# Fractional Photothermolysis: A New Concept for Cutaneous **Remodeling Using Microscopic Patterns of Thermal Injury**

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Background and Objectives: We introduce and clinically examine a new concept of skin treatment called fractional photothermolysis (FP), achieved by applying an array of microscopic treatment zones (MTZ) of thermal injury to the skin.

Study Design/Materials and Methods: Two prototype devices emitting at 1.5 µm wavelength provided a pattern of micro-exposures with variable MTZ density. Effects of different MTZ densities were tested on the forearms of 15 subjects. Clinical effects and histology were assessed up to 3 months after exposure. Treatment of photoaged skin on the periorbital area in an additional 30 subjects receiving four treatments over a period of 2-3 weeks was also tested. Tissue shrinkage and clinical effects were assessed up to 3 months after treatment.

Results: Pattern densities with spacing of 250 µm or more were well tolerated. Typical MTZ had a diameter of 100 µm and penetrated 300 µm into the skin. Reepithelialization was complete within 1 day. Clinical effects were assessed over a 3-month period. Histology at 3 months revealed enhanced undulating rete ridges and increased mucin deposition within the superficial dermis. Periorbital treatments were well tolerated with minimal erythema and edema. Linear shrinkage of 2.1% was measured 3 months after the last treatment. The wrinkle score improved 18% (P < 0.001) 3 months after the last treatment.

**Conclusions:** FP is a new concept for skin restoration treatment. Safety and efficacy were demonstrated with a prototype device. Further clinical studies are necessary to refine the optimum parameters and to explore further dermatological applications. Lasers Surg. Med. 34:426-438, 2004. © 2004 Wiley-Liss, Inc.

Key words: fractional; laser; photothermolysis; resurfacing; MTZ; non-ablative; photoaging; wrinkles; remodeling; rejuvenation

# INTRODUCTION

There is an increasing demand for an effective and safe laser treatment that repairs photoaged skin. Two treatment modalities, ablative skin resurfacing (ASR), and nonablative dermal remodeling (NDR), have been developed to address this demand. All currently available laser treatments, however, exhibit significant problems and these

laser systems typically operate safely and effectively only over a narrow, patient-dependent treatment range.

ASR with a pulsed CO<sub>2</sub> laser is generally considered to be the most effective laser treatment option for repair of most photodamaged tissue [1-8]. However, patients typically experience significant side effects following ASR treatment. Edema, oozing, crusting, and burning discomfort during the first week following treatment and prolonged erythema are common. In addition, patients may experience long lasting pigmentary changes, scarring, and infection. These side effects are unacceptable for many patients.

ASR with the Er:YAG laser was introduced as a gentler alternative to the  $CO_2$  laser [9]. The Er:YAG laser has a shallower absorption depth, which leads to less residual thermal damage and faster healing, but the Er:YAG is less effective for dermal collagen remodeling because the laser does not affect the dermis as significantly as the CO<sub>2</sub> laser. Studies indicate that the efficacy for treatment of rhytides and solar elastosis improves with increased thermal damage depth [9]. The most effective Er:YAG lasers for the treatment of rhytides use longer pulse durations to increase the residual thermal damage depth [10]. To enhance wound healing without sacrificing efficacy, a combined approach has become popular for ASR [11].

To overcome the problems associated with ASR procedures, so-called NDR techniques have emerged that selectively damage the dermal tissue to induce a wound response, but avoid damage to the epidermis [12–25]. In this technique, controlled dermal heating without epidermal damage is achieved by combination of laser treatment with properly timed superficial skin cooling. The wound response to thermally damaged dermal tissue results in formation of new dermal collagen and repair of tissue defects related to photoaging. The absence of epidermal

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We propose a new concept of skin rejuvenation called fractional photothermolysis (FP) that is specifically designed to overcome some of the previously mentioned problems. In contrast to ASR and NDR, which aim to achieve homogeneous thermal damage at a particular depth within the skin, FP creates microscopic thermal wounds and specifically spares tissue surrounding each wound. The schematics of the thermal damage patterns for ASR, NDR, and FP are illustrated in Figure 1. The microscopic treatment zones pictured in this cartoon form the building blocks for a macroscopic treatment effect. MTZs are intentionally made to be so small that they can barely be seen, if at all, without magnification. The macroscopic treatment effect of FP is characterized both by the arrangement and shape of these microscopic treatment zones within the skin. Laser parameters can be adjusted to produce different three-dimensional (3-D) MTZ shapes and depths [27]. In addition, similar to the use of microdots for reproduction of photographs in newsprint, it is in principle possible to achieve an arbitrary range of MTZ patterns. Thus, the skin can be treated primarily or "touched up" in a digital manner based on the density of the microscopic treatment zones.

In this report, we introduce the concept of FP and test the biological response to such treatment of human skin in vivo. Results are reported from clinical pilot studies that support the use of FP as a new, safe, and effective treatment modality.

New terms introduced in this paper are defined in Table 1.

