

Using Reflectance Confocal Microscopy to Observe In Vivo Melanolysis After Treatment With the Picosecond Alexandrite Laser and Q-Switched Nd:YAG Laser in Melasma

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Objectives: Melasma is an acquired type of hyperpigmentation that is characterized by the appearance of scattered light- to dark-brown macules and patches on the face. Recently, several lasers have been proposed as treatment options for melasma. In particular, the picosecond alexandrite laser is an ideal laser for selective photothermal melanolysis. The aim of our study was to compare the effectiveness in melanolysis of a single treatment of the picosecond alexandrite laser with that of the Q-switched Nd:YAG laser using reflectance confocal microscopy imaging of the melasma lesions.

Materials and Methods: We performed a split-face study using the picosecond alexandrite laser and Q-switched Nd:YAG laser in eight patients with melasma. Both melasma lesions and surrounding normal skin were examined under reflectance confocal microscopy 1 and 24 hours after treatment. The melanin intensity of each skin layer was investigated.

Results: At baseline, melasma has irregular melanin distribution and a higher melanin density than surrounding normal skin under reflectance confocal microscopy. After a single treatment with either the picosecond alexandrite laser or the Q-switched Nd:YAG laser, both melanin-induced reflectance and melanin index decreased.

Conclusion: Our findings suggest that it is feasible to assess the distribution of melanin by reflectance confocal microscopy and observe the melanolysis in melasma lesion after laser treatment. *Lasers Surg. Med.* 9999:1–7, 2018. © 2018 Wiley Periodicals, Inc.

Key words: melasma; Nd:YAG laser; picosecond alexandrite laser; reflectance confocal microscopy

INTRODUCTION

Melasma is an acquired, variable sized hyperpigmentation that is characterized by scattered light- to dark-brown macules and patches on the face. It predominantly affects the malar areas, forehead, and chin [1]. The etiology and pathogenesis of melasma are not yet fully understood. Its development is thought to be associated with genetic factors, sun exposure, pregnancy, and oral contraceptive uses [2]. There are various treatment modalities used for

melasma, including broad-spectrum protective sunscreens, and a wide variety of topical agents used alone or in combination. Several lasers have recently been proposed as alternative treatments for melasma with variable success [3–5]. Laser treatments in melasma could induce melanosome photothermolysis according to the thermal relaxation time. This cell damage requires a pulse duration of only nanoseconds, which is achievable with Q-switched lasers [6]. Repetitive low-fluence treatments (achieved over multiple sessions) of Q-switched lasers have been found to be efficacious in the treatment of melasma [7,8]. The 755 nm Q-switched alexandrite laser has also been used for cutaneous hyperpigmented lesions [7,8]. The picosecond alexandrite laser (PAL) has an ultra-short pulse duration compared to those of traditional lasers. This ultra-short pulse duration results in an intense photomechanical impact beyond the photothermal action, which selectively and effectively destroys the target while causing minimal damage to surrounding tissues [9].

Reflectance confocal microscopy (RCM) is a non-invasive tool for *in vivo* microscopic analysis of the skin. RCM allows visualization of skin in real-time at almost histologic resolution to a depth of 200–300 μm including epidermis and upper dermis [10]. Imaging is obtained by detecting singly backscattered photons from the optical section and contrast is due to the relative differences in refractive indices and sizes of cell organelles [11,12]. Strong signal and bright contrast is obtained particularly from keratin, collagen, and melanin [11]. Therefore, RCM is especially

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useful in the evaluation of pigment-related disorders such as hyperpigmentation [13]. Non-invasively, RCM would allow for assessment of dynamic changes in melanin content on target spots at sites of laser treatment. In melasma lesions, RCM images allow for identification and semi-quantification of the amount of melanin in each skin layer, including the epidermis (spinous layer and basal layer) and upper dermis before and after laser treatment.

Although PAL is an ideal laser for selective photothermal melanolysis, its use in the treatment of melasma has only recently been reported [9,14,15]. To establish the personalized/best laser treatment strategy for melasma, it is essential to study the dynamic changes in melanin and its surrounding structures during laser-skin interactions. No prior studies have used RCM to study *in vivo* melanolysis in human skin affected by melasma after laser treatments. In this study, we analyzed RCM imaging to investigate and compare the clinical improvement of melasma lesions after single treatments with PAL and Q-switched neodymium-doped yttrium aluminum garnet (QS Nd:YAG) lasers.

