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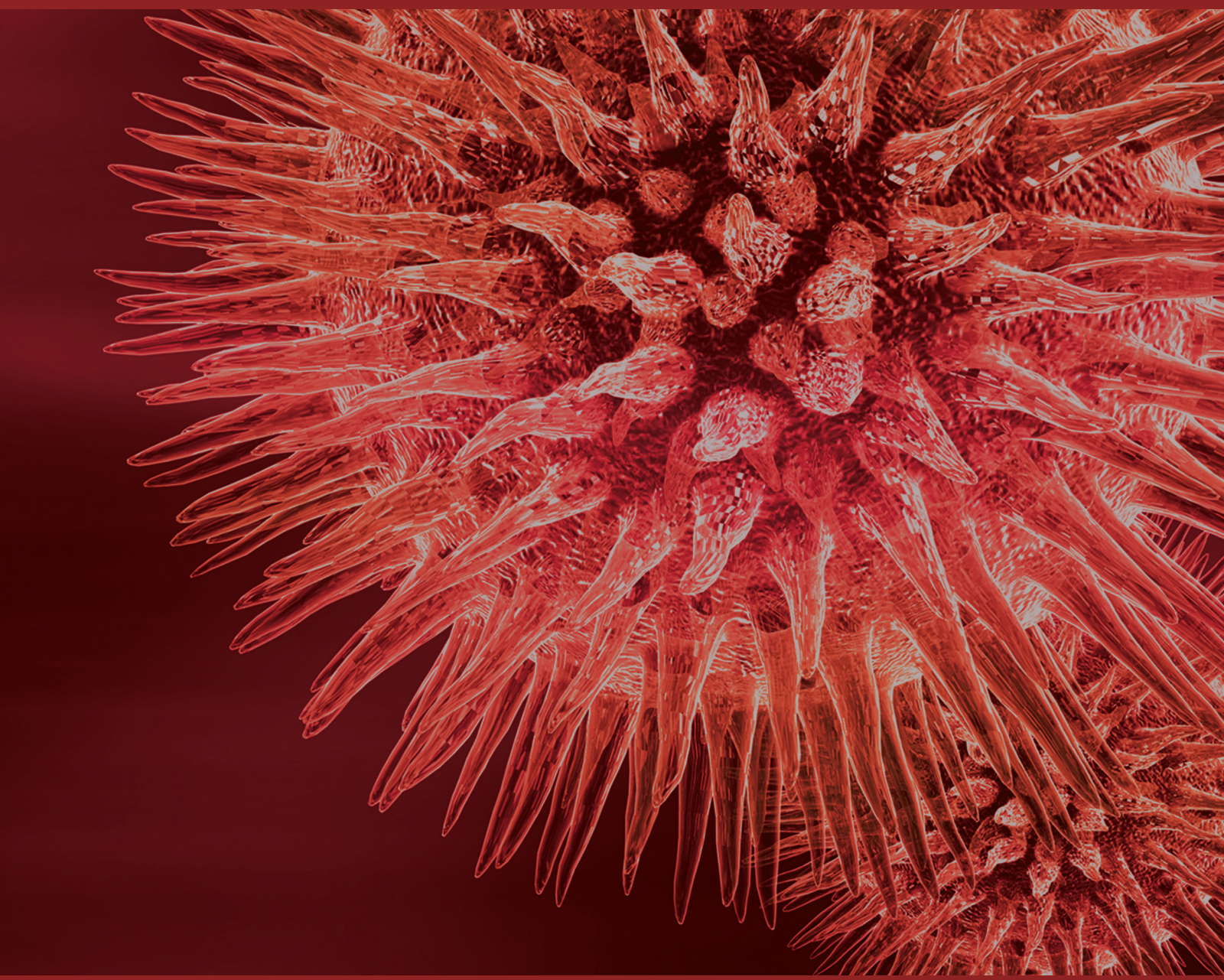
Lasers and Energy Devices for the Skin: Conventional and Unconventional Use

Guest Editors: Steven Paul Nistico, Andrea Chiricozzi, Federica Tamburi, and Giovanni Cannarozzo



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Hindawi Publishing Corporation
BioMed Research International
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http://dx.doi.org/10.1155/2016/9031091

Editorial

Lasers and Energy Devices for the Skin: Conventional and Unconventional Use

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Received 1 August 2016; Accepted 1 August 2016

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Laser technology represents nowadays an important advance in dermatology. Physical, technological, and clinical research is currently carried out in order to optimize laser-skin interaction and laser efficacy-safety profile.

The different dermatological indications may be summarized following the different spectral features of the laser sources used:

(i) UV laser and light sources: they have been used primarily for the treatment of inflammatory skin diseases and/or vitiligo, as well as striae. The mechanism of action is immunomodulatory. The XeCl excimer laser emits at 308 nm, near the peak action spectrum for psoriasis. Other UV nonlaser sources like the 355 nm have also been used for vitiligo and hypopigmentation disorders and various inflammatory diseases. The paper by N. Zerbinati et al. contributes to explaining the mechanism of action of the 355 nm laser.

(ii) Violet IPL spectra and low-power 410 nm LED and fluorescent lamps: both are used either alone or with ALA. Alone, the devices take advantage of endogenous porphyrins and kill *P. acnes*. After application of ALA, this wavelength range is highly effective in creating singlet O_2 after absorption by PpIX. Uses include treatment of AKs, actinic cheilitis, and BCCs.

(iii) Near-IR(A) (595, 755, and 810 nm): these wavelengths are used primarily to treat blood vessels and hyperpigmented lesions. They are positioned in the absorption spectrum for blood and melanin and will penetrate deeply enough to treat vessels up to 2 mm. Newer lasers, such as the Pulsed Dye 595 nm, may be indicated for the treatment of Port Wine Stains and infantile haemangiomas. Q switched lasers in this spectrum may be useful to treat multicoloured tattoos. The article by our group covers the nonconventional dermatological applications of this wavelength.

(iv) Near-IR(B) 940 and 1064 nm: these two wavelengths have been used extensively for lager and deeper blood vessels on the legs and face. Because of the depth of penetration (on the order of millimeters), they are especially useful in coagulation of deeper blood vessels and selective follicle denaturation for safe and effective hair removal. Q switched 1064 nm lasers are very effective on dark ink tattoos.

(v) Medium-IR lasers (1320–1540 nm): they heat tissue water, shrink collagen, and are widely used in cosmetology for antiageing purposes, treatment of striae, and acne scarring (nonablative fractional procedures).

(vi) Far-IR systems: represented mainly by the CO_2 and Er:YAG lasers. Dermatological applications are mainly surgical (warts, dermal nevi) and cosmetological thanks to their precision in ablation. Fractional CO_2 lasers guarantee a very precise epidermal and dermal heating that makes this device ideal for facial skin resurfacing (fine or moderate wrinkles,

dyspigmentation, and acne scarring) on facial skin. A novel application on keratosis pilaris has been herein covered by V. Vachiramon. Newer devices combine lasers to other energy sources such as radiofrequency in order to optimize antiageing dermatological procedures. In this special issue, these nonlaser systems are deeply covered (W. Manuskiatti et al. and D. H. Kim et al.).

By compiling these papers, we hope to enrich our readers and researchers with respect to the laser technology in the field of dermatology and cosmetology.

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Hindawi Publishing Corporation
BioMed Research International
Volume 2016, Article ID 7981640, 6 pages
http://dx.doi.org/10.1155/2016/7981640

Clinical Study

Nonconventional Use of Flash-Lamp Pulsed-Dye Laser in Dermatology

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Received 25 February 2016; Accepted 28 July 2016

Academic Editor: Marília G. de Oliveira

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Flash-lamp pulsed-dye laser (FPDL) is a nonablative technology, typically used in vascular malformation therapy due to its specificity for hemoglobin. FPDL treatments were performed in a large group of patients with persistent and/or recalcitrant different dermatological lesions with cutaneous microvessel involvement. In particular, 149 patients (73 males and 76 females) were treated. They were affected by the following dermatological disorders: angiokeratoma circumscriptum, genital and extragenital viral warts, striae rubrae, basal cell carcinoma, Kaposi's sarcoma, angiolymphoid hyperplasia, and Jessner-Kanof disease. They all underwent various laser sessions. 89 patients (59.7%) achieved excellent clearance, 32 patients (21.4%) achieved good-moderate clearance, 19 patients (12.7%) obtained slight clearance, and 9 subjects (6.1%) had low or no removal of their lesion. In all cases, FPDL was found to be a safe and effective treatment for the abovementioned dermatological lesions in which skin microvessels play a role in pathogenesis or development. Further and single-indication studies, however, are required to assess a standardized and reproducible method for applying this technology to "off-label" indications.

1. Introduction

Flash-lamp pulsed-dye laser (FPDL) is a nonablative laser technology that has gained an excellent reputation in the treatment of vascular lesions. It uses a rhodamine dye that is dissolved in a solvent and pumped by a flash-lamp producing an emission of a 595 nm wavelength, approximately near to the hemoglobin's and oxyhemoglobin's absorption peaks. It is therefore considered the most specific laser currently available for the treatment of superficial vascular lesions. Current indications of this technology have further been extended to include nonvascular lesions but with vascular structural involvement, which makes them amenable to being treated with such laser.

FPDL does not always represent the first-line treatment for all these lesions: in fact, many lesions can be successfully treated using different treatment methods, and others (viral warts and molluscum contagiosum) may sometimes disappear without external intervention; therefore, the specification of when FPDL could be beneficial is crucial. Dermatological lesions that represent the target may be considered typical vascular lesions, vascular dependent lesions, or nonvascular lesions, according to a recent classification [1].

Vascular lesions include not only port-wine stains, superficial hemangiomas, and telangiectasias in which dye laser is considered the gold standard therapy but also angiokeratomas and lesions typical of the Bourneville-Pringle syndrome that may be treated surgically or through cryotherapy.

Vascular dependent lesions can be divided into the following: viral infections such as verruca vulgaris and genital viral warts, inflammatory skin diseases such as example localized psoriasis and lupus erythematosus, connective tissue diseases such as hypertrophic scars, keloids, and striae rubrae, neoplastic dermatosis such as basal cell carcinoma, Kaposi's sarcoma and, angiolymphoid hyperplasia.

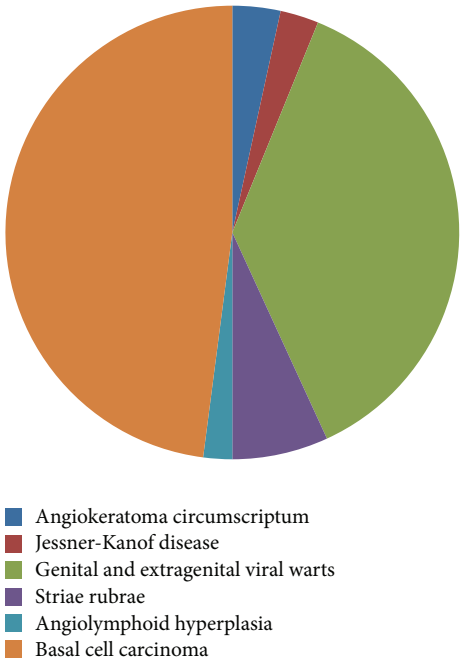
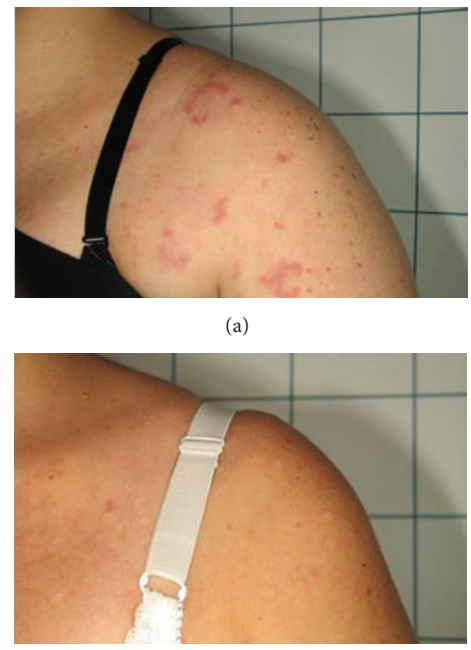


Figure 1: Type of lesions.



(a)

(b)

Figure 2: (a) A patient affected by Jessner-Kanof disease at baseline. (b) The disappearance of the lesions 12 months after the last PDL session.

Nonvascular lesions, on the other hand, include viral infections like molluscum contagiosum as well as other cutaneous conditions, for example, xanthelasma palpebrarum [1–13].

We selected a number of vascular dependent lesions and nonvascular lesions in an open study in order to verify the outcome of FPDL treatments.

2. Materials and Methods

A total of 149 patients affected by angiokeratoma circumscriptum (5), Jessner-Kanof disease (4), genital and extragenital viral warts (54), striae rubrae (10), angiolymphoid hyperplasia (3), basal cell carcinoma (70), and Kaposi's sarcoma (3) were selected for treatment. Figure 1.

Patients underwent different treatment sessions with the FPDL (Synchro Vas-Q, Deka MELA, Florence, Italy) as indicated by Table 1.

All patients underwent treatment after obtaining a detailed personal history (skin type, clinical manifestations, health conditions, previous medications, and life-style) and informed consent.

Five cases of angiokeratoma circumscriptum with presence of asymptomatic warty, keratotic nodules localized on the external genitals of adult men were treated using a 10600 nm CO_2 laser (Smartxide^2 Dot/RF, DEKA MELA, Florence), at 0.2–0.5 W, 10 Hz in order to obtain vaporization prior to three FPDL sessions.

Four patients with histological diagnosis of Jessner-Kanof disease, a benign yet chronic lymphocytic skin disorder, were treated in four FPDL laser sessions (Figure 2).

Fifty-four patients affected from viral warts (30 males and 24 females), between the ages of 6 and 75 years (mean 34.39 years old), were recruited with a total of 85 warts. The majority were children (range 6–18 years old) with localization on the hands (20 patients); in particular, lesions were located on the hands (palms, dorsum, and the fingertips), the periungual and subungual region, on the feet (12 patients) especially on the soles, in one case, on the fifth finger, and on the face (2 patients). Twelve of the older patients presented lesions on the periungual and subungual regions and eight of them had genital warts. All warts underwent at least one prior established treatment without success. All patients in the study were treated with the flash-lamp pumped-dye laser (three to seven treatment sessions every four weeks), preceded by curettage of the typical hyperkeratosis through salicylic acid 30% ointment, especially for the mosaic plantar warts (Figure 3).

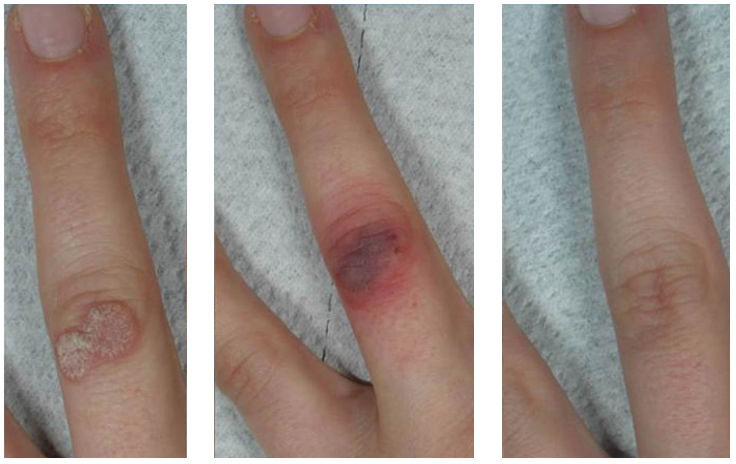
Ten patients with striae rubrae were also treated with the FPDL. Four to six sessions of FPDL were employed in the treatment of striae which had the following localizations: abdomen/groin (3 patients), axilla/anterior shoulder (4 patients), and buttock/upper thigh (3 patients).

Three young females affected by angiolymphoid hyperplasia whose diagnosis was based on clinical and histological findings were selected in the present study. Patients received a previous radiotherapy with no results. In all these cases, patients were treated in three sessions using FPDL in addition to ablative CO_2 laser (0.5–0.7 W) in order to obtain complete vaporization of the lesions.

Seventy patients (38 males and 32 females, aged 47 to 78) with superficial and nodular nonpigmented basal cell carcinoma (BCC), diagnosed after dermoscopic evaluation, were recruited. Most parts of the lesions were characterized by limited size (diameter < 1 cm) (Figure 4). The basal cell

Table 1: Specific PDL approaches for the different cutaneous disorders.