## MATERIALS AND METHODS

# **Forearm Study**

Fifteen healthy subjects of skin type II-VI received a single treatment with a 1.5 µm laser system (Reliant MTZ<sup>TM</sup>SR prototype, Reliant Technologies, Palo Alto, CA). The protocol was approved by an institutional review board and each subject was consented before participating in the study. Treatments were performed within mapped test sites on the inner forearm to test the effects of three different MTZ densities (400, 1.600, and 6.400 per  $cm^2$ ) for a constant MTZ energy of 5 mJ. These densities correspond to distances between MTZ centers of 500, 250, and  $125 \,\mu\text{m}$  and an average fluence within the test site of 2, 8, and 32 J/cm<sup>2</sup>. To avoid bulk heating of tissue, individual MTZs were interleaved and a delay of approximately 50 milliseconds between individual pulses was designed into the system. Exclusion criteria were history of keloid formation, history of isotretinoin (Accutane) use within the last 6 months, and current systemic infections. The age range of the subjects was 24-64 years with an average of 43 years. Each test site was marked with 4 micro-tattoos at the corners of an 8 imes $8\,\mathrm{mm^2}$  test site. Within each test site the central area of 7 imes7 mm<sup>2</sup> size was exposed. Hairs within the test sites were removed by shaving. No cooling or anesthesia was used to perform the exposures. The test sites including the control were evaluated before exposure and 1-3 days, 1 week, 1 month, and 3 months following exposure for clinical appearance, erythema (MEXAMETER MX 18, Courage & Khazaka GmbH, Köln, Germany) and transepidermal



Fig. 1. Conceptual comparison of ablative skin resurfacing (ASR), non-ablative dermal remodeling (NDR), and fractional photothermolysis (FP). A: ASR removes the epidermis and causes residual thermal damage within the dermis. Reepithe-lialization is delayed because of relative long migratory path lengths for keratinocytes that repopulate from skin appen-

dages. **B**: NDR creates a layer of thermal damage below the surface without causing epidermal removal or damage. **C**: FP is the distribution of microscopically small volumes of thermal damage, MTZ, within the skin. Epidermal repair is fast due to small wounds and short migratory paths for keratinocytes.

Term	Definition
MTZ	A microscopic treatment zone of thermal injury
Fractional photothermolysis	Formation of isolated non-contiguous micro-thermal
(FP)	wounds creating necrotic zones surrounded by zones of
	viable tissue in a geometrical pattern that is not
	dependent on chromophore distribution
Micro-epidermal necrotic	A spheroid or button-shaped collection of necrotic debris
debris (MEND)	located just below an intact stratum corneum overlying
	each dermal wound (i.e., over each MTZ)

**TABLE 1. Definitions of New Terms** 

water loss (TEWAMETER TM 210, Courage & Khazaka). Up to four additional test sites with the same exposure parameters were performed on the other medial forearm in order to obtain histology at selected times after exposure. Samples were stained for H&E, LDH viability stain (NBTC) [28], Fontana–Masson, and colloidal iron. Slides were read by a blinded dermatologist.

## **Periorbital Study**

Thirty subjects aged 30-70 years enrolled in a clinical trial to examine the safety and efficacy for the treatment of lateral periorbital wrinkles with a clinical prototype device (Reliant MTZ<sup>TM</sup>SR prototype, Reliant Technologies). The protocol was approved by an institutional review board and each subject was consented before participating in the study. The optical system of the device consists of a fiber laser source and an optical deflection and focusing system that concentrates the light into focused spots within the skin. This system differed from that used in the forearm study and consisted of a hand-held scanning device that delivered a fixed laser pattern at a selected spot density as the operator moved the handpiece across the skin. The laser handpiece was moved continuously across the skin at 0.5 cm/second. This device did not compensate for the speed of hand motion across the skin, but the exposure times were kept short, i.e., less than 5 milliseconds, such that blurring of the MTZ was small.

# **Subject Selection**

Of the 30 subjects recruited for participation, 22 were female and eight male, and all subjects identified as Fitzpatrick skin types II–III. Subjects were excluded if they reported any active local or systemic infections, a history of atopic dermatitis or keloid scarring. Additionally, if currently taking Accutane, allergic to Lidocaine, or a smoker, they were not eligible for inclusion.