MATERIALS AND METHODS

Subjects and Designs

Eight female volunteers with melasma were recruited and informed about the treatment. Participants were excluded if they had any facial skin condition that could interfere with the interpretation of melasma response to laser treatment. Participants were excluded if they had any of the following within 3 months of the study: facial treatments with laser, botox, fillers, or whitening creams or ointments, including hydroquinone or tretinoin. The average age of melasma patients was 49.00 ± 4.07 years (mean \pm SD, range 42–56 years). All subjects included in this study had Fitzpatrick skin type III. The baselines were measured using RCM (Table 1). The Ethics Committee (institutional review board approval #Dermapro IRB 1-220777-A-N-02-DICN16211) approved the study.

Intervention Protocol

After RCM was used to establish a baseline, participants received a single treatment of either PAL or low fluence QS Nd:YAG on the face, including the melasma lesion and surrounding normal skin. The treatments were performed in a split-face design. Briefly, patients were randomized to receive QS Nd:YAG treatment on one half of the face and PAL on the other half. We determined the appropriate fluence of the lasers to provide tolerable energy to the melasma lesions without topical anesthesia. The end point for the laser treatments was no visible skin change or mild temporary erythema described as low fluence laser toning. In this study, PAL (wavelength 755 nm, picosecond pulse duration, 0.8 J/cm^2 , 4.5–5.6 mm spot size, 5 Hz, 2–3 pass) (Picosure[®], Cynosure, Westford,

TABLE 1. The Characteristics of Melasma Patients at Baseline

No.	Age	Fitzpatrick skin type	Follicular plug	Follicular hyperpigmented ring	Melanocyte dendriticities	Melanophages		Dermal matrix reflectance	
						Melasma lesion	Surrounding normal skin	Melasma lesion	Surrounding normal skin
1	47	III	+	+	0	+	+	+	+
2	46	III	+	+	0	+	+	+	+
3	49	III	+	-	0	++	-	-	+
4	52	III	-	+	(Both ^a)	+	-	+	+
5	42	III	+	-	+ (Interfollicular)	-	+	++	+
6	51	III	-	-	0	+	-	+	+
7	56	III	+	-	0	+	-	+	+
8	49	III	+	+	++ (Both ^a)	+	+	+	+

Intensity values in this table are indicated as + signs; +, weak; ++, moderate; ++++, prominent.

^aBoth; follicular and interfollicular epithelium.

MA) and QS Nd:YAG (wavelength 1064 nm, 1.2–1.6 J/cm², 7–8 mm spot size, 5 Hz, 2–3 pass) (TRI-BEAM™, Jeisys, Seoul, South Korea) lasers were used. Two passes were performed on the the face.

RCM Imaging

The reflectance confocal microscopy (RCM) employed was a Vivascope 1500 (Lucid Technologies, Rochester, New York, NY), which is commercially available for clinical *in vivo* imaging. The power of RCM is 1.0–5.2 mV, with a mean power of 2.40 mV. Both melasma lesions and surrounding normal skin were examined before treatment and 1 and 24 hours after treatment.

Confocal Scoring

The pigment intensity of each skin layer (spinous layer, basal layer, and papillary dermis) was investigated 1 and 24 hours after treatment. This evaluation was performed by three blinded dermatologists using a qualitative scale of pigmentation with the following categories: much improved (+2); improved (+1); stationary (0); aggravated (–1); much aggravated (–2), in comparison with baseline.

Melanin Index

We measured the melanin index (MI) using the Mexameter® MX18 (C + K, Germany) before and after treatments.

Statistics

Statistical analysis was conducted using the SPSS® software program (IBM, Chicago, IL). The Shapiro–Wilks test or Kurtosis & Skewness was used for the normality test to determine whether variables followed a normal distribution. Statistical analysis of variables for parametric was conducted using the RM ANOVA. *P*-values <0.05 were considered statistically significant. The decrement rate (%) was defined according to the following formula: (Before treatment–After treatment)/Before treatment * 100.