Type of lesions	Patients	Sessions of PDL	Parameters	Combination with CO_2 laser
Angiokeratoma circumscriptum	5	3	Wavelength 595 nm Energy 6-7 J/cm^2 Spot size 12 mm Pulse 0.5–1.5 ms	X
Jessner-Kanof disease	4	4	Wavelength 595 nm Energy 6-7 J/cm^2 Spot size 12 mm Pulse 0.5–1.5 ms	
Viral warts	54	3–7	Wavelength 595 nm Energy 6-7 J/cm^2 Spot size 12 mm Pulse 0.5–1.5 ms	
Striae	10	4–6	Wavelength 595 nm Energy 6-7 J/cm^2 Spot size 12 mm Pulse 0.5–1.5 ms	
Angiolymphoid hyperplasia	3	3	Wavelength 595 nm Energy 6-7 J/cm^2 Spot size 12 mm Pulse 0.5–1.5 ms	X
Superficial and nodular basal cell carcinoma (BCC)	70	5	Wavelength 595 nm Energy 6-7 J/cm^2 Spot size 12 mm Pulse 0.5–1.5 ms	
Kaposi's sarcoma	3	4	Wavelength 595 nm Energy 6-7 J/cm^2 Spot size 12 mm Pulse 0.5–1.5 ms	

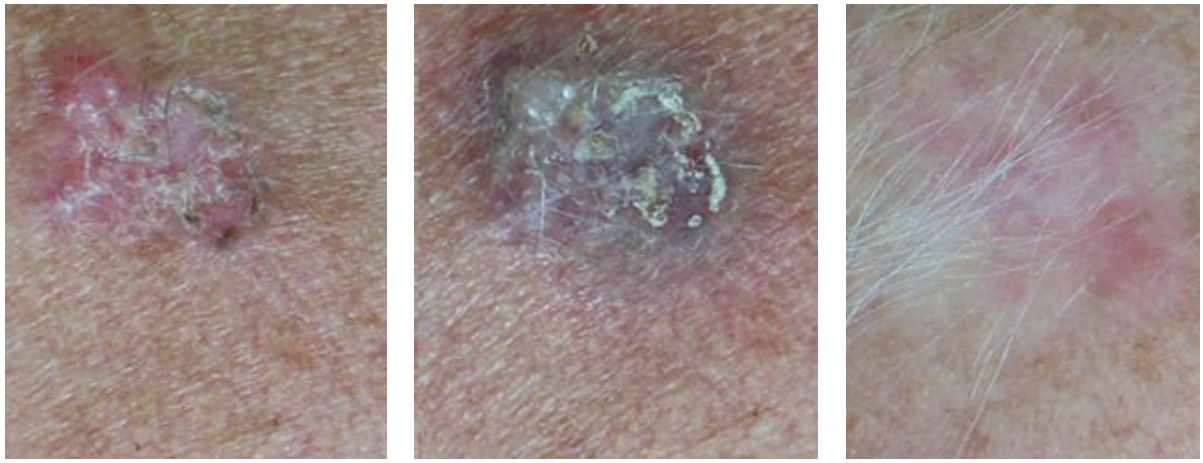


(a) (b) (c)

Figure 3: (a) A viral wart of the finger at baseline. (b) The typical immediate purpura after the PDL therapeutical session. (c) The excellent result achieved after 5 PDL treatments.

carcinomas were localized mainly on the face and neck area and in anatomically difficult areas as the nasal wings or the periocular zone. One patient had an allergy to anesthetics and five were cardiopathic, which limited the usage of surgical excision. All patients were subject to FPDL in five sessions with a 595 nm wavelength that enabled deeper penetration to the target (oxyhemoglobin or nuclear chromatin).

Three patients with classic Kaposi's sarcoma were treated with four FPDL sessions. The reason for treatments was due to the inability to undergo surgery or poor response to pharmacological treatments.



(a) (b) (c)

Figure 4: (a) A superficial basal cell carcinoma from the back of a woman at baseline. (b) The typical purpura due to PDL. (c) The disappearance of the lesion 12 months after the last PDL session.

Table 2: Global improvements.

Score 1	Score 2	Score 3	Score 4
Low or no removal of their lesion	Slight clearance	Moderate-good clearance	Excellent clearance
9 (6%)	15 (10%)	23 (15.4)	102 (68.4%)

Table 3: Subjective evaluations show that the vast majority of subjects were satisfied or very satisfied.

Unsatisfied	Not very satisfied	Satisfied	Very satisfied
2 (1.3%)	14 (9.3%)	23 (15.4%)	110 (73.8%)

A summary of the methodology used for the different indications is provided in Table 1.

Laser fluence settings for all diseases were 6–8 J/cm^2, a spot size of 12 mm, and a pulse duration of 0.5–1.5 ms.

Most of the lesions were treated without anesthesia. Its use has been limited as the procedure itself was not so painful and also because local anesthesia could cause oedema and hinder the "visual feedback processing" during treatment. An effective cooling device was always used during each laser session, improving comfort. Patients were instructed to avoid sun and cosmetics during the immediate postprocedural periods and to apply cool gauzes, emollient creams, and sunscreens until complete recovery. Daily application of cool wraps was useful to prevent the appearance of vesicles and blisters. An antibiotic ointment, gentamicin 0.1%, was also requested to be applied to the target areas for 7 days after each laser session, avoiding potential cutaneous superinfections.

3. Results

Results obtained were judged immediately and 4 weeks after the last session; treatment outcome was assessed by ranking the results into four categories, a quartile scale, of lesion clearance in comparison to baseline: 1 indicates no or low results (0–25% of the lesion area cleared), 2 indicates slight clearance (25–50% of the lesion area cleared), 3 indicates moderate-good clearance (50–75%), and 4 indicates excellent clearance (75%–100%).

Patients were asked for a subjective evaluation of the perceived overall results by means of the following score: unsatisfied, not very satisfied, satisfied, and very satisfied.

All patients observed global improvements (Figures 2–5 (a), (b), and (c)). All the lesions were completely removed except in the case of 7 patients with striae rubrae where lesions did not disappear at 12-month follow-up and two cases of BCC which showed a recurrence that was completely excised through surgery four weeks later.

102 patients (68.4%) achieved excellent clearance, 23 patients (15.4%) achieved good-moderate clearance, 15 patients (10%) obtained slight clearance, and 9 subjects (6%) had low or no removal of their lesion (Table 2).

Patients were asked for a subjective evaluation of the results: 110 patients (73.8%) were very satisfied, 23 (15.4%) were satisfied, and 14 (9.3%) were not very satisfied with the results, whereas only 2 patients (1.3%) were unsatisfied (Table 3); the low satisfaction rate was due probably to long-term purpura produced by the use of higher parameters which caused discomfort; dissatisfaction with the results was due to higher expectations in case of 2 patients with striae.

Relevant side effects, such as blisters, crusts, atrophy, and scars, were absent in all conditions; many patients showed typical FPDL-induced side effects like swelling and

(a) (b) (c)

Figure 5: (a) Kaposi's sarcoma of the penis, baseline. (b) A temporary little ulcer after 3 PDL sessions. (c) The final result 12 months after the last PDL session, with no side effects and a great satisfaction of the patient.

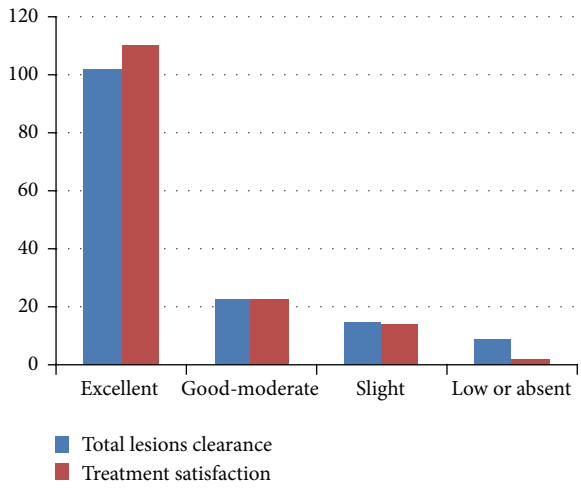


Figure 6: Results.

purpura, which disappeared three to ten days after treatment. Five patients reported long-lasting purpura (30 days). See Figure 6.

4. Discussion and Conclusion

Although intense flash-lamp pulsed-dye laser (FPDL) is normally used in vascular malformation therapy, we treated patients with a number of nonvascular indications.

The study evidences an overall patient improvement that may represent a valid alternative to other procedures.

A correct selection of patients using FPDL is the most useful strategy, as this technique is beneficial in selected patients, such as those with persistent and/or recalcitrant dermatological disorders with vessel involvement. Pediatric population, for example, may become alarmed by treatment and refuse painful procedures resulting in poor patient compliance [14]. Cardiopathic patients, subjects using anticoagulant drugs or unable to receive anesthesia, have great difficulty in undergoing surgical treatment and are thereby more likely to be candidates for FPDL treatment. Lastly, surgical devices are not recommended in certain areas, such as the face, neck, nose, nasal wings, groins, and anogenital areas, due to the risk of disfiguring scars or keloids.

We believe that the success of FPDL treatment lies in the fact that most of the lesions mentioned contain a large number of dilated blood vessels which represent the target of the device. The mechanism of action of FPDL is thereby based on specific destruction of abnormal vessels, components of the lesions themselves (angiokeratoma circumscriptum and striae rubrae), or a selective thrombosis of vessels with the consequent obliteration of the nutrient supply to the lesions (viral wart, angiolymphoid hyperplasia, Kaposi's sarcoma, and basal cell carcinoma).

Also, authors recently demonstrated that the 585 nm FPDL can decrease fibroblast proliferation and collagen type III deposition and furthermore may induce apoptosis and upregulation of extracellular signal-regulated kinase and p38 mitogen-activated protein kinase activity. Its use may be therefore extended to other dermatological conditions such as keloids [15–18].

In this study, similar to previous studies [19], different vascular or vascular dependent lesions were treated using FPDL laser alone or in combination with pulsed CO_2 laser. FPDL in all cases was reported to be a safe, well-tolerated, and effective treatment method and may be considered an alternative or a complementary treatment for resistant and/or recalcitrant lesions or when contraindications do not suggest the use of other therapies.

Its use, however, should be limited to selected cases in which labelled therapies have not proved effectiveness or when patients are unable to undergo such treatments. Also, off-label procedures must always be carried out carefully, with a strict follow-up in order to ensure safety.

The high cost of the procedure may represent a limit in its use, despite the excellent aesthetic outcome of results.

Future multicentrical studies on single indications, however, are desirable, with possible harmonization of the methods and parameters used.

Competing Interests

All authors declare to have no competing interests in this work neither financial nor personal.

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Hindawi Publishing Corporation
BioMed Research International
Volume 2016, Article ID 3415136, 6 pages
http://dx.doi.org/10.1155/2016/3415136

Research Article

Skin Treatment with Pulsed Monochromatic UVA1 355 Device and Computerized Morphometric Analysis of Histochemically Identified Langerhans Cells

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Received 27 February 2016; Revised 6 June 2016; Accepted 27 June 2016

Academic Editor: David Bernardo

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Fluorescent or metal halide lamps are widely used in therapeutic applications in dermatological diseases, with broadband or narrow band emission UVA/UVA1 (320–400 nm) obtained with suitable passive filters. Recently, it has been possible for us to use a new machine provided with solid state source emitting pulsed monochromatic UVA1 355 nm. In order to evaluate the effects of this emission on immunocells of the skin, human skin samples were irradiated with monochromatic 355 nm UVA1 with different energetic fluences and after irradiation Langerhans cells were labeled with CD1a antibodies. The immunohistochemical identification of these cells permitted evaluating their modifications in terms of density into the skin. Obtained results are promising for therapeutical applications, also considering that a monochromatic radiation minimizes thermic load and DNA damage in the skin tissues.

1. Introduction

Langerhans cells are immunocompetent cells located in the epidermis of the skin. These cells have been provided with cytoplasmic processes intermingled with keratinocytes; they do not contain keratin and do not form desmosomes with keratinocytes. They represent a pool of cells which are part of the peripheral immunosystem playing a specific role in the pathogenesis of some inflammatory diseases of the skin including psoriasis [1, 2]. Langerhans cells are directly involved in the starting step of the complex mechanism of detection, processing of antigens contacting epidermis, and presentation of processed antigens to lymphocytes, with a crucial role as peripheral sentinels.

The use of nonionizing radiations, particularly in the UV range as elective treatment targeting Langerhans cells of many skin inflammatory diseases, has been extensively studied and applied in clinical practice [3].

Many devices using sources emitting wave lengths in the UV (A, B, and C), in the visible or infrared range targeting Langerhans cells, have been extensively used in clinical applications concerning atopic dermatitis, mycosis fungoides, scleroderma, lupus erythematosus, and psoriasis. However, the wavelengths and energetic fluences have to be tuned to the specific absorption characteristics of the irradiated tissues.

As different new devices have been recently introduced, we have studied and evaluated the effects of a pulsed monochromatic UVA1 treatment on Langerhans cells. These cells were identified with immunohistochemical method as CD1a-positive cells for CD1a (Cluster of Differentiation 1a antigen). Langerin is also a Langerhans cells marker but is

expressed in mature cells. In histochemical preparations, the number of langerin-positive cells is lower than the number of CD1a-positive cells in the skin [4, 5], and therefore the evaluation of CD1a-positive cells was more suitable for morphometric evaluation of the UVA1 treatment effects on the eyelid skin here used.

2. Material and Methods

Eyelid samples from healthy subjects collected after surgical excision were irradiated (UVA1 355 nm) for 2 min, 3 min, and 4 min for a total transmitted energies, respectively, of 50, 75, and 100 J/cm^2 and maintained with control samples 100% UVA1 protected (0 J/cm^2) for 1 hour at 37°C in a culture medium optimized for skin [6].

For treatment, the energy UVA1 was produced by Alba 355 (EVLASER, Italy) based on a solid state laser (DPSS: Diode Pumped Solid State laser) used as an active medium and a coupled neodymium-doped yttrium orthovanadate (Nd:YVO4) crystal. The light emitted by the Nd:YVO4 at a wavelength of 1064 nm is sent to a crystal separator of 1064 nm harmonic components, particularly the second (532 nm) and the third (355 nm). By cutting off the second harmonic, the third harmonic emitted was filtered on. In this manner an emission of a single wavelength of 355 nm was obtained, as the UVA1 monochromatic wavelength used in this study.

After treatment, samples were fixed with a paraformaldehyde 4% solution PBS buffered, in order to obtain not only a good morphology but also the preservation of Langerhans cells specific antigens. Skin samples were then processed for microscopy through treatments of dehydration, paraffin embedding, microtome sectioning, rehydration, hematoxylin and eosin staining, last dehydration, and mounting on slides for observation. Particular care was used for a correct orientation of specimens in the embedding and sectioning procedures in order to have sections perfectly perpendicular to the skin surface.

Microscopic observations and digital recordings were made at a light microscope Carl Zeiss Axiophot provided with a 5-megapixel CCD camera Nikon DS-Fi2.

2.1. Immunohistochemistry. Antigenicity of the specific markers molecules, in particular CD1a (Cluster of Differentiation 1a), was well preserved with the fixative we have used, so immunohistochemistry with anti-CD1a antibody permitted the identification of Langerhans cells in the skin [7]. Tissue sections for immunohistochemistry were reacted with mouse monoclonal antibody anti-CD1a (DAKO, Agilent Technologies, USA). Slides were baked for 1 hour at 50°C, deparaffinized, and hydrated through a series of ethanol solutions at decreasing concentration to water. Slides were then treated with 0.05% Tween 20 in tris buffered saline for 5 minutes to permeabilize tissues and washed in tris buffered saline. Slides were heated in steamer in 1 mmol/L EDTA buffer (pH 8.0) or 10 mmol/L sodium citrate buffer (pH 6.0) for 20 minutes for antigen retrieval and treated with 3% hydrogen peroxide for 10 minutes to quench endogenous peroxidase activity. After washing with tris buffered saline, the slides were incubated with the primary antibody for 30 minutes at room temperature and then incubated with Envision1 (DAKO Corp.) anti-mouse labeled polymer conjugated with horseradish peroxidase for 20 minutes at room temperature. The immunolabeling reaction was revealed using DAB1 (Sigma-Aldrich, USA), and the sections were counterstained with hematoxylin. All immunohistochemical staining procedures were performed on an Autostainer Plus DAKO (DAKO Corp.).

2.2. Image Analysis. The selective immunocytochemistry staining not only permitted the identification of Langerhans cells but also permitted the application of a computerized morphometric analysis using ImageJ (NIH), a well-known software recognized as standard tool by international scientific community.

Computerized morphometric analysis was performed on immunostained sections with a thickness of 8 μm, with a sampling area of 4 sqmm for each skin sample. Each section was digitally recorded at the microscope through a 5 Mpixels ccd camera at the same magnification and in the identical conditions of the color temperature of the light source. Resulting microphotographs were used for ImageJ morphometric analysis with the following steps:

(1) Equalization of the image extended to the level limits of 0 and 255 in order to detect and acquire, at the same extent for all slides, the finest structural stained details for the best selection of Langerhans cells in their whole extension.