## **Treatment Procedures**

Subjects were randomly divided into four different treatment groups of varying energies and wavelengths at a constant MTZ density. Group 1: 1,480 nm, 6 mJ per MTZ; Group 2: 1,550 nm, 6 mJ per MTZ; Group 3: 1,535 nm, 10 mJ per MTZ; and Group 4: 1,535 nm, 12 mJ per MTZ. The treatment side of the face was chosen and subsequently mapped with a grid of carbon micro-tattoos superficially applied to the skin. Six tattoos were placed in triangular

formations within the treatment region and three control tattoos were placed in the adjacent non-treatment region at precise distances of 10 mm using a fixed template. Each laser treatment covered approximately 10 cm<sup>2</sup> of the lateral periorbital area. Subjects were offered local anesthesia in the form of a topical mixture of 1% lidocaine and 4% tetracaine (Lasercaine Forte, Unit Dose Pharmacy and Packaging, Phoenix, AZ). One treatment consisted of ten passes with the device to create a final MTZ density of 2,500 per cm<sup>2</sup>. Five passes were made in one direction and five passes were made in a perpendicular direction. The average fluence per pass was 1.5-3 J/cm<sup>2</sup>, depending on the energy per MTZ, which varied from 6 to 12 mJ. Each pulse had a duration of 1.5-5 milliseconds and produced a single MTZ. The rate of MTZ deposition was approximately 120 per second. The subjects were evaluated immediately post-treatment for any side effects and asked to score the intensity of pain felt during treatment. During each treatment visit, standardized photographic documentation, skin temperature measurements, and skin reflectance spectral measurements were collected. Subsequent laser treatments were provided at 4-7 days intervals based on subject scheduling. All subjects received four laser treatments.

The subjects were evaluated for side effects and changes 1 day following first treatment, 1 week following final treatment, and 1 month following final treatment. Pain was subjectively reported and scored by the subject during and after every treatment according to the following scale: 0, no pain and 10, bee sting. Erythema, edema, hyperpigmentation, hypopigmentation, and scarring were examined and scored at every visit by the investigator according to the following scale: 0, none; 1, mild; 2, noticeable; 3, moderate; 4, significant; 5, very significant; 6, severe. Changes in skin surface characteristics on treatment and non-treatment sides of the face were evaluated by both the subject and study investigators at 1 month and 3 months. Appearance of wrinkles and quality of skin texture were assessed by the investigator and the subject according to the following scale: 0, no improvement; 1, very slight improvement; 2, mild; 3, noticeable; 4, moderate; 5, very significant; 6, total improvement.

Distances between the corners of a triangle formed by three tattoos within the treatment region and within the control region were measured from subject photographs using Adobe Photoshop measurement tools. Six tattoo measurements were made within the treatment region and each measurement was repeated three times at every follow-up study visit (n = 18 per subject per visit). Similarly three replicate measurements were made in the control region (n = 9 per subject). After normalization to a reference ruler, each measurement was compared to the corresponding pre-treatment measurement. Average percentage change with respect to pre-treatment measurement was recorded for all nine reference distances. The average percentage change in treatment area distances and the average percentage change in control distances were calculated. Relative skin shrinkage was reported as the percent change in the average distances between tattoos in the treatment region divided by average distances between tattoos in the untreated region.

Two independent dermatologists conducted blinded analysis of the pre-treatment and 1 month and 3 months follow-up study photographs (n = 12 at 3 months, n = 2 at 1 month). Each dermatologist was asked to score pretreatment and 3 months photographs according to the Fitzpatrick wrinkle grading scale (Table 2). The subject's before and after photographs were included as a set and the investigator was asked to score each according to the Fitzpatrick wrinkle grading scale. Then the dermatologist was asked to select the photograph within the set, which appeared as "better overall." The dermatologist was asked to assign separate improvement scores for appearance of wrinkle, pigmentation, and texture (Table 2).

## **Statistical Analyses**

Statistical analyses were performed using the SAS Statistical package, version 8.10 (SAS Institute, Cary, NC). Study investigator scored assessments of treatment regions were reported for appearance of wrinkles and quality of skin texture as mean score changes. Paired tests were used to compare the mean values of pre-treatment and 3 months follow-up for both tattoo distances and Fitzpatrick wrinkle scores. Sample size calculations were performed based on the variance of tattoo shrinkage to confirm the appropriate population sizes for each treatment group.  $\chi^2$  tests and Pearson correlation coefficients were used to assess the relationships between post-treatment erythema and treatment groups as well as pain and treatment groups.

#### RESULTS

#### **Forearm Study: Clinical Results**

All forearm exposures were performed without the need for any anesthesia using a prototype device that produced a

**TABLE 2. Blinded Investigator Scoring Standards** 

Fitzpatrick wrinkle score (1–9)	Improvement scores (0–3)
Class I, scores 1–3 Class II, scores 4–6 Class III, scores 7–9	0, none 1, minor; 1–33% 2, moderate; 34–66% 3, marked; 67–100%

pattern of micro-thermal wounds (MTZ) at various densities. The sensation during the exposure of individual test sites was typically compared to a prickling sensation, and there was pronounced pulse to pulse variability in the intensity of each sensation.