RESULTS

Comparing the Reflectance Confocal Microscopy Findings of Melasma Lesions and Surrounding Normal Skin at Baseline

On the 30-fold magnified dermoscopic images with Vivacam, follicular plugs and hyperpigmented rings were observed in 6 (75%) and 4 (50%) of the melasma lesions from eight subjects, respectively (Fig. 1A). These hyperpigmented rings on dermoscopy were observed as hyper-reflectant rings on RCM in 4 (50%) of the melasma lesions (Fig. 1B). The RCM evaluation of melasma showed an irregular melanin distribution and higher melanin density compared to those of surrounding normal skin. We evaluated the melanin at all levels of the skin (epidermis, upper dermis). In the epidermis, we observed densely aggregated melanosomes in the keratinocytes of the spinous layer. At the dermo-epidermal junction (DEJ) level, there were papillary rings around the dermal

papillae composed of sequences of brighter structures and dendritic melanocytes. This is thought to comprise activated melanocytes and basal keratinocytes receiving packed melanosomes. Prominent dendritic melanocytes were present in the basal layer of the epidermis in three (37.5%) of eight subjects (Table 1, Fig. 2A–C). There were also variable-sized polygonal refractile structures in the papillary dermis. These structures were consistent with melanophages filled with melanin (Fig. 2D). In surrounding normal skin, there was less melanin in the epidermis and DEJ than there was in the melasma lesions.

RCM Findings After PAL and QS Nd:YAG Treatment

After the baseline RCM evaluation, a single treatment of PAL or QS Nd:YAG was employed in a split-face design on the melasma and surrounding normal skin. Subjects were examined after 1 and 24 hours after treatment (Fig. 3).

After PAL treatment, there was a decrease in melanin-induced reflectance in the spinous layer and basal layer (Table 2). The melanin index was significantly decreased at 1 and 24 hours after laser treatment. The decrement rates were 19.04% and 7.76%, respectively (Table 3).

In contrast, treatment with QS Nd:YAG led to slight or non-significant improvement in the spinous layer and aggravated findings in the basal layer (Table 2). However, the melanin index was significantly decreased 1 and 24 hours after laser treatment, and decrement rates were 14.23% and 5.78%, respectively (Table 3).

After the treatments, a decrease of melanin-induced reflectance was shown in melasma lesion and surrounding normal skin. However, there was more improvement in the surrounding normal skin than in the melasma lesions. The melanin index decreased at 1 hour after both treatments in surrounding normal skin.

RCM Findings of Melanocyte Dendrites After Laser Treatment

We evaluated the increase in melanocyte dendricities compared to that at baseline in melasma and defined it as the activation of melanocytes. After 1 hour of PAL or QS Nd:YAG treatment, 37.5% of subjects in both groups demonstrated melanocyte activation in the basal layer. One and two subjects showed melanocyte activation 24 hours after PAL and QS Nd:YAG treatment, respectively (Table 4). In addition, 37.5% of subjects showed perifollicular reflectance accentuation in the basal layer and upper dermis after treatment with QS Nd:YAG and PAL lasers, respectively.

DISCUSSION

In this split-face study, we observed dynamic changes in the amount and distribution of melanin in each layer of human skin after a single treatment with either PAL or QS Nd:YAG. And, by observing the bright epidermal dendritic cells on RCM images, we were able to identify the dynamic response of melanocytes in the melasma lesion. After a single treatment with PAL or QS Nd:YAG, the reflectance of melanin pigment decreased in the melasma lesions (and

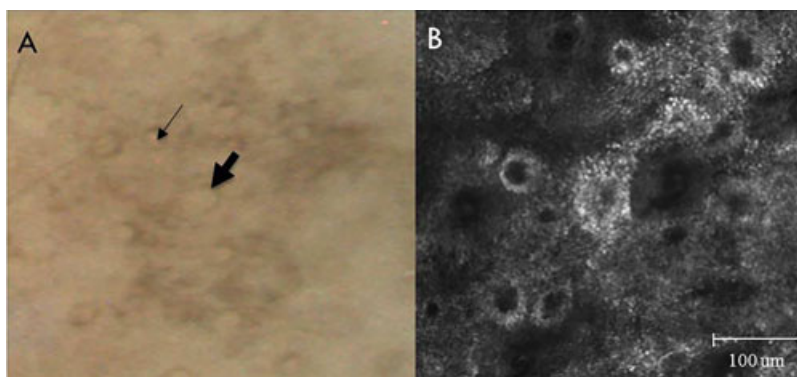


Fig. 1. (A) Dermoscopic images of follicular plugs (thin arrow) and follicular hyperpigmentation rings (thick arrow) prior to laser treatment (Subject No. 08). (B) Corresponding reflectance confocal images at the spinous layer.

in surrounding normal skin) through melanolysis in the absence of clinical changes.