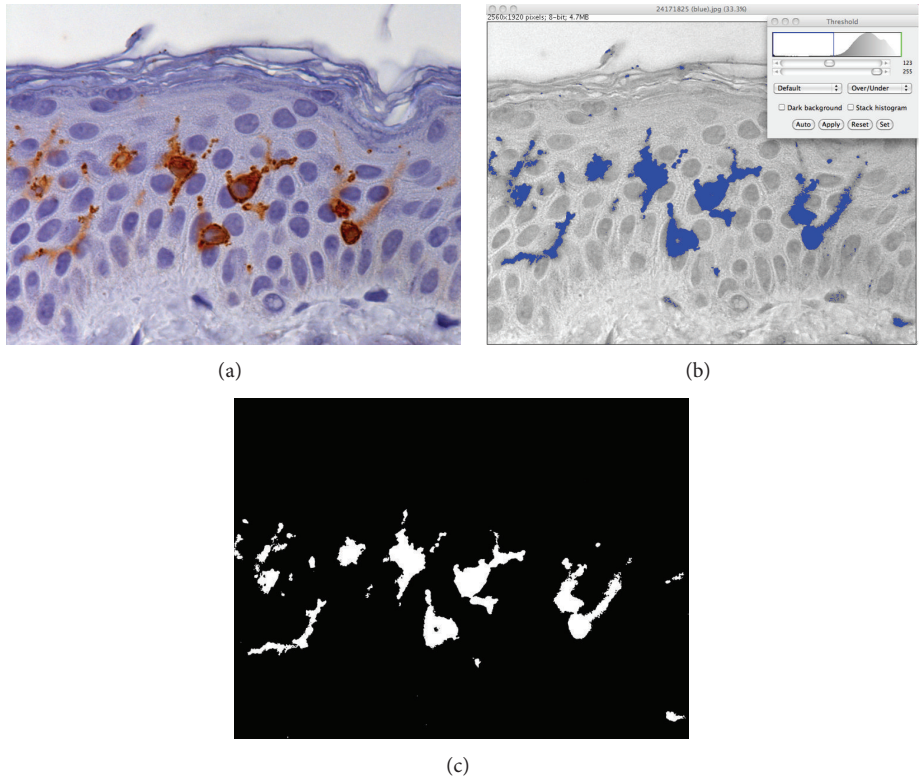
(2) Segmentation of the continuous tone image is defining the most suitable threshold segmentation value for obtaining a binary image representing faithfully the Langerhans cells over a background of keratinocytes. For maintaining the full reliability of the specimens comparisons, the threshold values were the same for all slides.

(3) Measurements and collection of data and quantitative evaluation were performed. All results are presented in graph as mean percentage ± standard deviation in Figure 3. Statistical analysis was performed comparing samples with Student's t-test. Differences were considered significant at $p < 0{,}01$.

3. Results

Skin samples were UVA1 irradiated with energy fluences of 50, 75, and 100 J/cm^2, and controls were 100% UVA1 protected (0 J/cm^2). They were processed for histology and histochemistry all together in each step of the procedures, in order to minimize possible differences due to technical reasons and to assure a reliable comparative analysis.

In Figure 1, immunostaining and image analysis of a control section are reported. In Figure 1(a), Langerhans cells, body, and cytoplasmic processes, labeled by specific antibody for CD1a, appear clearly visible (brown) on the background of keratinocytes faintly stained with hematoxylin. Figure 1(b) is representing the segmentation of the continuous tone



(a) (b)

(c)

Figure 1: More relevant steps of the image analysis procedure illustrated on a skin control sample. (a) Immunohistochemical analysis identified CD1a-positive Langerhans cells (brown) on a background of keratinocytes faintly hematoxylin stained. (b) Continuous tone image with labeled CD1a-positive Langerhans cells, blue masked following segmentation (inset), ready for digital conversion. (c) Binary image resulting from digitalization where white areas are constituting the pool of pixels to be evaluated.

image through a passband digital filter with related blue mask. Figure 1(c) shows the digitalized image where white pixels are constituting the areas to be measured as percentage of the 2560 × 1920 pixels standard reference frame. This methodological way has been applied with the same segmentation parameters to all sections obtained from controls (Figures 2(a), 2(b), and 2(c), 100% protected) and from specimens irradiated with energy fluences of 50, 75, and 100 J/cm^2, respectively (Figures 2(d)–2(l)). Finally (Figures 2(c), 2(f), 2(i), and 2(l)), the white areas of the digital images representing CD1a-positive Langerhans cells were measured, as percentage of the full area of the same reference frame.

The results of the measurements are reported as Figure 3, where the immunoreactivity for CD1a, covering 8,34% (SD ± 1,12) of the frame in controls, appears to be reduced to 4,37% (SD±1,24) at 50 J/cm^2, to 3,18% (SD±0,86) at 75 J/cm^2, and to 0,73% (SD ± 0,48) at 100 J/cm^2. Statistical analysis between samples showed significative differences ($p < 0{,}01$, asterisks in Figure 3). The reduction of the CD1a labeled Langerhans cells areas is related to the energetic fluence, with a maximal reduction after treatment with energetic fluence of 100 J/cm^2.

4. Discussion and Conclusions

The fluorescent lamps have low irradiance (5–10 W/cm^2) and delivery low UVA dose in long time (10–30 J/cm^2). High-output metal halide source has a much greater irradiance (80 W/cm^2) and can irradiate medium or high doses in treatment sessions of 50 min. Conventional machines for UVA treatment emit wavelengths mainly between 340 and 400 nm but may also produce scattered radiation >530 nm including infrared irradiation (780–3000 nm). Conventional UVA phototherapy may be accompanied by extensive heat load predominantly generated by infrared irradiation (780–3000 nm) and/or insufficient cooling systems of the phototherapy devices.

Light therapy lamps with halogen-metal band confined to the very high irradiance UVA1 (340–400 nm) have experienced a growing success and interest [8]. Unlike UVA2 and UVB (290–340 nm), the biological effects of UVA1 on the skin are mainly related to oxidative stress, mediated by aerobic photooxidation with the intermediate formation of reactive oxygen species such as singlet oxygen, superoxide anion, and hydroxyl radicals [9–11].

About 37% of Caucasian UVA1 irradiation penetrates deeply in the skin up to 60–90 mm and therefore these wavelengths can effectively modulate the activity of immune and inflammatory cell populations resident or patrolling the epidermis and the dermis.

UVA1 may induce apoptosis of T lymphocytes, especially T helper, not only through late mechanisms dependent on a synthesis *de novo* of some proteins but also through mechanisms that are early, preprogrammed, and independent

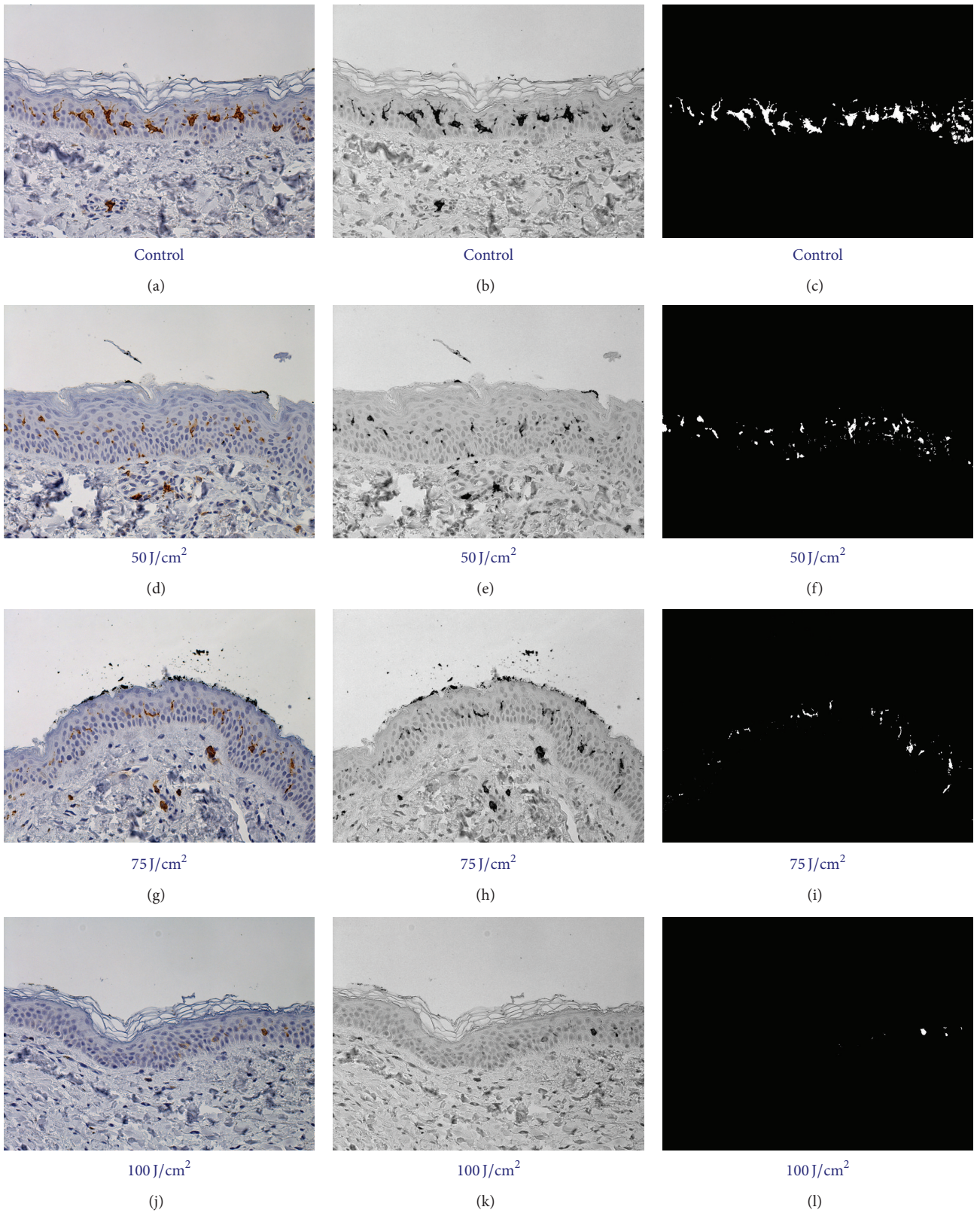


Figure 2: The same procedure of Figure 1 was applied to all samples: control (a, b, and c), and samples irradiated with fluences of 50, 75, and 100 J/cm^2, respectively (d–l). Columns identify original images (a, d, g, and j), segmented images (b, e, h, and k), and digital images (c, f, i, and l).

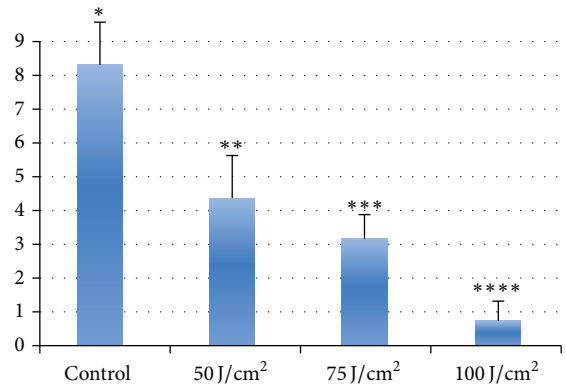


Figure 3: Percentage of CD1a-positive Langerhans cells in skin control and treated with monochromatic UVA1 355. Results of the measurements, where the units represent the percentage areas occupied by CD1a-positive Langerhans cells in controls and irradiated, respectively, over the same reference areas. Student's t-test results: $*$ versus $**$, $**$ versus $***$, and $***$ versus $****$, for all $p < 0{,}01$.

of the UVA1 proteins synthesis. This is causing the production of PGE2 and thromboxane in the cells of Langerhans but does not alter the number and function and induces migration in satellite lymph nodes [3].

The recent availability of a machine emitting pulsed monochromatic UVA1 355 nm wavelength (Alba 355) allowed evaluating the effects of such irradiation on CD1a-positive Langerhans cells, considering that a monochromatic radiation minimizes collateral effects in the irradiated tissues. The radiation used (not a broad or narrow band but a monochromatic wave) and the very short time of single pulses permitted a much better control of the energy transfer to the skin. In this way, at the same time, it minimizes thermal surcharge of tissues and risks related to DNA damage, with pyrimidine dimers formation [12]. Our experimental model has been eyelid skin fragments freshly excised and maintained in short term organotypic culture (one hour, comprehensive of the short time of irradiation). The protocols for UVA1 355 nm phototherapy of skin samples involved the irradiation of single specimens with 50, 75, and 100 J/cm^2, respectively, and specimens 100% protected as control.

As the most common and reliable antigen for the histochemical identification of Langerhans cells in the epidermis, we have considered the CD1a antigen, a transmembrane glycoprotein structurally related to the Major Histocompatibility Complex (MHC) protein antibody currently used as specific marker of these cells [13]. CD1a, also expressed on macrophages on the connective tissue, is highly specific for Langerhans cells in the epidermis and used in routine dermatological diagnostic practice. Our *in vitro* experimental condition of the skin samples and the short time following irradiation did not permit considering the possible migration of these cells in the dermis and towards lymph nodes.

Our results are demonstrating significant modifications of the percentage of anti-CD1a labeled cells in the epithelium of skin treated with specific 355 nm monochromatic wavelength, with an effect energy-transfer dependent, minimizing collateral adverse effects on DNA differently from wide spread UVA applications in dermatological diseases [11].

Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

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Hindawi Publishing Corporation
BioMed Research International
Volume 2016, Article ID 6939018, 7 pages
http://dx.doi.org/10.1155/2016/6939018

Research Article

Thermal Response of *In Vivo* Human Skin to Fractional Radiofrequency Microneedle Device

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Received 7 December 2015; Accepted 5 April 2016

Academic Editor: Steven P. Nistico

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Background. Fractional radiofrequency microneedle system (FRMS) is a novel fractional skin resurfacing system. Data on thermal response to this fractional resurfacing technique is limited. *Objectives*. To investigate histologic response of *in vivo* human skin to varying energy settings and pulse stacking of a FRMS in dark-skinned subjects. *Methods*. Two female volunteers who were scheduled for abdominoplasty received treatment with a FRMS with varying energy settings at 6 time periods including 3 months, 1 month, 1 week, 3 days, 1 day, and the time immediately before abdominoplasty. Biopsy specimens were analyzed using hematoxylin and eosin (H&E), Verhoeff-Van Gieson (VVG), colloidal iron, and Fontana-Masson stain. Immunohistochemical study was performed by using Heat Shock Protein 70 (HSP70) antibody and collagen III monoclonal antibody. *Results*. The average depth of radiofrequency thermal zone (RFTZ) ranged from 100 to 300 μm, correlating with energy levels. Columns of cell necrosis and collagen denaturation followed by inflammatory response were initially demonstrated, with subsequent increasing of mucin at 1 and 3 months after treatment. Immunohistochemical study showed positive stain with HSP70. *Conclusion*. A single treatment with a FRMS using appropriate energy setting induces neocollagenesis. This wound healing response may serve as a mean to improve the appearance of photodamaged skin and atrophic scars.

1. Introduction

The concept of fractional photothermolysis (FP) has been developed to overcome the limitations of ablative and nonablative resurfacing systems [1]. Subsequently, FP has been considered as a new technique for facial rejuvenation by inducing homogeneous, fractional thermal damage at a particular depth of the skin, followed by collagen remodeling process. This technique creates microscopic noncontiguous columns of thermal injury in the dermis (referred to as microscopic thermal zones or MTZ), surrounded by zones of viable tissue. Therefore, the reepithelization process can occur faster with fewer side effects, when comparing to those of ablative skin resurfacing system [2–4].

Application of fractional technology to nonablative skin rejuvenation techniques has potential to provide efficacy while maintaining advantages of minimal to no downtime. However, despite these safety advantages the clinical efficacy profile does not match that of the full ablative process, especially with respect to moderate-to-severe rhytides and atrophic scars. Subsequently, fractional delivery systems for ablative lasers, including CO_2 and Er:YAG, have also been developed in an attempt to match the clinical results achieved with traditional ablative lasers. Ablative fractional lasers (AFLs) appear to lead to a more robust wound healing response and accompanying dermal fibrosis, which may explain the rapid and significant clinical effects that can be achieved with ablative versus nonablative devices. However,

AFLs are associated with a higher risk of postinflammatory hyperpigmentation (PIH), compared with that of nonablative fractional lasers (NAFLs) [4, 5].

Fractional radiofrequency (RF) system is one of novel fractional resurfacing techniques. It creates controlled thermal damage in the dermis and stimulates wound healing response, initiating collagen remodeling process by using thermal production from tissue impedance and subsequent heat diffusion to deeper tissue. An advantage of fractional radiofrequency is that it causes less epidermal disruption by only 5%, compared with 10–70% of that of fractional ablative laser systems [6]. The healing process is faster with minimal downtime. Therefore, this technique may be an alternative choice of facial rejuvenation and atrophic scars, especially in patients with darker skin complexion.

Recently, fractional radiofrequency microneedle system (FRMS) was introduced [7, 8]. By using insulated microneedle electrodes penetrating through the epidermis to directly deliver radiofrequency pulse at the upper dermal level while sparing the epidermis, this technique has shown to provide equivalent efficacy with less side effects, comparing with traditional ablative fractional resurfacing procedures [9].