Treatments with MTZ spacing of 250 and 500 µm (i.e., medium and low MTZ densities) were very well tolerated. Induration due to edema became palpable and minor erythema was noted within a few minutes after exposure. These sites demonstrated clinically normal skin color after 1-2 weeks. Spectrophotometer measurements (Fig. 2) show minimal erythema that resolved within 1 week for the low and moderate MTZ density and persisted for 3 months for the high MTZ density. Clinically, a slight bronzing appeared within a 2-3 days and resolved within 2 weeks. Epiluminescence microscopy revealed that this bronzing was due to a regular pattern of brown spots (Fig. 3) which matched the pattern of laser exposures provided by the device. Individual brown spots resolved in a process of superficial desquamation. Comparing Figure 3A.D. the texture of treated skin sites appeared smoother and there was apparent decrease in dermatoglyph relief during the first 3 months. This clinical response seemed to correlate with repair of MTZ wounds at the microscopic level as described below. Low and medium MTZ densities did not produce any significant side effects in any subject and produced no measurable change in transepidermal waterloss (TEWL) due to treatment.

These results from low and medium MTZ densities were in marked contrast to the high MTZ density sites (125  $\mu$ m separation) which produced significant side effects. Immediate whitening was followed within 1 day by oozing and crusting and significant elevations for TEWL. Within 1–2 weeks TEWL measurements returned to normal levels and these high-density treatment sites exhibited sloughing, formation of confluent granulation tissue, and elevated erythema. The epidermal separation observed in the high MTZ density sites confounded erythema measurements by spectrophotometry (Fig. 2) thus creating artificially low measurements for the 1 day and 1 week follow-up visits. The erosions due to high MTZ density treatment resolved within 2 weeks, but resulted in hyperpigmentation for darker skin types.

## **Forearm Study: Histologic Results**

Figure 4 depicts the histology that corresponds to the individual brown spots observed by epiluminescence microscopy. In panel A (left), the regular pattern of brown spots is apparent in a subject with type VI skin at 1 day post exposure and medium spot density exposure. A biopsy sample taken from a skin site treated identically in the same subject (Fig. 4B) shows well defined, cylindrically shaped clear areas within the papillary and reticular dermis. These clear areas represent complete loss of cell viability (LDH stain) that results from individual laser pulses. Little or no loss of epidermal viability is apparent at 1 day, but overlying each dermal wound is a spheroid or button-shaped collection of necrotic debris located just below an intact stratum corneum. These button-shaped foci



Fig. 2. Average erythema values from 15 subjects as measured by spectrophotometry. Erythema resolves quickly following FP treatment when densities are low and moderately spaced. High-density treatments result in erythema that persists for up to 3 months.

(MEND, for *micro-epidermal necrotic debris*) seen in histology correspond to the brown spots observed via epiluminescence microscopy. Each MEND structure measured approximately  $40 \times 80 \ \mu m$  in cross section.

To further characterize cutaneous healing response, LDH viability staining was performed on biopsies taken from multiple treatment sites after various times. Figure 5A shows that immediately after treatment both epidermal and dermal cell necrosis are present within a sharply defined area of about 100 µm in diameter extending up to 400 µm deep. One day following treatment, however, no epidermal defect was apparent by LDH staining (Figs. 4B, 5B). Loss of dermal cell viability was co-localized with loss of collagen birefringence as assessed by cross polarization microscopy (data not shown). Homogenization of dermal matrix also appeared to demarcate each thermal damage zone in the dermis corresponding to individual MTZ using both Fontana-Masson and H&E staining (Fig. 5D-F, small arrows). Loss of LDH stain corresponding to MTZs within the dermis was still evident 1 week after treatment (data not shown). After 3 months, however, there was no evidence of the MTZ detected by LDH staining in either dermal or epidermal compartments (Fig. 5C).

Within 24 hours of exposure, the epidermal defects were repaired in a relatively rapid process of keratinocyte movement involving the effective extrusion of damaged epidermal components by viable keratinocytes at the lateral margins of the MTZ. This process of epidermal elimination appears to be responsible for the formation of the MEND structure. Importantly, the stratum corneum was histologically spared and remained intact overlying each MEND until they were sloughed after 1 or 2 weeks (Fig. 5F, large arrows). Fontana–Masson staining indicates that MENDs contain melanin (Fig. 5D,E) among other components. The pigment content of MEND is enhanced for darker skin types (compare Fig. 5D,E). Close examination of the basal layer in these photomicrographs demonstrates realignment of keratinocytes at the margins of the MTZ starting in the basal layer, where cell shape changes are apparent (arrows, Fig. 5D), consistent with conversion from a stationary cuboidal cell to a spindleshaped migratory cell phenotype [29,30].

The basement membrane within each MTZ contained a characteristic cleft that appears immediately following laser exposure and is located beneath the damaged basal keratinocyte layer (Fig. 5F). This clefting persisted for up to 2 weeks and was associated with a loss of reticulin staining (data not shown). The dermal portion of the MTZ appeared to be repaired within the first month. For low and moderate MTZ densities, little or no inflammatory cell infiltrates or granulation tissue was observed. This is in marked contrast to high MTZ densities for which granulation tissue formation and marked inflammatory response were observed. While no changes in LDH staining were observed



Fig. 3. Cross polarized photomicrograph (epiluminescence) images of forearm skin showing surface healing response in a single subject following FP treatment. A: Pre-exposure, (**B**) 1 day post-exposure, (**C**) 1 week post-exposure, (**D**) 3 months post-exposure. Individual brown spots are visible after 1 day and begin to slough by 1 week.