In melasma lesions, electron micrographs and light microscopy revealed numerous melanosomes in keratinocytes and melanocytes; the melanocytes were enlarged with increased numbers of melanosomes and prominent dendrites [16]. Therefore, Grimes et al. [16] suggested that melasma is a consequence of hyperactive melanocytes that cause excessive melanin deposition in the epidermis and perivascular melanophages in the dermis. Other ancillary histological findings in melasma were increased solar elastosis and numbers of mast cells or increased microvasculature. RCM consistently detected epidermal and upper dermal pigmentation, similar to the histological studies of melasma lesions [17,18]. Kang et al. [17] reported that the degree of solar elastosis was more severe in the melasma lesion than in perilesional normal skin. In RCM images, solar elastosis is characterized by less refractile, ragged, and lacy structures at varying degrees within the dermis. However, we only identified two cases (25%) of suspected solar elastosis in the papillary dermis at baseline. After the laser treatments, fine hyper-refractile dermal matrix changes were observed in three patients (37.5%). One potential reason for these changes is that laser treatments increased the collagen reflectance due to increased

collagen cross-linking and reorganization of the fine dermal matrix structure. However, additional research is needed.

Melasma is classified based on the depth of pigmentation into epidermal, dermal, and mixed types. A wood lamp examination can be used to determine the subtypes of melasma. However, others [16,19] have suggested that wood light examinations and histopathology are poorly correlated. Alternatively, melasma can be classified based on the melanin distribution observed using RCM [19,20]. Based on RCM images, melasma is classified into epidermal and mixed types [4,18,19,21]. Liu et al. [21] recruited 200 melasma cases and classified their types by RCM images. Of the 200 patients, 143 (71.5%) cases were classified as the epidermal type and the other 57 (28.5%) cases were classified as mixed type. In our study, when comparing melasma lesion and surrounding normal skin, higher melanin density were observed in the epidermis in all the melasma lesions. And 37.5% showed similarly small amounts of dermal melanophages in both areas. 50% had more dermal melanophages in the melasma lesions than there were in the surrounding normal skin. This finding suggests that half of our cases are epidermal type with increased melanin only in the epidermis and half were mixed type with increased melanin in both the epidermis

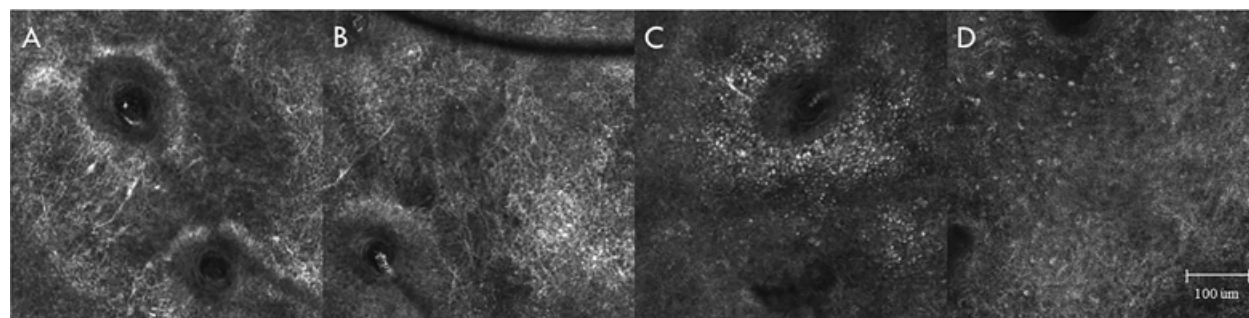


Fig. 2. Activated melanocyte dendrites in a melasma lesion prior to treatment; (A and B) dendrites from subject #04 in the basal layer; (C) dendrite and accentuating melanosomes around the follicles of subject #08 in the spinous layer; (D) melanophages filled with melanin from subject #03.