The current available information on the wounding response of human skin to FRMS treatment is limited. Several of the previous studies on wound healing responses to FRMS only investigated in the Western subjects [3, 10]. In fact, racial differences in skin pathophysiology have been well documented [11]. The high risk of pigmentary alterations and scarring following any procedure that produces inflammation of the skin continues to influence physicians to exercise caution with this group of patients.

The purpose of this study was to investigate the histological response after FRMS treatment with varying energy settings and pulse stacking techniques in dark-skinned individuals by using histopathology and immunohistochemistry to identify zones of denatures collagen, inflammatory infiltration, neoelastogenesis, and neocollagenesis, as well as any side effects to pigmentation and textural alteration, throughout the 3-month study.

2. Materials and Methods

2.1. Study Design. Two female participants (skin phototype IV), aged 43 and 45 years, prescheduled for abdominoplasty by a plastic surgeon, were enrolled in the study. Both patients had no underlying skin disease, no history of topical medication use within 1 month, and no history of laser treatment within 6 months on their abdomen skin prior to the study. This study was approved by the Siriraj Institutional Review Board (SIRB) and written informed consent was obtained from all study participants.

2.2. Fractional Radiofrequency Device. Study subjects were treated with a FRMS (INTRAcel system, Jeisys Inc., Korea). The handpiece of this system consisted of 49-gauge needle electrodes in a 1×1 cm^2 area. Each electrode was 1.5–2.0 mm in length. While the proximal 0.5 mm of each electrode was insulated with a biocompatible material, the uncovered distal part emitted RF pulse to the treated area. Treatment levels can be adjusted ranging from the test level to level 5 (Table 1). The study subjects were treated with different energy levels at 6 different time periods, including immediately, 1 day, 3 days, 1 week, 1 month, and 3 months prior to their prescheduled abdominoplasty to follow up on the chronological evolution of the *in vivo* wounding responses (Table 2).

Table 1: Treatment parameters.

Energy	Voltage (watt)	Radio wave (millisecond)	Time of electrodes existing in the skin (millisecond)
Test level (needle insertion only)	0	0	250
1	50	30	280
2	50	50	300
3	50	80	330
4	50	100	350
5	80	50	300

2.3. Treatment Regimens. Prior to surgery, 30 squares, size 1.5×1.5 cm^2, were tattooed on each patient's abdominal wall as shown in Figure 1. The study participants were scheduled to receive 6 treatment sessions, immediately, 1 day, 3 days, 1 week, 1 month, and 3 months before their prescheduled abdominoplasty. At each visit, each participant received FRMS treatment using 5 different energy levels on 5 test areas (Table 2). Preoperatively, the treated area was cleaned with 2% chlorhexidine solution. Lidocaine 2.5% and prilocaine 2.5% cream (a eutectic mixture of local anesthetic, AstraZeneca LP, Wilmington, DE) was applied under occlusion for an hour before treatment.

Immediately before abdominoplasty, 30 biopsy specimens from the marked test sites and one from a controlled untreated site were collected by using a 4 mm punch biopsy (Stiefel Laboratories, NC, USA). Each of the specimens was placed into 10% (v/v) neutral buffered formalin overnight before paraffin embedding. The specimens were sectioned vertically into 5 μm thick slices and stained with hematoxylin and eosin (H&E), Verhoeff-Van Gieson (VVG) elastic, colloidal iron, and Masson-Fontana stain for histopathologic analysis.

Immunohistochemical study was performed by using Heat Shock Protein 70 (HSP70) antibody (Thermo Scientific, MA, USA) for evaluation of inflammatory response and neocollagenesis. The staining method for HSP70 was done in the following steps. Antigen retrieval was performed by incubation in citrate buffer (pH 6.0) at 95°C for 40 minutes in water bath and neutralized endogenous peroxidase activity with 3% hydrogen peroxidase followed by block nonspecific binding with 2% bovine serum albumin for 20 minutes [12]. The histological examination of each specimen was evaluated by using light microscope and was further recorded by using a DP 72 microscope digital camera (Olympus Corporation, Tokyo, Japan).

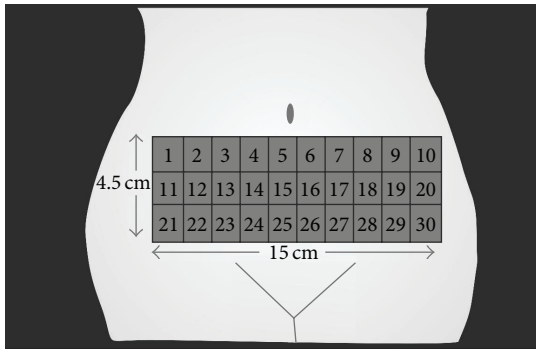


Figure 1: Illustration showing 30 of $1.5 \times 1.5\,cm^2$ squares, tattooed on each patient's abdominal wall.

Table 2: Sequenced treatment plan of all test areas illustrated in Figure 1.

Visiting time (before surgery)/parameters	3 mo	1 mo	1 wk	3 d	1 d	Imm
Test level (needle insertion only) 1 pass	1	6	11	16	21	26
Level 2 1 pass	2	7	12	17	22	27
Level 2 2 passes	3	8	13	18	23	28
Level 4 1 pass	4	9	14	19	24	29
Level 5 1 pass	5	10	15	20	25	30

Imm = immediately before abdominoplasty, d = day, wk = week, and mo = month.

2.4. Outcome Measures. The zones of denatured collagen or radiofrequency thermal zone (RFTZ) and neocollagenesis of all specimens were evaluated by 2 experienced dermatopathologists (Penvadee Pattanaprichakul and Suchanan Hanamornroongruang) who were blinded to the corresponding treatment protocol and tissue labeling. The effects of varying energy settings and pulse stacking on the depth of RFTZ and neocollagenesis were described.

Side effects associated with each treatment such as hypo- and hyperpigmentation, pinpoint bleeding, purpura, and textural change were recorded.

3. Results

3.1. Depth of RFTZ. The average depth of RFTZ ranged from 100 to 300 μm, correlating with energy levels. The maximum depth of RFTZ was 300 μm at a site treated with energy level 5 (80 watts, wave duration of 50 milliseconds (ms), and electrode insertion duration of 300 ms), whereas the minimum depth of RFTH was 100 μm at a site treated with energy level 2 (50 watts, wave duration of 50 milliseconds (ms), and electrode insertion duration of 300 ms). RFTZ was not detectable on a site treated with test level (electrode insertion only).

Table 3: Qualitative grading of mucin production after treatment.

Energy	Imm	1 d	3 d	1 wk	1 mo	3 mo
Test level	0	0	0	0	0	0
2	0	0	0	0	+	+
2/2 passes	0	0	0	0	++	++
4	0	0	0	0	++	++
5	0	0	0	0	++	++

Imm = immediately before abdominoplasty, d = day, wk = week, mo = month, 0 = no significant staining detected, + = minimally positive staining, and ++ = moderately positive, compared to nontreated site.

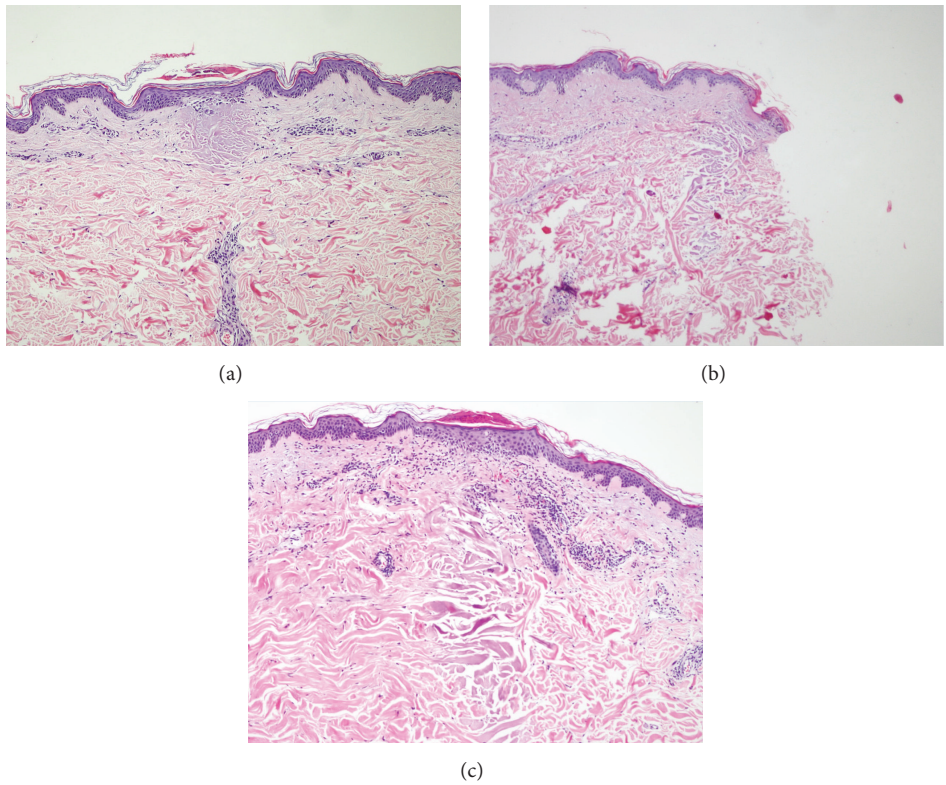
Figure 2 demonstrates confined zone of denatured collagen or radiofrequency thermal zone (RFTZ). The confined zone of denatured collagen from the specimens was demonstrated in the specimens treated with energy starting from level 2 (50 watts, wave duration of 50 milliseconds (ms), and electrode insertion duration of 300 ms). Inflammatory cell infiltrations were also noted. However, this inflammatory response was disappeared by 3 months following treatment in both patients (Figure 3). There was no evidence of collagen denaturation observed at the site treated with electrode insertion alone and the site treated with energy level 1 (50 watts, wave duration of 30 milliseconds (ms), and electrode insertion duration of 280 ms).

3.2. Neocollagenesis. In order to identify the evidence of neocollagenesis, HSP70 immunohistochemical study was used to investigate wound healing cascade. HSP70 staining started showing positive staining at superficial perivascular inflammatory cells at energy level 2 (50 watts, wave duration of 50 milliseconds (ms), and electrode insertion duration of 300 ms) by 1 day after treatment, peaked at 7 days, and was not evident at 1 month and beyond. A finding of dermal coagulation zone, the presence of inflammatory response (positive staining of HSP70), together with detection of dermal mucin production (positive colloidal iron stain), confirmed an evidence toward neocollagenesis.

3.3. Inflammatory Cells. Tissue response to FRMS through 6 time periods was demonstrated in Figure 3. Within the first 24 hours after treatment, predominant neutrophils infiltration around RFTZ was observed. Lymphocytic infiltration was found as early as 3 days after treatment and persisted up to 1 month.

3.4. Mucin Production. The amounts of mucin at 1 month and 3 months were increased, compared with that of the untreated site, demonstrated by colloidal iron stain. The results are shown in Table 3 and Figure 4.

3.5. Neoelastogenesis. The well-formed elastic fibers were also increased at 1 and 3 months after treatment demonstrated by positive stain of VVG (Figure 5).



(a) (b)

(c)

Figure 2: Demonstration of confined zone of denatured collagen or radiofrequency thermal zone (RFTZ), immediately after treatment. (a) RFTZ is initially found at treatment level 2 (50 watts, wave duration of 50 milliseconds (ms), and electrode insertion duration of 300 ms). (b) RFTZ at treatment level 4 (50 watts, wave duration of 100 milliseconds (ms), and electrode insertion duration of 350 ms). (c) RFTZ at treatment level 5 (80 watts, wave duration of 50 milliseconds (ms), and electrode insertion duration of 300 ms), H&E stain; original magnifications: 10x.

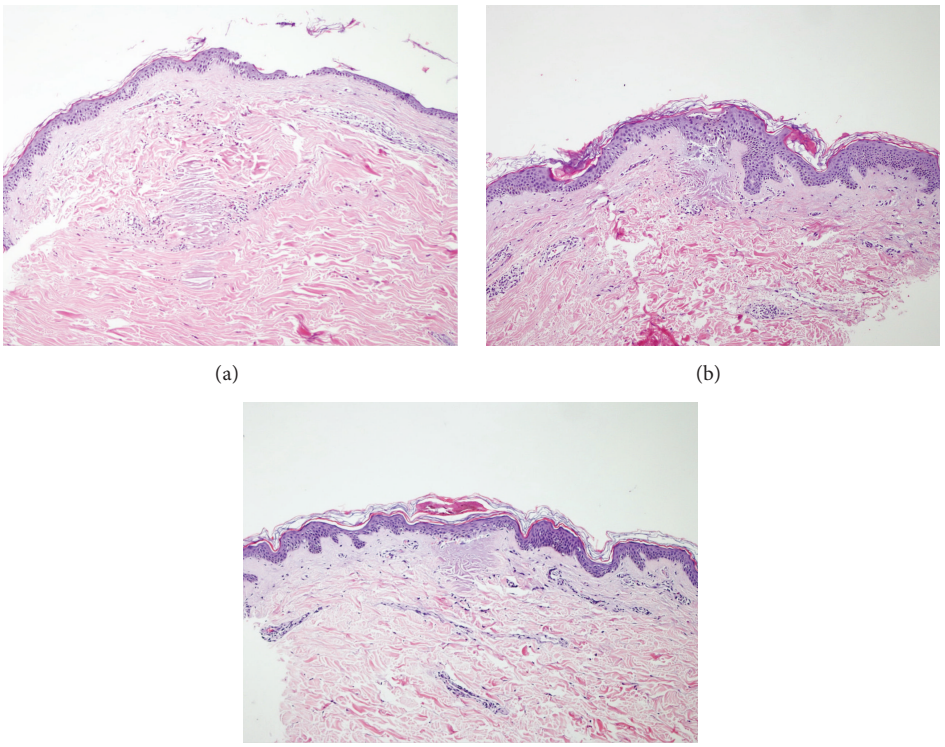
3.6. Melanin Pigment. The density of melanin incontinence gradually decreased from the time immediately after treatment until it became completely disappeared at the 3-month follow-up visit.

3.7. Adverse Effect. Pinpoint bleeding was observed immediately after treatment and stopped after compression for 2-3 minutes. Mild hyperpigmentation at the test sites treated with energy level 5 in both subjects was the only adverse effect observed in this study. This pigmentary change was observed at 1 month after treatment and disappeared at the 3-month follow-up visit.

4. Discussion

The present study demonstrated the potential application of FRMS to induce neocollagenesis by creating multiple microscopic zones of denatured collagen or radiofrequency thermal zone (RFTZ) intervening with normal tissue in the dermis as the result of the fractional thermal response. In addition, the current study showed that the depth of RFTZ could be controlled by the radio wave duration and time of electrodes existing in the skin. The formation of denatured collagen zones was not detectable at the site treated with electrode insertion alone and the site treated with low energy level (energy level 1). However, zone of collagen denaturation was initially observed at energy level 2 (50 watts, wave duration of 50 milliseconds (ms), and electrode insertion duration of 300 ms). This observation implies that an induction of neocollagenesis requires an optimum level of dermal heating and physical insertion of the electrode only without a sufficient thermal influence may not be able to stimulate a process of new collagen formation.