Fig. 4. Correlation of clinical and histological characteristics of FP in a subject with type VI skin, 1 day post-exposure with MTZs spaced on a 250  $\mu$ m grid. A: Cross-polarized photomicrographs of skin surface; (B) LDH viability stain. Cylindrically shaped clear areas in the dermis represent loss of cellular

metabolism at laser impact sites (i.e., MTZs). Brown spots visible in (A) are due to collections of melanin and micro-epidermal necrotic debris (MEND) structures measuring approximately  $40\times80~\mu m$  just beneath an intact stratum corneum.



Fig. 5. Histology composite from the forearm study. **A**–**C**: LDH staining of treated skin shows healing response at 1 hour (A), 1 week (B) and 3 months (C) post-exposure. Clear areas represent loss of cell viability. Note epidermal repair is complete within 24 hours. **D**, **E**: Fontana–Masson stain showing formation of MEND structure and epidermal MTZ wound response. In (D) arrows indicate keratinocyte morphologic changes in a subject with type II skin, 1 day post exposure. Dark areas represent melanin. In (E) MEND is nearly extruded in a subject with type IV skin, 1 week post-exposure.

**F**: H&E stain depicts microanatomy of the MTZ at 1 day post exposure. Small arrows show homogenization of dermal matrix components below a clefted basement membrane and large arrows show MENDs just below an intact stratum corneum. **G**, **H**: Colloidal iron stain; (G) is pretreatment;(H) is a replicate adjacent site on the same subject, 3 months post-treatment with moderate MTZ density and demonstrates increased mucin content in the superficial dermis and enhanced rete ridge patterning.

in 3 months biopsies, enhanced mucin staining (colloidal iron) within the papillary and superficial reticular dermis was observed versus matched unexposed control skin procured at the same time (Fig. 5G,H). An increased undulating rete ridge pattern was also apparent in treated skin versus control sites (Fig. 5H,G). These trends in histological appearance were observed by blind inspection of samples, but were not quantified.

## **Periorbital Study**

To further investigate the clinical effects of FP as it applies to skin resurfacing, the lateral periorbital area of 30 subjects was treated with a scanning laser handpiece that generated an array of microscopic thermal wounds at a constant density. Topical anesthesia (Lasercaine) was used before treatments for most subjects, as necessary. Treatments were well tolerated by all subjects with an overall average pain intensity score of  $3.2\pm0.9$  (scale of 0-10). Pain levels were directly correlated with laser treatment energies over a range of 6-12 mJ per pulse. First pass pain levels were usually lower than subsequent passes and were variously described as "feeling like a hot brush" or "a mild prickling" sensation. Subsequent passes were perceived as more of a burning discomfort or a dull pain. All pain sensations generally subsided within 10-60 minutes and no significant differences in pain were noted by subjects with successive treatment visits. Immediately following treatment, skin surface temperatures were elevated by an average of 1-2°C.

Post-treatment edema scores ranged from 0 to 3 (scale of 0-6) and typically subsided within 6-24 hours. At 1 day, about a third of the subjects had noticeable erythema. Approximately 10% of subjects had mild edema and noticeable erythema that persisted for up to 1 week. Table 3 indicates that FP treatment of facial skin is well tolerated with minimal side effects. Erythema, edema, and pain correlated directly with laser energies for pulse energies in the range of 6-12 mJ/pulse. Similarly, the shortest wavelength (1,480 nm) was associated with slightly increased erythema and edema levels. No evidence of hyper- or hypo-pigmentation was noted in any subject.

TABLE 3.	Clinical	Side	Effects	of Periorbital	FP
Treatment	t				

Characteristic	Mean/SD
Erythema	
1 day post-treatment	$1.44\pm0.6$
1 week post-treatment	$0.714\pm0.7$
1 month post-treatment	$0.08\pm0.3$
3 months post-treatment	0
Edema	
1 day post-treatment	$0.8\pm0.7$
1 week post-treatment	$0.12\pm0.3$
1 month post-treatment	0
3 months post-treatment	0
Pain	
Average pain intensity $(0-10)$	$3.2\pm0.97$

During the first 2 weeks following treatment, however, the skin had a slight "bronzing" similar to the results seen in the forearm study. Epiluminescence microscopy revealed this discoloration was due to thousands of individual brown spots on the skin surface (MENDs) which disappeared by desquamation within 2 weeks. Simple face washing appeared to accelerate this process.