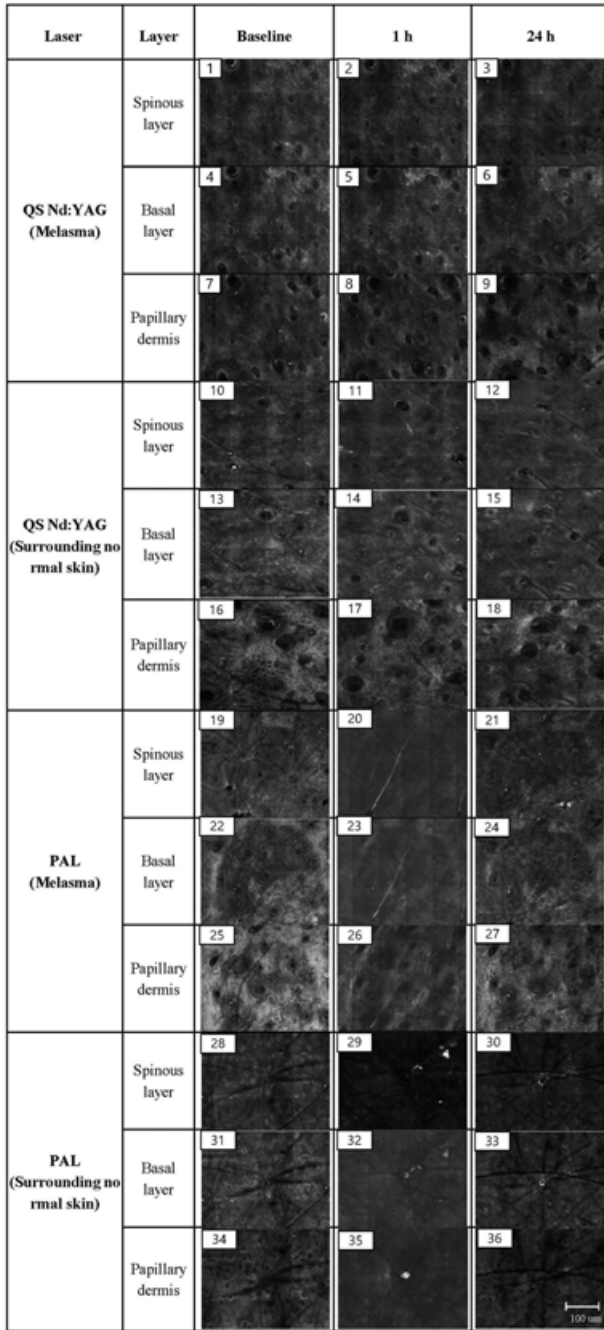


Fig. 3. RCM images before and after treatments with the PAL (Subject #05) and QS Nd:YAG lasers (Subject #03).

and the dermis. As in previous studies, we found no pure dermal type. Therefore, we supposed that dermal melanin should not be considered the main treatment target for melasma. And RCM analysis may become an adjuvant method of classifying melasma. In addition, RCM analysis is helpful in assessing melanocyte activity at baseline in melasma patients. Kang et al. [18] detected activated melanocytes in 6 of 26 melasma patients by RCM, which is a finding that was only previously recognized through electron microscopy studies.

TABLE 2. Improvements in Melanin Reflectance After Laser Treatment (Much Improved [+2], Improved [+1], Stationary [0], Aggravated [-1], Much Aggravated [-2])

Laser	Spinous layer		Basal layer	
	1 h	24 h	1 h	24 h
PAL				
Melasma	0.375	0.083	0.375	0.083
Surrounding normal skin	0.583	0.417	0.625	0.333
QS Nd:YAG				
Melasma	0.042	0.000	-0.125	-0.083
Surrounding normal skin	0.042	0.125	0.125	0.167

Improvement scoring; +2, much improved; +1, improved; 0, stationary; -1, aggravated; -2, much aggravated.