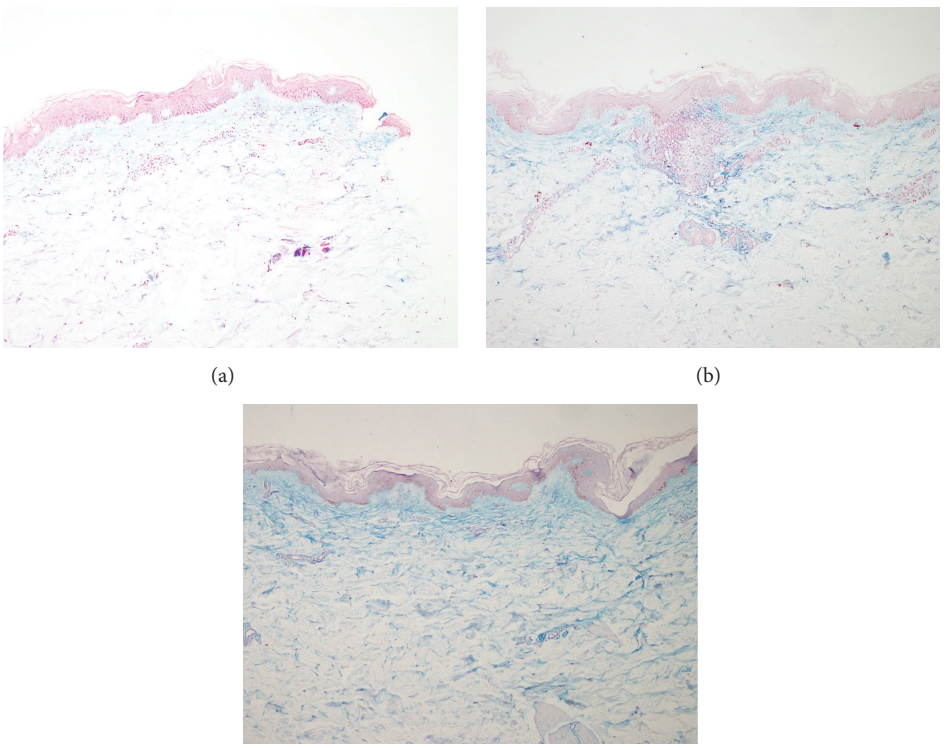
Recently, numerous studies have shown the protective effects of HSPs on different organs subjected to different environmental stress including radiation, oxidative damage, heavy metals, and thermal stress. In skin, cells of epidermis and dermis express HSPs, which confer resistance to damage caused by stressors [13, 14]. The heat shock response of cells is thought to have a role in ameliorating cellular injury and preventing programmed cell death. The protective effects of HSPs are likely mediated by the ability of these proteins to function as molecular chaperones, preventing inappropriate protein aggregation and facilitating transport of immature proteins to target organelles for packing, degradation, or repair [15]. However, these proteins show different expression patterns depending on the types of stressor and cell. Earlier studies have shown that intense pulsed light (IPL) and laser treatments induce expression of several Heat Shock Proteins (HSPs) including HSP47, HSP70, and HSP72 by dermal dendritic cells [16, 17]. It is believed that activation of these cells may be the underlying mechanism of collagen



(a) (b)

(c)

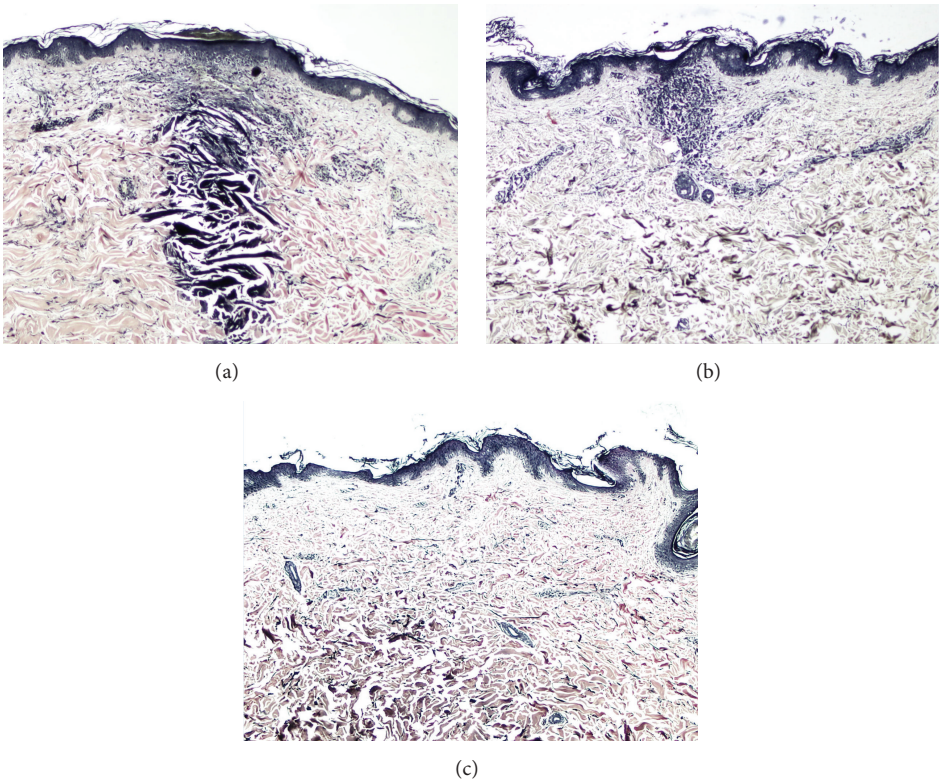
Figure 3: Demonstration of tissue response following treatment. (a) The inflammatory cells are found within 24 hours around RFTZs at 1 day after treatment. (b) Three days. (c) One week. (H&E stain; original magnifications: 10x.)



(a) (b)

(c)

Figure 4: Demonstration of increasing amount of mucin around RFTZ starting at 1 month after treatment. (a) One week. (b) One month after treatment. (c) Three months. (Colloidal iron stain; original magnifications: 10x.)



(a) (b) (c)

Figure 5: Neoelastogenesis. (a) Immediately after treatment, enlarged and clumped elastic fibers were found, representing denatured elastic fibers from heat. (b) There is increasing amount of smaller size elastic fibers around zones of denatured collagen compared to nontreated site at 1 month after treatment. (c) Three months after treatment. (Verhoeff-Van Gieson stain; original magnifications: 10x.)

deposition [3, 15]. Similar to a finding of the present study, an expression of HSP70 was detectable following Nd:YAG [18] and CO_2 [19] laser irradiation. Additionally, a previous study revealed that the extent and duration of HSP70 induction were varied with laser pulse durations and radiant exposures [19].

Zones of denatured collagen had been replaced by newly formed collagen fiber by 3 months after a single FRMS treatment. This corresponds with the presence of an increasing amount of mucin deposition surrounding RFTZs from 1- to 3-month follow-up visits. In addition, an increased amount of well-formed elastic fiber, representing neoelastogenesis, was demonstrated by Verhoeff-Van Gieson stain at 1- and 3-month follow-up visit. Similar to the present study, earlier studies on FRMS treatment used a fractional radiofrequency device with a different parameter setting to create fractionated tissue response [8, 20]. Both neoelastogenesis and neocollagenesis have been demonstrated following FRMS treatment. However, most of the study subjects of the aforementioned studies had skin phototypes I-II and were followed up for a shorter period of time, compared to our study. Taken together, the evidences of the present study suggest that thermal response to FRMS treatment induces wound healing cascade leading to long-term dermal remodeling with increased collagen synthesis.

Hyperpigmentation was the only adverse effect observed in both patients (100%) on the area treated by using high energy level and resolved by 3 months after treatment. This finding correlated with the results from melanin staining, demonstrating moderate amounts of melanin pigments immediately after treatment that nearly disappeared by 3 months. A previous study using a similar bipolar microneedle radiofrequency device in patients with a majority of skin phototypes II and III reported no evidence of PIH in all 15 subjects treated. The physician should be aware that hyperpigmentation can develop as an adverse effect following a FRMS treatment, especially in dark-skinned patients. A limitation of the present study was the small number of subjects ($n = 2$). However, the histopathological and immunohistochemistry findings in both subjects were identical.

In summary, the present study demonstrated an evidence of neocollagenesis following microscopic thermal heating to the dermis by using FRMS at an optimum heating level. Further studies, enrolling a larger number of volunteers, are required to optimize parameter setting for maximizing clinical efficacy and minimizing adverse effects.

Competing Interests

The authors declare that they have no competing interests.

Acknowledgments

This research project is supported by the Faculty of Medicine Siriraj Hospital, Mahidol University. The authors wish to

thank Dr. Nittaya Lektrakul for her assistance in illustration drawing and Mrs. Phassara Klamsawat and Ms. Pornsuk Yamlexnoi for their assistance in recruiting subjects and managing the database.

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Hindawi Publishing Corporation
BioMed Research International
Volume 2016, Article ID 1928540, 6 pages
http://dx.doi.org/10.1155/2016/1928540

Clinical Study

Fractional Carbon Dioxide Laser for Keratosis Pilaris: A Single-Blind, Randomized, Comparative Study

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Received 15 February 2016; Accepted 18 April 2016

Academic Editor: Steven P. Nistico

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Objective. Keratosis pilaris (KP) is a common condition which can frequently be cosmetically disturbing. Topical treatments can be used with limited efficacy. The objective of this study is to evaluate the effectiveness and safety of fractional carbon dioxide (CO_2) laser for the treatment of KP. *Patients and Methods*. A prospective, randomized, single-blinded, intraindividual comparative study was conducted on adult patients with KP. A single session of fractional CO_2 laser was performed to one side of arm whereas the contralateral side served as control. Patients were scheduled for follow-up at 4 and 12 weeks after treatment. Clinical improvement was graded subjectively by blinded dermatologists. Patients rated treatment satisfaction at the end of the study. *Results*. Twenty patients completed the study. All patients stated that the laser treatment improved KP lesions. At 12-week follow-up, 30% of lesions on the laser-treated side had moderate to good improvement according to physicians' global assessment ($p = 0.02$). Keratotic papules and hyperpigmentation appeared to respond better than the erythematous component. Four patients with Fitzpatrick skin type V developed transient pigmentary alteration. *Conclusions*. Fractional CO_2 laser treatment may be offered to patients with KP. Dark-skinned patients should be treated with special caution.

1. Introduction

Keratosis pilaris (KP) is a common disorder of keratinization. It is characterized by multiple tiny follicular keratotic papules that may have surrounding erythema. Hyperpigmentation can sometimes occur especially in individuals with dark-complexioned skin. KP mainly involves the extensor arms, back, anterior thighs, face, and buttock. The exact prevalence is difficult to estimate but could be found up to 50% of the general population [1, 2]. Although KP has no impact on general health, its influence on the quality of life arises especially for those with lesions on the exposed areas. Treatment options include emollients, keratolytics, and topical steroids when necessary [3]. However, the result is highly variable and recurrence following treatment discontinuation is often problematic.

Over the past decade, attempts to eradicate KP through various laser and light-based therapy have been investigated. This includes 532 nm potassium titanyl phosphate laser; 595 nm pulsed dye laser; 1064 nm Q-switched Nd:YAG laser; long-pulse 1064 nm Nd:YAG laser; combination of 595 nm pulse dye laser, long-pulse 755 nm alexandrite laser, and microdermabrasion [4–9]. To the best of our knowledge, there has never been a report on the effectiveness of fractional carbon dioxide laser (CO_2) for the treatment of KP. We hypothesize that fractional CO_2 laser could remove the keratotic component and brown pigmentation of KP. The objective of this study is to evaluate the efficacy and safety of fractional CO_2 laser for the treatment of KP.

2. Materials and Methods

This study is a prospective, randomized, single-blinded, intraindividual comparative study. The study protocol has been approved by Mahidol University Institutional Review Board for Human Subject Research (Protocol number 015821). The study confirmed the guidelines of the declaration

of Helsinki. All study subjects had obtained the inform consent at the enrollment.

2.1. Patient. Subjects were recruited from an outpatient dermatology clinic at a university-based hospital (Ramathibodi Hospital, Mahidol University, Bangkok, Thailand). Healthy patients aged 18 years or older with the presence of KP on both sides of arms were eligible. Patients with history of keloid or hypertrophic scar and those who were pregnant or lactating were excluded from the study. Patients who received topical medications or emollients within 1 month or had performed any laser treatment or dermabrasion for KP within past 6 months were also excluded. In addition, we eliminated patients with prior history of medication affecting keratinization (e.g., isotretinoin and acitretin) within the past 3 years from this trial.

After enrollment, informed consent had been obtained and demographic data was recorded. Using the table of randomization (a table of random numbers), the left side and right side of the arms were randomly allocated to receive laser treatment. One side received fractional CO_2 laser therapy (side A) and the other (side B) did not receive laser treatment.

2.2. Treatment Regimens. Fractional CO_2 laser therapy using a 10,600 nm eCO_2 laser (Lutronic Corporation, Goyang, Republic of Korea) was performed to the lesions on side A. This was a single session laser treatment. The settings were the pulse energy of 24–30 mJ and spot density of 300 spots/cm^2 in static mode; 2 passes were delivered using a 300-density tip. To minimized pain, local anesthetic cream (a eutectic mixture of local anesthetics, Astra Zeneca LP, Wilmington, DE) was applied under occlusion for 30 minutes before the laser treatment and air cooling with a cold air cooling device (CRIOjet AIR Mini CRIO Medizintechnik GmbH, Birkenfeld, Germany) at a cooling level of 4 was used during the laser therapy.

After the laser treatment, petrolatum ointment was applied to the lesions on side A twice a day for 5 days. To avoid confounding effect of petrolatum ointment, the lesions on side B also received petrolatum ointment twice daily for 5 days. The patients were appointed for follow-up on the 4th and 12th week after the last treatment (Figure 1).

2.3. Outcome Evaluation. Standard digital photograph was taken at baseline, 4 weeks, and 12 weeks after the last treatment. Two dermatologists who did not perform the laser procedure evaluated the response through digital images. Global improvement score, keratotic papules, hyperpigmentation, and erythema were evaluated using the grading system for improvement. The grading scale is as follows: grade −4, >75% worsening; grade −3, 51–75% worsening; grade −2, 26–50% worsening; grade −1, 1–25% worsening; grade 0, no change; grade 1, 1–25% improvement (minimal); grade 2, 26–50% improvement (moderate); grade 3, 51–75% improvement (good); grade 4, >75% improvement (excellent).

Patient satisfaction was assessed at the end of study (12th week of follow-up). They were asked to rate the overall improvement and satisfaction by grading score, that is, 0, unsatisfied; 1, poor; 2, fair; 3, satisfied; 4, extremely satisfied. Pain score was also evaluated immediately after laser treatment using 10-point visual analogue scale (VAS, 0–10), in which 0 indicated no pain at all and 10 indicated extreme pain. Possible adverse events (e.g., erythema, burning sensation, hyperpigmentation, hypopigmentation, scarring, and infection) were recorded at every visit.

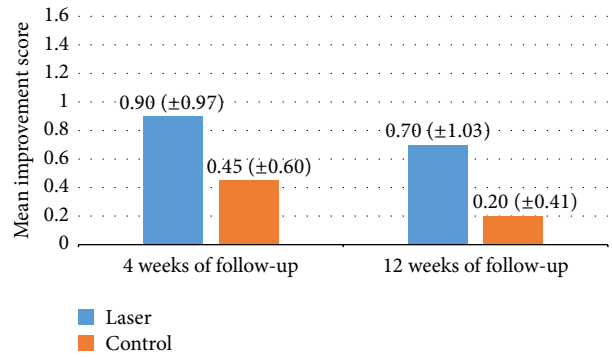

Figure 1: Mean improvement score at 4-week and 12-week follow-up.

2.4. Statistical Analyses. Categorical variables were expressed as percentages. Continuous variables (e.g., physician grading score and VAS) were expressed as mean ± standard deviation or median (range). t-test was used to compare continuous data between two dependent samples. All analyses were performed using STATA version 13 (Stata Corp., College Station, Texas, USA). A p value of 0.05 or less was considered statistically significant.

3. Results

Twenty-four eligible patients with KP on both arms were enrolled in this study. Four patients lost to follow-up at 12 weeks after treatment for reasons unrelated to laser treatment (i.e., inconvenient follow-up schedule). Twenty patients completed the 12-week study and were included in the analysis (20 arms on side A and 20 arms on side B). Twelve were men (60%) and 8 were women (40%). The median age of subject was 27 years old (19–32). The median age of KP onset was 15 years old (10–22). Eight patients were Fitzpatrick skin type III, 5 patients were Fitzpatrick skin type IV, and 7 patients were Fitzpatrick skin type V.

3.1. Global Assessments. At 4 weeks after treatment, grade 2 or more improvement was achieved in 4 arms on side A and 1 arm on side B ($p = 0.097$). At 12 weeks of follow-up, 6 arms on side A achieved grade 2 or more improvement but no KP lesion on side B achieved such response ($p = 0.02$). None of the patients experienced worsening of the lesions (Table 1).

In terms of mean improvement score, the score was 0.90 (±0.97) on side A and 0.45 (±0.60) on side B ($p = 0.08$) at 4 weeks after treatment. The mean improvement scores on side A and side B at 12 weeks of follow-up were 0.7 (±1.03) and 0.2 (±0.41), respectively ($p = 0.05$) (Figures 1 and 2). There was

Table 1: Global assessment by nontreating dermatologists.

Grading	4 weeks of follow-up, $n = 20$		12 weeks of follow-up, $n = 20$	
	Side A	Side B	Side A	Side B
0 (no improvement)	8	12	13	16
1 (1–25% improvement, minimal)	8	7	1	4
2 (26–50% improvement, moderate)	2	1	5	—
3 (51–75% improvement, good)	2	—	1	—
4 (>75% improvement, excellent)	—	—	—	—

Side A: fractional CO_2 laser treated side.
Side B: control side.

Figure 2: Photograph of patient before (a, d); at 4-week follow-up (b, e); at 12-week follow-up (c, f). Left arm was treated with fractional CO_2 laser (a, b, c) and right arm was served as control (d, e, f). Left arm showed grade 3 improvement at 12-week follow-up (c).

no statistically significant difference according to sex, age, or disease duration.

3.2. Keratotic Papules. The evaluators assessed overall improvement of keratotic papules but the actual number was not counted. Similar to global assessments, higher grade of improvement (grade 2 or more) was found in 4 arms on side A and 1 arm on side B at 4 weeks of follow-up. At 12 weeks of follow-up, at least grade 2 improvement was achieved in 5 arms on side A and 1 arm on side B. No study subjects experienced worsening of keratotic papules (Table 2).

3.3. Hyperpigmentation. Grade 2 or more improvement in hyperpigmentation was achieved in 5 patients on side A at 4 weeks of follow-up. At 12 weeks of follow-up, there were 6 arms on side A that achieved grade 2 or more improvement.