Mild to moderate improvement in wrinkles and skin texture was reported both by subjects and by study investigators after 1 month and 3 months as shown in Table 4. For the untreated side of the face, all subjects and investigators reported no change in the appearance of wrinkles and quality of skin texture. On the treated side, no subjects were observed to have worse skin characteristics following treatment and only 1 of 28 subjects was rated as having no improvement in appearance of wrinkles at the 1 month follow-up visit on the treated side of the face. Twelve percent of the subjects demonstrated mild improvement in the appearance of wrinkles (investigator scores), 30% demonstrated noticeable improvement. and 54%demonstrated moderate to significant improvement. Similarly, 53% of subjects showed moderate improvement in the quality of skin texture. At the 3-month follow-up visit, only two out of 15 subjects were ranked to have no improvement in the quality of skin texture. Noticeable improvement reported at the 1-month follow-up visit was generally sustained through the 3-month examination. At 3 months, 34% of subjects showed moderate improvement or better in appearance of wrinkles and 47% of subjects showed moderate improvement or better in quality of skin texture. Independent blinded dermatologists selected the posttreatment photographs as "better" overall in 96% of the subject photographs sets. The Fitzpatrick wrinkle score improved by an average of 0.9±0.7 points from pretreatment to the 3-month follow-up visit. This change

TABLE 4. Observed Improvement in the Appearanceof Wrinkles and the Quality of Skin Texture

Characteristic	1 month	3 months
	(n = 28)	(n = 15)
Improvement in		
appearance of wrinkles		
By subject	$2.9 \pm 1.5$	$2.1\pm1.6$
By investigator	$2.5\pm1.2^{*}$	$1.6\pm1.3^{**}$
Improvement in quality		
of skin texture		
By subject	$3.2\pm1.3$	$2.1\pm1.6$
By investigator	$2.7 \pm 1.2^*$	$1.8\pm1.4^{**}$

\*One month Pearson correlation coefficient between subject and investigator wrinkle score r = 0.58, P = 0.002; texture score r = 0.79, P = 0.001.

\*\*Three months Pearson correlation coefficient between subject and investigator wrinkle score r = 0.74, P = 0.001; texture score r = 0.74, P = 0.002.

Scale 0-6 (0, no improvement; 1, very slight improvement; 2, mild; 3, noticeable; 4, moderate; 5, very significant; 6, total improvement).

demonstrated statistical significance in the paired t-test analysis.

Figure 6 demonstrates before and after photographs of three representative subjects who received treatment in the periorbital area. There is noticeable improvement in class I and II rhytides and an improvement in dyschromia. The panel of blinded dermatologists scored a set of 14 clinical photographs of treatment sites before and 3 months after treatment. These results are shown in Table 5 and demonstrate that there was a mild to moderate improvement in skin texture, periorbital wrinkles, and photodamage consistent with the investigator and subject scoring data presented above.

Table 6 shows the relative linear shrinkage along the triangular-shaped sides of the microtattoo patterns of treated skin compared to control sites. Although only the 1 week data were statistically significant there was a trend of immediate shrinkage within the first week followed by an apparent relaxation after 1 month that was followed by a retightening by 3 months. This sequence of skin shrinkage is similar to that reported for ablative resurfacing.

# DISCUSSION

Although various techniques are currently used for skin resurfacing, the application of a pattern of multiple individual microscopic thermal wounds has not been previously reported. Previous work from our group [27] demonstrated that arrays of microscopic photothermal lesions can be created within human skin in vitro using a low power infrared laser device focused at high numerical aperture, at a laser wavelength absorbed by tissue water. In the current study, we investigate the biological and clinical effects of producing these microscopic thermal wounds (MTZ) in human skin in vivo.

Laser microbeams have been used in experimental biology and ophthalmology for decades [31,32], but no therapeutic microbeam devices have been developed for skin surgery or dermatology. By adjusting focal depth and pulse energy, different tissue compartments can be arbitrarily chosen as primary sites for photothermal lesions. For this study, recent advances in semiconductor infrared lasers, fiber lasers, miniaturized optics, and computerdriven beam controllers were used to develop a functional FP system for clinical use in skin resurfacing. We believe this novel technique will offer a greater degree of freedom to treat photo-damaged skin, and propose to call the general approach FP.

FP is distinct and yet similar to the well-known process of selective photothermolysis (SP) originally described over 20 years ago [33]. Both SP and FP cause small, spatially limited zones of photothermal effects within tissue due to local energy deposition. Widespread clinical use of SP for decades has shown that this type of injury is very well tolerated; the same is true of FP in this study. In any photothermal process, including SP and FP, distribution of thermal excitation is proportional to the product of the local optical energy density times the local optical absorption coefficient [34]. While SP relies on selective absorption of a largely uniform optical field by pigmented target structures, FP relies on optical foci within a largely uniform medium. It should be noted that SP and FP are conceptual descriptions of idealized situations (Table 7). In practice, neither the medium nor the optical field is ever completely homogenous.

