang CL, Sung KC, Chen HY: Effect of low frequency ultrasound on the in vitro percutaneous absorption of clobetasol 17-propionate. *Int J Pharm* 191:33–42, 1999
- Herd R, Dover JS, Arndt KA: Skin resurfacing: Laser. In: Freedberg IM, Eisen AZ, Wolff K, Austen KE, Goldsmith LA, Katz SI, Fitzpatrick TB (eds). *Fitzpatrick's Dermatology in General Medicine*. McGraw-Hill Health Professions Division, New York, 1999
- Jacobson D, Bass LS, Vanderkam V, Achauer BM: Carbon dioxide and erbium:YAG laser resurfacing. *Clin Plast Surg* 102:509–515, 2000
- Lee AR, Tojo K: Characterization of skin permeation of vitamin C. theoretical analysis of penetration profiles and differential scanning calorimetry study. *Biol Pharm Bull* 46:174–177, 1998
- Lee WR, Shen SC, Lai HH, Hu CH, Fang JY: Transdermal drug delivery enhanced and controlled by erbium:YAG laser: A comparative study of lipophilic and hydrophilic drugs. *J Control Release* 75:155–166, 2001
- Lee WR, Shen SC, Wang KH, Hu CH, Fang JY: The effect of laser treatment on skin to enhance and control transdermal delivery of 5-fluorouracil. *J Pharm Sci* 91:1613–1626, 2002
- Lloyd JR: The use of microdermabrasion for acne: A pilot study. *Dermatol Surg* 27:329–331, 2001
- Lupo MP: Antioxidants and vitamins in cosmetics. *Clin Dermatol* 19:467–473, 2001
- Manaloto RMP, Alster T: Erbium:YAG laser resurfacing for refractory melasma. *Dermatol Surg* 25:121–123, 1999
- Marcellis GN, Ellis DAF: Laser facial skin resurfacing: Discussion of erbium:YAG and CO_2 lasers. *J Otolaryngol* 29:78–82, 2000
- Nelson JS, McCullough JL, Glenn TC, Wright WH, Liaw LL, Jacques SL: Mid-infrared laser ablation of stratum corneum enhances *in vitro* percutaneous transport of drugs. *J Invest Dermatol* 97:874–879, 1991
- Seckel BR, Younai S, Wang KK: Skin tightening effects of the ultrapulse CO_2 laser. *Plast Reconstr Surg* 102:872–877, 1998
- Semenzato A, Austria R, Dall'Anglio C, Bettero A: HPLC determination of ionic compounds in cosmetic emulsions: Application to magnesium ascorbyl phosphate. *J Chromatogr A* 705:385–389, 1995
- Tan MH, Spencer JM, Pires LM, Ajmeri J, Skover G: The evaluation of aluminum oxide crystal microdermabrasion for photodamage. *Dermatol Surg* 27:943–949, 2001
- Tsai RY, Wang CN, Chan HL: Aluminum oxide crystal microdermabrasion: A new technique for treating facial scarring. *Dermatol Surg* 21:539–542, 1995
- Zhang L, Lerner S, Rustrum WV, Hofmann GA: Electroporation-mediated topical delivery of vitamin C for cosmetic applications. *Bioelectrochem Bioenerg* 48: 453–461, 1999