Table 2: Assessment of keratotic papules by nontreating dermatologists.

Grading	4 weeks of follow-up, $n = 20$		12 weeks of follow-up, $n = 20$	
	Side A	Side B	Side A	Side B
0 (no improvement)	11	15	8	14
1 (1–25% improvement, minimal)	5	4	7	5
2 (26–50% improvement, moderate)	1	1	3	1
3 (51–75% improvement, good)	3	—	2	—
4 (>75% improvement, excellent)	—	—	—	—

Side A: fractional CO_2 laser treated side.
Side B: control side.

Table 3: Assessment of pigmentation by nontreating dermatologists.

Grading	4 weeks of follow-up, $n = 20$		12 weeks of follow-up, $n = 20$	
	Side A	Side B	Side A	Side B
0 (no improvement)	13	14	13	16
1 (1–25% improvement, minimal)	2	6	1	4
2 (26–50% improvement, moderate)	5	—	5	—
3 (51–75% improvement, good)	—	—	1	—
4 (>75% improvement, excellent)	—	—	—	—

Side A: fractional CO_2 laser treated side.
Side B: control side.

Table 4: Patiens' satisfaction at 12 weeks of follow-up.

Patients' grading	Side A, $n = 20$	Side B, $n = 20$
0 (unsatisfied)	—	5
1 (poor)	8	11
2 (fair)	5	4
3 (satisfied)	5	—
4 (extremely satisfied)	2	—

Side A: fractional CO_2 laser treated side.
Side B: control side.

Majority of lesions on side B showed no improvement in terms of pigmentation at both the 4th and 12th week of follow-up (Table 3).

3.4. Erythema. Grade 3 improvement in erythema was achieved on 2 arms of side A at 4 weeks of follow-up but no lesions on side B achieved similar response. At 12 weeks, grade 2 and grade 3 improvement were found in 2 patients each on side A. None of KP lesions on side B achieved grade 2, 3, or 4 improvement at 12 weeks. No patients had worsening of erythema after laser.

3.5. Patient Satisfaction. All patients rated the lesions as improved on side A. Improvement of seven lesions on side A (35%) was marked as satisfied or extremely satisfied whereas no lesion on side B was rated as satisfied or extremely satisfied ($p = 0.08$) (Table 4).

3.6. Adverse Events. Pain was observed on the laser-treated side in all patients. The mean pain score was 4.2 (±2.6).

Figure 3: Hypopigmentation occurred on the laser-treated side at 4-week follow-up.

Erythema and burning sensation were also observed in all laser-treated lesions. The median duration of erythema was 2 days (1–5) and median duration of burning sensation was 24 hours (1–72). Two patients experienced hyperpigmentation on laser-treated side at 4 weeks of follow-up, both of which had spontaneous improvement at 12 weeks after treatment. Hypopigmentation developed on 2 lesions of side A: the first lesion spontaneously improved at 4-week follow-up and another lesion improved without any treatment at 12-week follow-up (Figure 3). No infection or scarring was observed. All patients who developed hyperpigmentation or hypopigmentation were Fitzpatrick skin type V.

4. Discussion

KP is a very common condition among the general population but few treatment options with promising and sustained results exist. Although it is often asymptomatic, it can cause pruritus in some patients. In addition, the affected skin resembling gooseflesh resulting in unsightly appearance may lead to psychological distress. Treatment options are variable due to various aspects of the lesions. In most cases, reassurance and general skin care focusing on avoiding skin dryness are required. Emollients, keratolytic agents, topical steroids, chemical or mechanical peels, vitamin D3 analogues, and topical or systemic retinoids may be used [8]. Various laser or light-based therapies targeting different components of the lesion have been tried with variable success rate [4, 5, 10, 11]. Despite the advantage of low cost, topical treatments have limited success rate, as they may not target all components of KP (e.g., hyperkeratosis, hyperpigmentation, and erythema). In addition, irritation from high-concentrated keratolytic agents can be intolerable to some patients. According to the histopathologic nature of KP, epidermal hyperkeratosis, hypergranulosis, and plugging of individual hair follicles are seen [3, 12]. Mild superficial perivascular lymphocytic inflammatory infiltrates can be found which reflex the erythematous component of some KP lesions. In Fitzpatrick skin types III–V (i.e., Asian skin), brown pigmentation is often seen [7]. In this study, the rationale for the use of fractional CO_2 is to target the excess keratinous plug and the brown pigments.

In this study, we demonstrated that a single session of fractional CO_2 laser treatment results in moderate to good improvement of KP lesions in some patients by global assessment. Although merely 30% of the patient had moderate to good improvement at the end of the study, to the patients' perception, all stated that the laser treatment improved the appearance of their lesions.

Taking particular elements of KP into account, keratotic papules and hyperpigmentation appear to respond better than erythematous components. Evidently, higher numbers of patients rated their keratotic lesions and hyperpigmentation as moderate or good response on both 4 weeks and12 weeks of follow-up. This result supports our hypothesis that fractional CO_2 laser treatment could eradicate excess keratosis and pigmentation.

Regarding the laser parameter in this study, the pulse energy of 24–30 mJ with a spot density of 300 spots/cm^2 was delivered into 2 passes. This setting was capable of causing maximum ablative depth of approximately 530–580 μm and the width of coagulative zone is 243 μm per dot, whereas the epidermal thickness of the shoulder and upper extremities is approximately 81–135 μm [13, 14]. With this setting, we believe that certain parts of each keratotic papule would be destroyed. However, due to disparity in size and depth of keratotic papules, absolute ablative depth may be variable. Hence, some may be completely ablated and others may only be partially ablated.

There was marginal degree of improvement on KP lesions of control side (side B). This is probably due to the emollient effect from petrolatum ointment. As also demonstrated the mean improvement score of side B at the 12th week of follow-up was less than that of the 4th week of follow-up. Therefore, emollients are proven beneficial in KP when given continuously. Cessation of emollient may cause gradual recurrence. This same effect also occurred on side A, where the mean improvement score at the 4th week was better than the 12th week. For this reason, application of emollient should be advised to all patients, regardless of laser therapy.

Previous studies have evaluated the effectiveness of various lasers intended to attack specified targets such as 532 nm potassium titanyl phosphate laser and 595 nm pulsed dye laser aimed to reduce KP-associated erythema [4, 5, 10]. According to a study by Alcántara González et al., all 10 patients with keratosis rubra pilaris or keratosis pilaris atrophicans faciei achieved more than 75% improvement in erythema after 2–7 sessions of 595 nm pulsed dye laser [10]. In patients with pronounced pigmentary component of KP, 1064 nm Q-switched Nd:YAG laser may be used [6, 7]. Park et al. evaluated the efficacy of 1064 nm Q-switched Nd:YAG laser on pigmented KP, 41.7% of patients showed more than 50% improvement in dyspigmentation [6]. Recently, Saelim et al. successfully treated KP with long-pulsed 1064 nm Nd:YAG laser [9]. A significant improvement in global assessment, erythema, and keratotic papules was noted after 3 sessions of long-pulsed 1064 nm Nd:YAG laser at 4-week interval. The proposed mechanism of long-pulsed 1064 nm Nd:YAG laser was to reduce the size of the affected hair follicles. According to a study by Lee et al., the combination of 595 nm pulsed dye laser, long-pulsed 755 nm alexandrite laser, and microdermabrasion to target 3 components of KP resulted in marked improvement of KP in 51.7% of patients [8]. Therefore, the most suitable choice for the treatment of KP mainly depends on the pronounced and problematic component. Combination of different lasers or modalities may be most beneficial to patients with multiple elements of KP. However, lasers hold several limitations such as pain, stinging sensation, erythema, purpura, hyper- and hypopigmentation, and high cost [4–10]. In addition, recurrence of the lesions may occur after treatment cessation.

The limitations of this study are the small sample size and the short follow-up time of 3 months. Therefore, a conclusion cannot be drawn as to how long the laser effect would last and whether recurrence would occur. Moreover, we did not count the actual keratotic lesions and skin roughness was inaccessible through the evaluation by 2D photography. Finally, our study was performed in Asian subjects with Fitzpatrick skin types III–V; hence, this laser setting cannot be applied to all skin types.

In conclusion, we demonstrated that fractional CO_2 laser can be used as an alternative treatment for KP in some patients, particularly in the presence of marked keratotic components. However, special caution should be given to patients with greater Fitzpatrick skin type, as pigmentary alteration can occur as a sequel. Further studies are needed to find the optimum parameter, appropriate frequency, and suitable treatment sessions of fractional CO_2 laser for KP.

Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

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Hindawi Publishing Corporation
BioMed Research International
Volume 2016, Article ID 6016943, 6 pages
http://dx.doi.org/10.1155/2016/6016943

Clinical Study

27.12 MHz Radiofrequency Ablation for Benign Cutaneous Lesions

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Received 2 February 2016; Accepted 17 March 2016

Academic Editor: Steven P. Nistico

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As surgical and/or ablative modalities, radiofrequency (RF) has been known to produce good clinical outcomes in dermatology. Recently, 27.12 MHz RF has been introduced and has several advantages over conventional 4 or 6 MHz in terms of the precise ablation and lesser pain perception. We aimed to evaluate the clinical efficacy and safety of 27.12 MHz RF for the treatment of benign cutaneous lesions. Twenty female patient subjects were enrolled. Digital photography and a USB microscope camera were used to monitor the clinical results before one session of treatment with 27.12 MHz RF and after 1 and 3 weeks. Treated lesions included telangiectasias, cherry and spider angiomas, skin tags, seborrheic keratoses, lentigo, milium, dilated pore, acne, piercing hole, and one case of neurofibroma. For vascular lesions, clinical results were excellent for 33.3%, good for 44.4%, moderate for 11.1%, and poor for 11.1%. For nonvascular lesions (epidermal lesions and other benign cutaneous lesions), clinical results were excellent for 48.3%, good for 45.2%, moderate for 3.2%, and poor for 3.2%. No serious adverse events were observed. Mild adverse events reported were slight erythema, scale, and crust. The 27.12 MHz RF treatment of benign vascular and nonvascular lesions appears safe and effective after 3 weeks of follow-up.

1. Introduction

Electrosurgery is defined as the use of heat generated in body tissue through tissue resistance to high-frequency alternating current for the destruction and removal of diseased tissue or for cutting through normal tissue with minimal bleeding [1]. Since electrosurgery had been introduced in dermatologic practice in the 1950s, it became a dermatologic treatment for skin cancer [2]. Although electrosurgery for skin cancer was replaced by Mohs micrographic surgery, it continues to be used routinely for treatment of a variety of benign dermatologic conditions and for epilation, the process of removing unwanted hairs [3, 4].

Radiofrequency (RF) delivers rapidly alternating currents from a probe tip to tissue, which produces a thermal effect on target by resistive heating or impedance to current flow [5]. Thermal effect of RF current on tissue is dependent on both the electrical properties of the tissue and the electrode configuration [6]. During RF procedure, temperature increases over 60°C (70–90°C) at electrode-tissue interface causing collateral thermal damage. To achieve minimal scarring, RF device should have high-frequency power with low intensity, tissue heating during the procedure should be minimal, and the diameter of electrode in contact with the tissue should be as small as possible [7, 8].

The range of designated frequencies used for RF in dermatology has been increasing since 1925 from 0.5 MHz to 1.7 MHz to 3.35 MHz to 4 MHz. Then, in 1985, the FCC (The Federal Communications Commission) introduced the ISM

rules for industrial, scientific, and medical applications with frequencies of 6.758 MHz, 13.56 MHz, and 27.12 MHz [9].

Previously, we had conducted the histological evaluation of thermal effect of 27.12 MHz RF on ex vivo human hair follicle tissue. The results showed that thermal damage extended over several hundred micrometers (100–400 μm) from the center of the inserted probe [4]. Although 27.12 MHz RF has been studied for hair removal, there is no clinical study of the treatment for benign vascular and epidermal lesions. In this study, we evaluated the clinical efficacy and safety of a first 27.12 MHz RF treatment to ablate benign vascular and epidermal lesions.

2. Materials and Methods

2.1. Study Design and Study Population. This pilot study was approved by the "comité d'éthique de la recherche du Centre de Recherche du CHU de Québec-Université Laval" for the protection of human subjects.

The study subjects were patients presenting benign cutaneous lesions treated at Académie Dectro. Patients who had undergone concomitant treatments for their benign cutaneous lesions including laser, IPL, and electrosurgery/radiosurgery within the previous 6 months were excluded and not eligible. Patients with keloid, bleeding tendency, nevus, and immunosuppression were also excluded. Thus, 20 patients were enrolled after obtaining a written informed consent for participation in this research. Patients completed a self-administered questionnaire to collect detailed personal history (skin type, skin symptoms, health condition, and drugs). Mean patient age was 47.2 years old (range 18–72) and all were female with Fitzpatrick skin phototypes from II to IV.

2.2. Procedures. Each patient took one session of treatment with a 27.12 MHz RF device (Eclipse, Dectro International, QC, Canada). According to manufacturer's protocol, two operation modes were used: TeleFlashTM mode (pulses in thousandth of a second) and MultiCoagulationTM mode (slow heating followed by TeleFlashTM mode). Peak temperature in the skin at the moment of RF treatment approximates 75°C (167°F) (manufacturer's data). No anesthetic procedure was used during the procedure. Patients were instructed to use a moisturizer (Action de Gala, Dectro International, QC, Canada) several times for a few days after treatment to promote wound healing and to minimize skin dryness.

2.3. Objective and Subjective Evaluations. Photographs were taken using identical digital camera (Nikon D3100) and USB microscope (M2, Scalar Corporation, Tokyo, Japan) settings, lighting, and subject positioning at baseline and 1 week and 3 weeks after the treatment. A dermatologist performed the clinical evaluation. The clinical results were scored as excellent (complete reduction), good (more than 75% reduction), moderate (more than 50% reduction), or poor (less than 50% reduction).

The subjects were surveyed during the last follow-up (3 weeks after the treatment) to determine their overall level of satisfaction with treatment results using the following scale: very satisfied, satisfied, slightly satisfied, and unsatisfied. Relative pain scores associated with the treatment were evaluated using 10 cm visual analog scales (VAS), with 0 being "no pain" and 10 being "extremely painful." The occurrence of adverse events was evaluated at each visit.

Table 1: Characteristics of patients and treated lesions.

Characteristics	Frequency (n)	Percentage (%)
Age		
10–29	2	10
30–49	7	35
>50	11	55
Skin type		
II	1	5
III	18	90
IV	1	5
Type of lesion		
Vascular lesion		
Telangiectasia	22	32.8
Cherry angioma	14	20.8
Spider angioma	1	1.5
Epidermal lesion		
Skin tag	13	19.4
Seborrheic keratosis	8	11.9
Lentigo	1	1.5
Miscellaneous		
Milium	2	3.0
Dilated pore	3	4.5
Acne	2	3.0
Piercing hole	1	1.5
Neurofibroma	1	1.5
Location of lesion		
Face & neck		
Periocular area	3	4.5
Nose	12	17.9
Others	24	35.8
Torso		
Back	5	7.5
Chest	8	11.9
Abdomen	7	10.4
Axilla	3	4.5
Extremities		
Upper extremities	1	1.5
Lower extremities	5	7.5

3. Results

A total of 20 female patients were enrolled and completed the treatment session with a 27.12 MHz RF device. Selected baseline characteristics of the study population are described in Table 1. One patient was lost to follow-up. The total number of treated lesions was 67 in 20 patients (mean 3.3 per patient). There were 36 vascular lesions, including 22 telangiectasias, 13 cherry angiomas, and 1 spider angioma. There were 31