Prior to this study, nothing was known about clinical response to FP. Experimental treatment of the face using moderate MTZ density in this study was effective, well tolerated, and safe. Clinical use of FP can be based on this study, but further details about optimal FP lesion size, spacing, depth, shape, number of treatments, and treatment interval remain to be refined. The ability to arbitrarily adjust size, depth, and density of photothermal lesions during FP suggests that clinical response can be finely adjusted. For example, "feathering" at the edge of a treated area can be accomplished by decreasing MTZ density to avoid leaving a noticeable treatment border. In SP, therapeutic response is primarily adjusted by wavelength, fluence, and pulse duration. In FP, response is primarily adjusted by MTZ shape, depth, and pattern. Like SP, FP produces an adjustable 3-D microscopic thermal burn. The comparison raises interesting questions about the impact of microscopic thermal pattern density. On one extreme, it is certain that a few microscopic thermal lesions will have essentially no therapeutic effect. On the other extreme, tightly packed FP lesions would create a 2-D burn, equivalent in many ways to laser resurfacing or, if the epidermis is spared, to very aggressive non-ablative laser rejuvenation. It is apparent from this study that both efficacy and side effects are dependent on the shape and location of individual MTZs and on the pattern in which the MTZs are arranged.

Potentially, the efficacy of FP may also be more consistent than that of the lasers now used for non-ablative remodeling. With pulsed lasers, conflicts arise between obtaining sufficiently high power, treating with a large spot size, and producing unwanted bulk heating. The principle of FP overcomes these problems by allowing tight beam focusing to achieve high local irradiance in each MTZ, while keeping average irradiance to a low level that avoids bulk heating of skin. For example, the pulsed mid-infrared lasers now used for non-ablative remodeling (e.g., at wavelengths 1.32, 1.4, or 1.5 µm) heat very large volumes of dermis compared with each MTZ used for FP. It would be unsafe to drive the temperature of dermis to the point of thermal cell necrosis in such large volumes. In practice, this limits the ability of physicians to set the fluence of a nonablative laser at a consistently safe and effective level. In contrast, we have shown in this study that FP MTZs, which clearly produce cell necrosis and collagen denaturation, are very well tolerated at moderate and lower MTZ densities, which were also found to be effective. High local irradiance during FP essentially "guarantees" a biological effect, while the tight spatial confinement of each MTZ provides a wide safety margin, similar to that of SP.

The forearm study results demonstrate that photothermal tissue necrosis involving epidermal and dermal compartments down to the mid-reticular dermis was clinically well tolerated when MTZ grid spacing was above



Fig. 6. Three subjects before (**A**, **C**, and **E**) and after (**B**, 1.5 months post-treatment; **D**, 3 months; and **F**, 1 month) FP on the lateral periorbital areas. Note effacement of class I and II rhytides, and improvements in dyschromia and skin texture.

TABLE 5. Blinded Grading of Pre-Treatment andFollow-Up Photographs

Fitzpatrick wrinkle score	
Pre-treatment	$5.1\pm1.9$
Post-treatment	$4.2\pm1.6$
Mean score improvement	$0.9\pm0.7$
Improvement score (0–3)	
Appearances of wrinkles (0–3)	$1.6 \pm 1.3^*$
Pigmentation improvement (0–3)	$1.9\pm1.4^*$
Texture improvement $(0-3)$	$1.7\pm1.3^*$

\*Paired *t*-test analysis demonstrated statistical significance (P < 0.001).

TABLE 6. Measurement of Linear Skin ShrinkageBetween Micro-Tattoos Placed in the PeriorbitalRegion

Follow-up	Number (n)	% Shrinkage in treatment area, relative to control
1 day	23	$1.7 \pm 4.6 \ (P = 0.083)$
1 week	20	$2.9 \pm 4.2 \; (P = 0.006)$
1 month	18	$0.6 \pm 3.0 \; (P = 0.430)$
3 months	7	$2.1\pm3.7\;(P{=}0.197)$

twice the diameter of individual MTZs (i.e.,  $>200 \ \mu$ m). Considerations were made to assure that the effects observed were related to MTZ density and not to non-specific bulk heating by adjusting the timing between energy pulses. Thousands of MTZ wounds clinically appeared as a mild bronze discoloration that presented within the first few days and resolved within 2 weeks of treatment by mild desquamation. Side effects commonly seen in ablative resurfacing procedures [2–4,35] were not observed in either the forearm or periorbital studies reported here. Presumably, these same effects could be elicited by increasing MTZ density to a very high percentage of the skin.

The application of a 3-D, microscopic pattern of individual laser wounds, in contrast to a continuous layer of thermal damage, produced rapid healing in this study. The epidermal component of each MTZ is rapidly repaired within the first few hours after exposure in a process that has not been previously described in the literature. The histologic analysis indicates rapid healing by keratinocyte migration into each epidermal MTZ. In less than 1 day, there was micro-reepithelialization to produce melanincontaining, spheriod-shaped structures (MENDs). Formation of MENDs occurs within hours in a process that does not require cell mitosis but is likely to involve transition of bordering keratinocytes from a resting state to a wound phenotype in an effort to close a breached basement membrane, similar to macroscopic wound repair processes [29,36]. MENDs collect beneath the corneal layer within 24 hours where they persist for up to 2 weeks, correlating with observed clinical bronzing.

Based on histology, lack of TEWL changes, and clinical observations following FP treatment, it appears that skin barrier function is preserved, achieved both by a relative sparing of the stratum corneum during laser exposure and the fact that collections of MENDs do not seem to alter normal corneocyte biology. This deserves further study. Preservation of barrier function following treatment may explain the lack of clinically evident oozing and crusting and the absence of skin infections noted. As MTZ density approaches a critical value (in this study, near 125  $\mu$ m center-to-center MTZ separation), the dose-response curve for FP appears to steepen. We noted this effect in the forearm study at high MTZ density. Further studies are planned to investigate the MTZ wound repair phenomenon in more detail.

The effects of FP on epidermal pigment are of interest because current non-ablative treatments in the infrared region have little or no effects on epidermal pigmented lesions, and ablative techniques remove the complete pigmented layer which results in prolonged pigmentary perturbation [35]. In our study, there were several dark skinned subjects, who demonstrated little or no significant pigmentation abnormalities after FP at low or medium MTZ densities per treatment. Histology revealed that there is a localized, well-controlled melanin release and transport mechanism using MENDs as a "vehicle." Unwanted localized accumulations of pigment (e.g., solar lentigines) appear to be effectively removed in a precise and gradual manner as observed in the periorbital study (see Fig. 6C-F and Table 5). Further investigation regarding this aspect of FP may offer new concepts for treatment of dyschromia.

The dermal component of MTZ repair involves removal and replacement of necrotic cellular materials and denatured extracellular matrix in a relatively prolonged process that does not appear to involve a classical "inflammatory" phase of wound repair. While histology samples from biopsies taken both 1 day and 1 week post exposure show little evidence of significant inflammatory cell infiltration, the immediate erythema and induration observed clinically following treatment (Table 3) are consistent with the generation and release of wound mediators in both epidermal and dermal compartments. Remodeling of matrix components was apparent by 3 months, at which time increased mucin staining was observed in the

TABLE 7. Key Differences Between Selective Photothermolysis (SP) and FP

Characteristic	$Selective \ photothermolysis \ (SP)$	FP
Optical field in medium	Homogeneous	Focused beam
Optical properties of medium	Local absorbers	Homogenous
Confined thermal damage	Target chromophore	Optical focus regions

superficial dermal compartment. Furthermore, we noted increased undulating rete ridge patterns in treated skin relative to control skin. Effacement of rete ridges is characteristic in aged skin [37-39]. Both enhanced rete ridge patterning and increased mucin deposition are desirable markers for skin rejuvenation [40,41]. We speculate that the clinical efficacy of FP for skin resurfacing will depend in part on the dermal remodeling phase of MTZ repair, as this wound response appears to coincide with clinical improvement in photoaged skin. In this study, 92% of subjects demonstrated improvement in the appearance of wrinkles and skin texture; both study and blinded investigator scores showed significant improvements in photoaged skin parameters (Tables 4 and 5). In addition, small, but reproducible, skin shrinkage was observed as measured by microtattoo placement. Skin shrinkage was still evident 3 months following treatment; however, longer follow-up may be required to document the full effects of this treatment. As shown in previous studies [8,9,13,42], we hypothesize that skin tightening may be a factor in the improvement of wrinkle appearance.

Pain and discomfort experienced by subjects in this study were minimal (Table 3). Treatment of large surface areas with MTZ wounds, which are applied in a serial manner, offers advantages compared to homogeneous treatments where tissue damage is provided simultaneously. It is remarkable that while pronounced localized tissue damage occurs, the exposure could be performed without anesthesia. The lack of temporal and spatial summation of nociceptor activation [43,44] may partially explain this observation and offers considerably more options to minimize the need for anesthesia during FP.

## CONCLUSIONS

FP is a promising new modality that, based on this study, produces a consistent level of efficacy for treatment of photoaged skin with significantly reduced side effects. Current treatment modalities, such as AR and NDR, cause thermal injury in a 2-D layer. In contrast, FP creates microscopic, 3-D patterns. The building blocks of this concept are the microscopic lesions, MTZs, which can be placed with the desired density on the skin to adjust the level of treatment. We have demonstrated that an array of MTZs is a promising new treatment for skin remodeling. We designed a device to deliver these MTZs with adjustable parameters and tested it in two complementary clinical studies. Histology revealed complete epidermal and dermal necrosis sharply confined to MTZs of sub-millimeter size. In spite of marked localized damage, these MTZ wounds were very well tolerated and epidermal repair was completed in less than one day. Side effects typically observed for ablative techniques, such as marked erythema and oozing were absent for all but the most extreme parameters. It was demonstrated that this device and technique can be safely and effectively used to treat periorbital skin and improve clinical markers of photoaging. The results of the study suggest that it is possible to perform FP on all skin types including dark pigmented skin. Further studies are required to optimize treatment parameters and protocols. Optimizing the use of FP for photoaged skin, and for potential applications, such as treatment of dyschromia, acne, and scars remain to be explored.

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