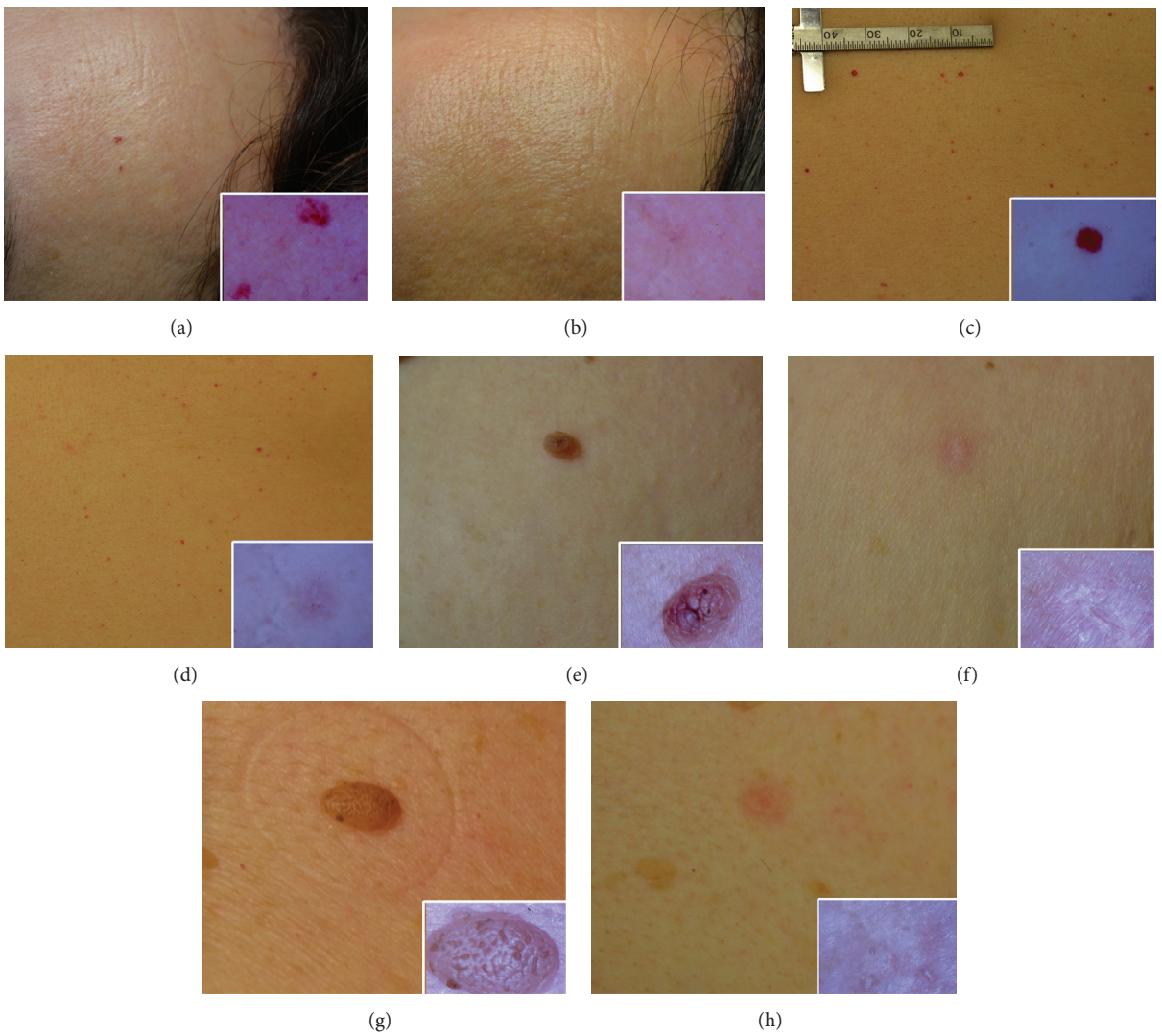

Figure 1: Representative photographs of vascular lesions treated with radiofrequency. Cherry angioma on the forehead at baseline (a) and the end of treatment (b). Cherry angioma on the abdomen at baseline (c) and the end of treatment (d). Skin tag on the axilla at baseline (e) and the end of treatment (f). Seborrheic keratosis on the anterior chest at baseline (g) and the end of treatment (h). Insert: appearance of the lesion under USB microscope.

nonvascular lesions (epidermal and other benign cutaneous lesions), mostly epidermal lesions, including 13 skin tags, 8 seborrheic keratoses, 1 lentigo, and 9 others (Table 2).

Representative photographs of positive clinical treatment outcomes for different skin lesion types treated with RF are presented in Figure 1. The clinical treatment outcomes of vascular lesions indicated that 12 cases (33.3%) showed excellent results; 16 cases (44.4%) showed good results; 4 cases (11.1%) showed moderate results; and 4 cases (11.1%) showed poor results (Table 2). Lesions situated on the face showed good response to the treatment. Two of the subjects showing moderate improvement had a medical history of photodamaged skin and rosacea, respectively. The anatomic sites of cases which showed poor results were nasal ala and upper eyelid, which are generally prone to pain. Besides, nasal ala is generally difficult to approach.

The clinical treatment results of nonvascular lesions had similar outcome to those of vascular lesions, which showed 15 cases (48.3%) with excellent, 14 cases (45.2%) with good, 1 case with moderate, and 1 case with poor results (Table 2). The epidermal lesion that showed poor result was a case of lentigo on the lateral canthus area, which remained erythematous at the end point of treatment. Moderate outcome was observed in the case of piercing hole.

Surveys evaluating overall satisfaction with treatment revealed that, for vascular lesions, 34 cases (94.4%) were very satisfied and 2 cases (5.6%) were satisfied. For nonvascular lesions, 26 cases (83.9%) were very satisfied and 5 cases (16.1%) were satisfied (Table 2).

In this study, no anesthesia procedure was used. The treated area usually became erythematous and swollen within several minutes after the treatment. In most patients, minimal

Table 2: Clinical improvement and adverse events in response to 27.12 MHz radiofrequency.

Type of lesion	Number of cases	Clinical improvement					
		Objective improvement				Overall satisfaction	
		Excellent	Good	Moderate	Poor	Very satisfied	Satisfied
Vascular lesion							
Telangiectasia	22	3	13	4	2	20	2
Cherry angioma	13	8	3	0	2	13	0
Spider angioma	1	1	0	0	0	1	0
Total	*36*	*12 (33.3%)*	*16 (44.4%)*	*4 (11.1%)*	*4 (11.1%)*	*34 (94.4%)*	*2 (5.6%)*
Epidermal lesion							
Skin tag	13	10	3	0	0	13	0
Seborrheic keratosis	8	4	4	0	0	8	0
Lentigo	1	0	0	0	1	0	1
Miscellaneous (dilated pore, milium, neurofibroma, acne, and piercing hole)	9	1	7	1	0	5	4
Total	*31*	*15 (48.3%)*	*14 (45.2%)*	*1 (3.2%)*	*1 (3.2%)*	*26 (83.9%)*	*5 (16.1%)*

Type of lesion	Adverse events		Pain
	Serious	Mild* (percentage)	Mean VAS score
Vascular lesion			
Telangiectasia	None	10 (45.5%)	3.16
Cherry angioma	None	9 (69.2%)	3.27
Spider angioma	None	None	0
Mean	*None*	*52.7%*	*3.11*
Epidermal lesion			
Skin tag	None	7 (53.8%)	4
Seborrheic keratosis	None	7 (87.5%)	4.19
Lentigo	None	1 (100%)	3
Miscellaneous (dilated pore, milium, neurofibroma, acne, and piercing hole)	None	4 (44.4%)	3.78
Mean	*None*	*61.3%*	*3.95*

Clinical evaluation was performed by a dermatologist. Clinical results were assessed by physical examination, photographic follow-up, and USB microscope (M2, Scalar Corporation, Tokyo, Japan). The results were described as excellent (complete reduction); good (more than 75% reduction), moderate (more than 50% reduction), and poor (less than 50% reduction). VAS: 10 cm visual analog scales. *Adverse events reported were slight erythema, scale, and crust.

discomfort was reported during the RF procedure and it disappeared immediately after. The mean relative pain score using VAS was 3.11 for the treatment of vascular lesions and 3.95 for the nonvascular lesions (Table 2). Subjects reported higher pain score in the pain-sensitive area such as nostril, nasal ala, and eyelid. During the procedure, subjects reported the gradual decrease of pain intensity. Serious adverse events were not reported. As mild adverse events, slight erythema, scale, or crust was found at the end point of treatment in 19 vascular (52.7%) and 19 nonvascular (61.3%) lesions (Table 2).

4. Discussion

In the present study, we observed that clinical results following a first 27.12 MHz RF treatment to treat vascular, epidermal, and other benign cutaneous lesions were excellent for 40.3%, good for 44.8%, moderate for 7.4%, and poor for 7.4% after 3 weeks of follow-up. Regarding the patients with moderate and poor results, it is not excluded that a second course of treatment could lead to satisfactory results. However, it is recommended to wait a minimum of three weeks between treatments in order that skin recovers.

Many diverse treatments can be chosen for benign cutaneous lesions, such as surgical excision, CO_2 laser [10], Er:YAG laser [11], trichloroacetic acid (TCA), and cryosurgery [12] for ablating epidermal lesions. Also, pulsed dye, alexandrite, KTP, and Nd:YAG lasers and intense pulsed light (IPL) can be used for cutaneous vascular lesions [13]. RF ablation is an effective surgical treatment for benign cutaneous lesions. In many countries, RF continues to be the preferred method and has been used along with electrosurgery. RF ablation procedure has been shown to be quick, bloodless, and leading to good cosmetic outcomes [5, 7, 14].

In this study, we evaluated the efficacy and safety of 27.12 MHz RF for the treatment of benign cutaneous lesions. Benign vascular anomalies, especially on exposed sites, are associated with psychological distress [15, 16]. Traditionally, electrosurgery has been used to treat benign skin tumors such as spider angiomas, telangiectasias, and cherry angiomas [17]. In 1983, selective photothermolysis by Anderson and Parrish dramatically transformed the trend of the treatment for cutaneous vascular lesions [18]. Lasers with various wavelengths or IPL are now considered the gold standard treatment for many cutaneous vascular anomalies [19]. These techniques are based on the selective absorption of a brief radiation pulse that generates and confines heat at certain pigment targets and are also good and safe methods to reduce the number and size of hairs [20]. Though vascular lasers proved to be fairly effective, the high cost for devices and maintenance is an obstacle in real practice, whereas RF is inexpensive, is easy to handle, and needs little maintenance. Herein, we observed that 27.12 MHz RF effectively treats various vascular lesions with no serious adverse events. We consider that 3 weeks of follow-up was sufficient to conclude the complete recovery of the skin after the intervention, making a fourth follow-up visit unnecessary for patients. Because the size of treated vascular lesions was less than 1 mm, treatment outcomes were very good. However, thick and deep vessels, for example, located on thighs, were hard to treat, requiring multiple treatment sessions.

Although many therapeutic options exist for acne, relapse often occurs after treatment is stopped. Lee et al. reported that selective sebaceous gland electrothermolysis using a high-frequency electrical current can be a safe and effective method of achieving consistent remission in acne [21]. In our cases, one patient with inflammatory acne on both cheeks and mandible was treated with RF. The number of inflammatory comedones had decreased without any anti-acne treatment three weeks after one session of RF treatment. Because the number of cases is limited, more cases are needed to evaluate the efficacy of RF on acne.

One patient had a dermal neurofibroma on the shoulder. Multiple cutaneous neurofibromas are usually found in individuals with von Recklinghausen disease or neurofibromatosis type 1. Although dermal neurofibromas do not become malignant, neurofibroma with pain and disfiguring features can be treated with surgical excision or CO_2 lasers. Kim et al. reported multiple cutaneous neurofibromas treated with 4 MHz RF ablation and excision in 16 neurofibromatosis type 1 patients [22]. Although dermal neurofibroma was incompletely removed in our case, the patient was satisfied with the clinical result and the procedure was quick and simple with no bleeding. RF can be an alternative option for the treatment of multiple cutaneous neurofibromas.

RF using 27.12 MHz has several advantages over conventional 4 MHz RF. Because the sensation perceived by the patient seems to be less intense as the frequency increases, a frequency of 27.12 MHz will be better for pain control. Also, higher frequency has a more efficient power absorption rate by water molecules, meaning that less power is needed to destroy the target. Furthermore, the rapid change from positive to negative polarity—27 million cycles per second—allows more precise and fast electrocoagulation.

5. Conclusions

RF ablation using 27.12 MHz was very effective and safe for benign vascular and nonvascular cutaneous lesions with minimal adverse events. Most lesions were easily treated with no anesthesia during the procedure. The time for the procedure did not exceed a few minutes for each lesion. All patients rarely complained about pain during the procedure and pain control was not needed. Further clinical trials are needed to clarify its indications for benign cutaneous lesion treatment.

Competing Interests

This study was made possible through a research contract between the CHU de Québec-Université Laval and Dectro International, which received funds from the CNHR to appoint independent researchers (Lucie Germain and Danielle Larouche). Clément Beaumont and Raymonde Piquette are employees of Dectro International; Clément Beaumont is the Director of Dectro International and provided the equipment manufactured by the company; Raymonde Piquette is an International Trainer at "Académie

Dectro" and she administered the radiofrequency treatments. Dong Hyun Kim is a dermatologist who was on sabbatical at the LG's Lab. Dong Ju Hyun voluntarily contributed to the writing of the paper.

Acknowledgments

This work was financed by the Canadian National Research Council (CNRC), Dectro International, and Canadian Institutes of Health Research (CIHR).

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Hindawi Publishing Corporation
BioMed Research International
Volume 2016, Article ID 3853754, 7 pages
http://dx.doi.org/10.1155/2016/3853754

Clinical Study

The Efficacy and Safety of Fractional CO_2 Laser Combined with Topical Type A Botulinum Toxin for Facial Rejuvenation: A Randomized Controlled Split-Face Study

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Received 12 December 2015; Accepted 27 January 2016

Academic Editor: Steven P. Nistico

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Objective. We evaluated synergistic efficacy and safety of combined topical application of Botulinum Toxin Type A (BTX-A) with fractional CO_2 laser for facial rejuvenation. *Methods*. Twenty female subjects were included for this split-face comparative study. One side of each subject's cheek was treated with fractional CO_2 plus saline solution, and the other side was treated with fractional CO_2 laser plus topical application of BTX-A. Patients received one session of treatment and evaluations were done at baseline, one, four, and twelve weeks after treatment. The outcome assessments included subjective satisfaction scale; blinded clinical assessment; and the biophysical parameters of roughness, elasticity, skin hydration, transepidermal water loss (TEWL), and the erythema and melanin index. *Results*. BTX-A combined with fractional CO_2 laser sides showed higher physician's global assessment score, subject satisfaction score, roughness, skin hydration, and skin elasticity compared to that of fractional CO_2 plus saline solution side at 12 weeks after treatment. TEWL and erythema and melanin index showed no significant differences between two sides at baseline, one, four, and twelve weeks after treatment. *Conclusion*. Topical application of BTX-A could enhance the rejuvenation effect of fractional CO_2 laser.

1. Introduction

Fractional CO_2 laser has now been considered as the gold standard for skin rejuvenation [1]. Nevertheless, when it is used for facial rejuvenation, it often requires multiple therapy sessions which will be a kind of psychological and economic burden for the patients. Besides that, fractional CO_2 laser is still reported to have a variety of complications, such as postinflammatory hyperpigmentation, prolonged erythema, skin swelling, infection, and scarring [2, 3]. How to reduce adverse reactions of fractional CO_2 laser, while enhancing its rejuvenation effect, has become one hot topic in dermatological research field.

Botulinum Toxin Type A (BTX-A) is one of seven neurotoxins produced by *Clostridium botulinum*. BTX-A can result in muscle fiber atrophy and subsequent clinical flaccid paralysis [4]. Consequently, its cosmetic use by subdermal injections in treating wrinkles induced by muscle hyperactivity is widespread [5]. Some physicians have observed a face-lifting effect after intradermal injection of BTX-A to the mid and lower face [6, 7]; however the underlying mechanisms still remain unclear. In 2012, Oh et al. studied the *in vitro* effects of BTX-A on normal fibroblasts and found that BTX-A has a significant effect in increasing the level of collagen production and downregulating its degradation [8]. In 2014, our research group further confirmed the direct antiphotoaging

potential of BTX-A in UVB-induced premature senescence of human dermal fibroblasts *in vitro* through decreasing senescence-related proteins [9]. Whether BTX-A shows its direct skin rejuvenation effects has not been studied *in vivo* as yet.

Recent studies have also reported the transepidermal delivery of drugs, stem cells secreted factors, and even bone marrow mesenchymal stem cells using a fractional ablative laser [10–12]. According to the results of previous literatures, we can speculate that BTX-A can pass through the micropores created by fractional CO_2 laser and penetrate into the dermis, thereby executing various biological effects. In present 12-week clinical study, BTX-A solution was topically administrated following fractional CO_2 laser; we then focused on the effects of BTX-A on the efficacy and adverse effects after fractional CO_2 laser.

2. Material and Methods

2.1. Subjects. This was a single-center prospective pilot study. All subjects provided written informed consent, and this study was approved by the Institutional Review Board of the First Affiliated Hospital of Nanjing Medical University. Twenty female subjects with Fitzpatrick phototypes III and IV were enrolled in the study after signing an informed consent form. Patients were aged 21 to 53 (mean age 35.6). Exclusion criteria were keloids and hypertrophic scars, cancer lesions, warts or skin infections in the area to be treated, viral herpes infections during the previous 6 months, collagen disease, and autoimmune disease. Individuals who had taken systemic isotretinoin, BTX-A injection, or any facial rejuvenation procedures during the previous 12 months, had used nonreabsorbable fillers, were undergoing treatment with antineoplastics, corticosteroids, or anticoagulants, or were diabetic, pregnant, or breastfeeding were excluded.

2.2. Treatment Protocol. A topical anesthetic cream (2.5% lidocaine and 2.5% prilocaine; Tsinghua Ziguang Co., Beijing, China) was applied for 30 minutes before treatment and then completely removed. A fractional CO_2 laser (Acupulse, Lumenis, Inc., Santa Clara, CA, USA) was used for the fractional laser treatment. We treat both cheeks' area of subjects with DeepFX microscanner handpiece of the fractional ultrapulsed CO_2 laser. The laser parameters were set: single pass, 0.12 mm spot size, pulse energy 10 mJ, density 5%, pulse size 10 × 10 mm, and repetition rate 300 Hz. The peak power and beam width of the fractional ultrapulsed CO_2 laser are 200 watt and 50–80 μs, respectively. Ten milliliters of BTX-A solution (diluted in saline solution, concentration at 5 μ/mL) was topically applied onto the fractional laser-treated sites of one randomly selected facial side for one hour, while saline solution was applied to fractional laser-treated sites of the another face side for one hour. The outcome assessments included the subjective satisfaction scale, improvement score according to blinded investigators (using standardized photography), and biophysical measurements. Measurements were conducted at baseline, one, four, and twelve weeks after the treatment. Subjects were permitted to apply their usual skin care products throughout the study. It was requested that subjects do not alter their usual skincare routine during the study period.

2.3. Outcome Assessment

2.3.1. Subjective Satisfaction Scale. Subjects completed a self-assessment questionnaire and rated their improvement on a scale from 0 (aggravated) to 4 (much improved). After treatment, subjects were asked to grade their intraprocedure pain on a 10 cm visual analog scale (VAS), with the end points designated as 0 (no pain) and 10 (the worst pain imaginable). The investigator subjectively graded edema after treatment on a scale from 0 to 4 (0 = absent, 1 = trace, 2 = slight, 3 = moderate, and 4 = prominent). The duration of erythema and crusting was investigated through interviews. Any adverse events and complications were recorded at the time of each treatment and at the follow-up visit.

2.4. Blinded Clinical Assessment. Standardized photographs were obtained at baseline and 1 month after the last treatment. Standardized close-up photographs were taken using a Visia photo stand (Canfield Imaging Systems, Fairfield, NJ) mounted with a high resolution digital camera (Canon EOS-40D, Canon Corp., Tokyo, Japan). Two dermatologists who were blinded to subject treatment group evaluated the serial photographs independently and performed clinical assessments on fine wrinkles, coarse wrinkles, roughness, mottled hyperpigmentation, laxity, and skin tone using a well-established grading scale of 0 ≤ 25% (minimal), 1 = 26–50% (fair), 2 = 51–75% (good), 3 = 75–90% (excellent), and 4 = 91–100% (clear) improvement. Average improvement scores were calculated as the mean of the grading scales of all categories.

2.5. Biophysical Evaluations. The parameters assessed were *in vivo* erythema, melanin, transepidermal water loss (TEWL), elasticity, skin surface roughness, and hydration, which were measured with respective probes (Courage and Khazaka Electronic GmbH, Cologne, Germany). All measurements were taken after subjects had undergone an acclimatization period of at least 20 minutes in an air-conditioned room under standardized conditions (22–25°C, 50% humidity). Each measurement was performed on the left and right cheeks of each subject.

2.6. Statistical Analysis. The results were analyzed with the paired t-test using SPSS 15.0 software (SPSS, Inc., Chicago, IL). $P < 0.05$ was considered to be significant.

3. Results

3.1. Objective Clinical Assessment. Figure 1 shows representative clinical manifestations of both treatment sides taken at indicated time points. During the evaluation periods, two blinded evaluators scores increased successively in both sides. Twelve weeks after the treatment, BTX-A side objective clinical assessment score value was 3.40 ± 0.42 which was significantly higher than that of saline solution side (2.70 ± 0.43) ($P < 0.05$).

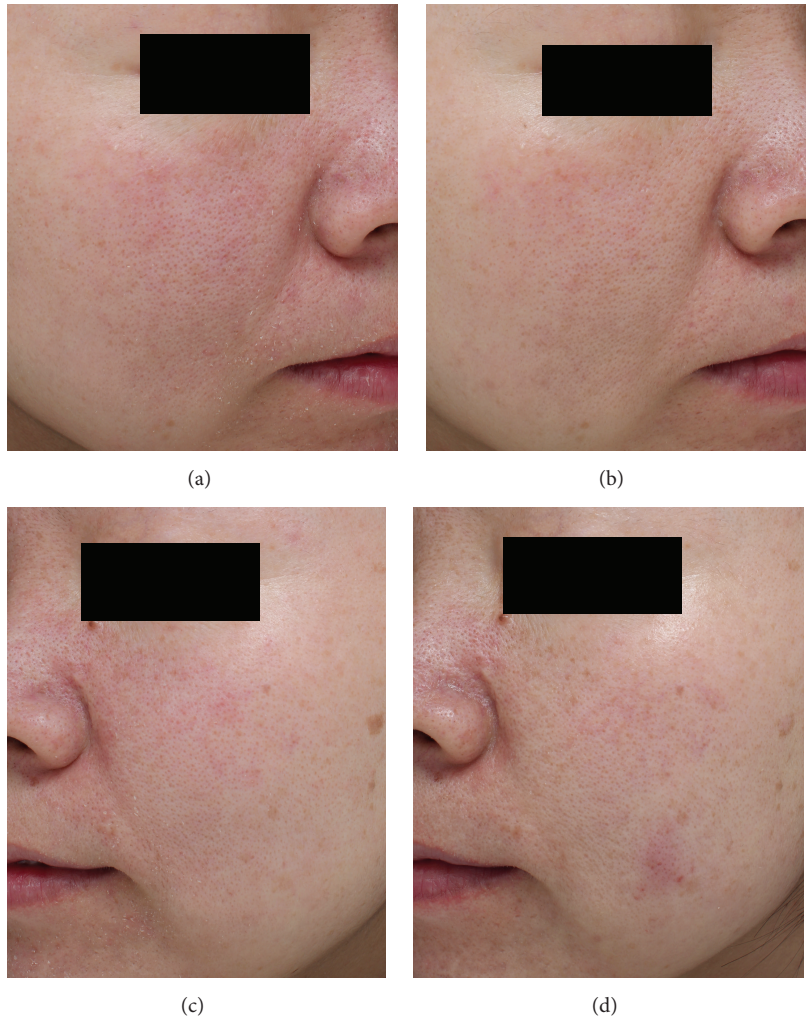

Figure 1: Representative clinical photographs: (a, c) baseline, (b) 12 weeks after the BTX-A + fractionated CO_2 laser treatment, and (d) 12 weeks after the saline solution + fractionated CO_2 laser treatment.

3.2. Subjective Satisfaction Scale. During the evaluation periods, the subjective satisfaction scores in both sides increased successively. Twelve weeks after the treatment, BTX-A side subjective satisfaction score value was 3.40 ± 0.53 which was significantly higher than that of saline solution side (2.70 ± 0.47) ($P < 0.05$).

3.3. Biophysical Analysis

3.3.1. Erythema Index (EI) and Melanin Index (MI). As shown in Figures 2(a) and 2(b), during the study, the average value of EI and MI in both sides achieved the peak value at one week after the treatment, while decreasing to nearly baseline four weeks after treatment. However, the differences between the two sides were not statistically significant at any given point of time after the treatment.

3.4. Elasticity. As shown in Figure 2(c), one week after the treatment, the skin elasticity of both sides was higher than that prior to the treatment, then decreasing to nearly baseline four weeks after the treatment. Twelve weeks after the treatment, the skin elasticity in BTX-A side increased and was higher than that of baseline; however, it remained nearly unchanged in saline solution treated side and the difference of elasticity between the two sides was statistically significant ($P > 0.05$).

3.5. TEWL. TEWL was as one of the noninvasive indices to evaluate the skin barrier integrity or function. As shown in Figure 2(f), the value of TEWL increased at one week after the treatment and then rapidly decreased to baseline at four weeks after the treatment. In BTX-A side, the average values even showed a lower level than baseline and are statistically significantly lower than that of saline solution side at twelve weeks after treatment ($P < 0.05$).

3.6. Hydration. Hydration content was also considered as one of the indices to evaluate the skin barrier. As shown in Figure 2(d), hydration increased from 56.08 ± 9.59 to 67.76 ± 3.38 in BTX-A side. The hydration values in BTX-A side

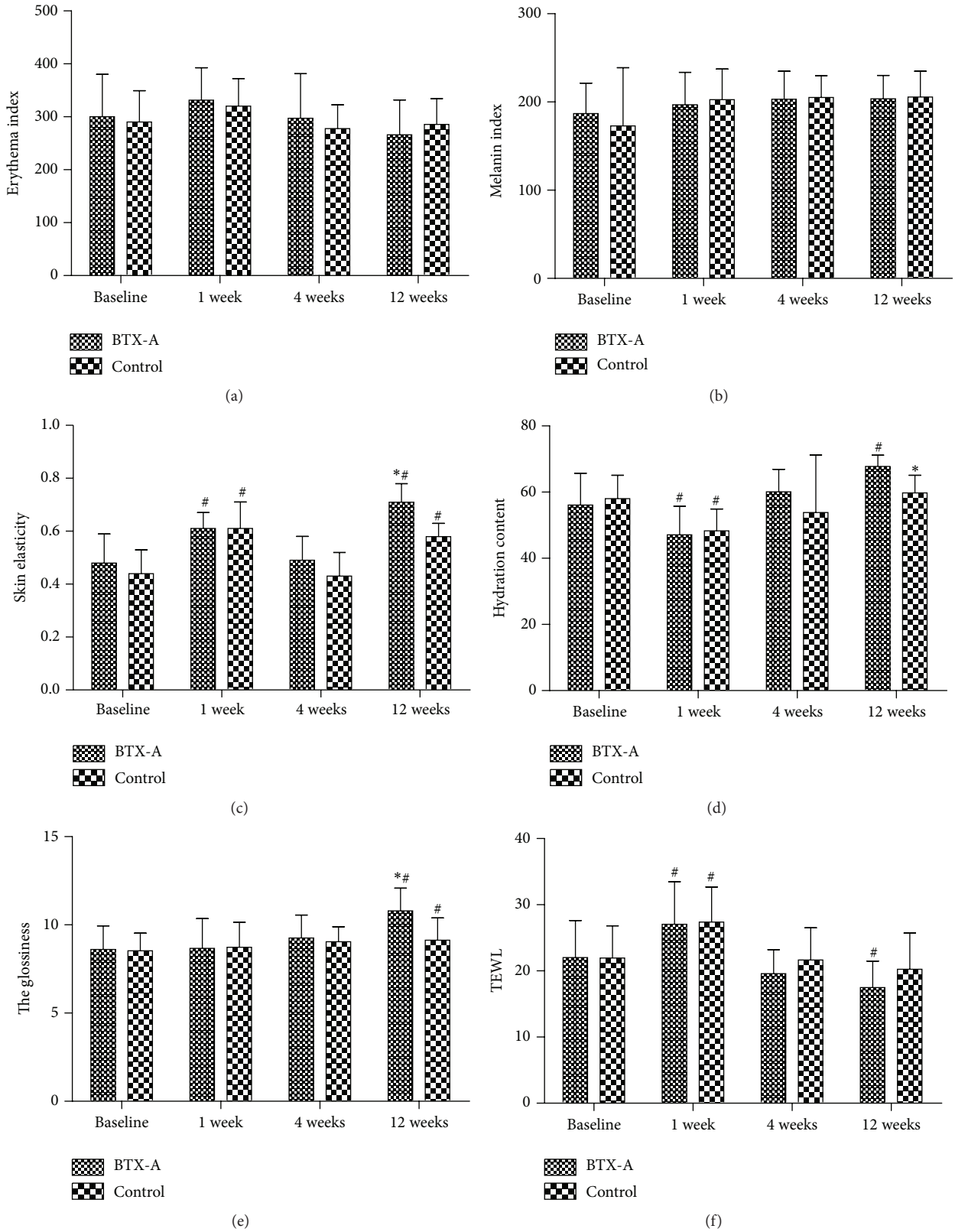

Figure 2: Skin measurements by biophysical evaluation methods were taken at baseline, 1 week, 4 weeks, and 12 weeks after treatment on both sides. (a) Objective measurement of erythema index, (b) melanin index, (c) overall elasticity ($R2$), (d) hydration, (e) glossy, and (f) TEWL. $^{*}P < 0.05$ compared with control side at same time point; $^{\#}P < 0.05$ compared with baseline.

Table 1: Comparison of adverse events on both sides after treatment ($\overline{x} \pm S$).

Group	Pain score	Edema score	Duration of incrustation (d)	Duration of erythema (d)
BTX-A treatment	5.03 ± 0.81	1.73 ± 0.58	7.43 ± 2.27	20.29 ± 1.75
Saline solution treatment	4.95 ± 1.03	1.97 ± 0.72	7.23 ± 2.64	20.45 ± 1.33

Note. No significant differences were observed on both cheeks ($P > 0.05$).

shown were significantly higher than that of saline solution side at twelve weeks after treatment ($P < 0.05$).

3.7. Skin Surface Roughness. As shown in Figure 2(e), skin surface roughness increased significantly (from 8.53 ± 1.0 to 9.13 ± 1.26) in saline solution side and more significantly increased in BTX-A side (from 8.61±1.33 to 10.79±1.29). The differences between the two sides were noted at twelve weeks after treatment ($P < 0.05$).

3.8. Comparison of Adverse Reactions. No serious or persistent side effects occurred during the course of study, and none of the subjects withdrew from the study because of any adverse event. No hypopigmentation or hypertrophic scarring was observed in any subject throughout the study period. As shown in Table 1, there was no significant difference of pain score, edema score, duration of crust, and duration of erythema between the two sides.

4. Discussion

Regarding erythema and melanin index, there are no significant differences between BTX-A side and saline solution control side up to 12 weeks after laser treatment in all subjects. Several reports demonstrated that BTX-A can be used for the treatment of facial erythema and flushing [13–15]. The clinical results were substantiated with corresponding decreases in cutaneous blood flow, as measured using laser Doppler flowmetry [16]. Although BTX-A cannot reduce erythema index after laser treatment, it at least did not induce excessive angiogenesis as shown in the present study. Besides that, there are not any significant differences between the two sides in regard to the pain score, edema score, duration of crusting, and duration of erythema, which verify that adjuvant BTX-A treatment may not help promote the recovery of laser-damaged skin and decrease downtime.

Although an increase in the level of TEWL and a decrease in the hydration content in the stratum corneum one week after the treatment were the signs of impaired skin barrier function, these two indices in both sides recovered to the baseline levels at four weeks after the treatment. Interestingly, at twelve weeks after the treatment, the TEWL was significantly lower, while hydration was significantly higher in BTX-A side than that of saline solution side. These results suggested that although BTX-A did not have an impact on skin recovery, it improves skin barrier function quality after laser treatment. A recent research indicated that the skin barrier function was enhanced after the injection of a new variety of BTX [17]. Besides that, Oh et al. also found that BTX-A could increase the viability of skin fibroblasts, suggesting its role in promoting cell growth and wound healing [8]. Moreover, some researchers have also proven that BTX-A could accelerate the healing process of several kinds of skin wounds [18–20], suggesting BTX-A may activate the proliferation and migration of human skin cells which are important for skin barrier function maintenance. In one word, our results in the present *in vivo* study further confirmed that better skin barrier function can be achieved by BTX-A topical treatment.

There have been many studies which have suggested that fractional CO_2 laser can significantly improve skin texture and wrinkles [21–23]. Similar to previous reports, the subjective satisfaction scores of skin elasticity and texture were significantly better than control sides. Besides that, we also confirmed by biophysical analysis that the skin elasticity and skin surface roughness in BTX-A sides were also higher than those of control sides 12 weeks after treatment. These results suggested that topical application of BTX-A can significantly enhance the facial rejuvenation effect of fractional CO_2 laser. Several *in vivo* histopathological researches have confirmed that depth of the microthermal zones of fractionated CO_2 laser, whose settings are similar to the present study, was limited to the middermis [24–26]. Skin permeation of small-molecule drugs, macromolecules, and nanoparticles mediated by a fractional carbon dioxide laser mostly target the skin dermis [27, 28]. In this consideration, we speculate that BTX-A solution used in the present study may take its action mainly on dermis rather than muscle fibers alone. Besides that, the best facial rejuvenation effect of topical BTX-A appeared 12 weeks after laser treatment which further supports our hypothesis. In our previous study, we found BTX-A has positive effects on photoaged fibroblasts *in vitro* by increasing collagen production, decreasing collagen degradation, and stimulating cell proliferation via decreasing senescence-related proteins [9]. However, up to date, it remains unclear with which cellular signal pathway BTX-A acts as an antiaging molecule. Its exact functional mechanisms deserve further basic in-depth researches.

One major shortcoming of this study is that it is merely a small sample prospective clinical study. A larger sample randomized controlled clinical trials need to verify the results of this study. To the best of our knowledge, our study is first to report the efficacy and safety of topical BTX-A markedly enhancing the facial rejuvenation effect of fractional CO_2 laser. We suggest that topical BTX-A can be used as an adjuvant therapy after fractional CO_2 laser. Its exact mechanism of action and how to optimize its clinical effects are still worth further researches.

Conflict of Interests

The authors report no conflict of interests.

Acknowledgments

This work was supported by Grants from the China National Natural Science Foundation (81573072 and 81301384) and the Priority Academic Program Development (PAPD) of Jiangsu Higher Education Institutions.

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