One study using electron microscopy found that, in melasma lesions, there were more stage IV melanosomes in the dendritic processes of the melanocytes than there were in surrounding normal skin [22]. They found that the melanosomes existed in membrane-bound clusters and were more densely packed in basal and suprabasal keratinocytes in melasma lesions. In this study using RCM, irregularly distributed aggregated melanosomes were found in all melasma patients. The distribution of melanin aggregates was uneven, so a therapeutic strategy of laser treatment must consider this irregular pattern. We suggest that fixed energy is difficult to achieve adequate photothermal melanolysis for clinical improvement and requires stepwise laser energy adjustments. In addition, we suspect that aggregated melanosomes may have higher absorbed photothermal energy density than do single melanosomes. Laser treatments can be locally aggressive and damage follicular melanocytes and may cause mottled hypopigmentation, which can occur after repeated laser treatments [17].

Several studies have also studied RCM findings after intense pulsed light (IPL) and QS Nd:YAG treatments and showed significant clinical improvement in hyperpigmentation lesions such as lentigines [23,24]. In this study, we used RCM imaging to determine whether subclinical *in vivo* melanolysis could be achieved, despite an absence of clinical improvements (in hyperpigmentation) after a single treatment. The laser treatment produced variable reductions in the melanin pigment of the basal layer level. Treatment with the QS Nd:YAG laser also led to pigment reduction, but less than that of the PAL. The melanin index decreased by 14.23% and 19.04% 1 hour after treatment with the QS Nd:YAG and PAL lasers, respectively. However, there were no significant changes in the Mexameter measurements in the surrounding normal skin. In contrast, in the RCM imaging, the melanin reflectance was improved and the pigment tended to decrease after laser treatments in normal looking skin. Therefore, we suggest that energy of both PAL and QS Nd:YAG lasers used in this study are more effective at inducing melanolysis in surrounding normal skin than they are in melasma lesions.

TABLE 3. Melanin Index After Treatment With PAL and QS Nd:YAG Lasers

Group	Time	Melanin index (mean \pm SD)	Decrement rate (%)	P-value
PAL				
Melasma	Baseline	176.21 \pm 14.40	—	—
	After 1 h	142.67 \pm 27.43	19.04▼	0.003*
	After 24 h	162.54 \pm 23.80	7.76▼	0.017*
	Baseline	131.20 \pm 19.71	—	—
Surrounding normal skin	After 1 h	129.60 \pm 16.98	1.22▼	0.562
	After 24 h	134.67 \pm 18.50	2.64△	0.144
QS Nd:YAG				
Melasma	Baseline	174.46 \pm 16.91	—	—
	After 1 h	149.63 \pm 35.80	14.23▼	0.004*
	After 24 h	164.38 \pm 19.27	5.78▼	0.003*
	Baseline	143.56 \pm 14.75	—	—
Surrounding normal skin	After 1 h	135.67 \pm 14.75	5.50▼	0.020*
	After 24 h	141.89 \pm 20.26	1.16▼	0.667

*P-values <0.05 are statistically significant.

In the treatment of melasma, there is a risk of melanocyte stimulation, with subsequent cutaneous inflammatory cascades causing post-inflammatory hyperpigmentation. Because of the interaction between the laser and the skin, it is necessary to monitor the response of the dynamic skin tissue to select the appropriate treatment laser intensity. Depending on the location and distribution of the melanosomes in individual lesions, the treatment intensity of the appropriate laser may vary. It is ideal to

TABLE 4. Melanocyte Activation After Treatment With PAL and QS Nd:YAG Lasers

No.	PAL		QS Nd:YAG	
	1 h	24 h	1 h	24 h
1	0	0	0	+
2	+	0	0	0
3	0	0	+	0
4	++	+	++	++
		(Both ^a)		(Both ^a)
5	0	0	0	0
6	0	0	+	0
			(Interfollicular)	
7	0	0	0	0
8	++	0	0	0
	(Follicular)			

Definition of activation; increased compared to baseline. Degree of activation: ++, much activated; +, activated; 0, stationary.

^aBoth; follicular and interfollicular epithelium.

determine an intensity that does not induce perivascular inflammation and does not stimulate melanocytes with excessive thermal stimulation. In RCM imaging, the PAL- and QS Nd:YAG-treated lesions did not have any unusual features reminiscent of perivascular inflammatory cell infiltration or papillary edema (data were not shown). We observed melanocyte activation by observing changes in melanocyte dendriticities with RCM. Three of eight patients with PAL or QS Nd:YAG laser treatment experienced increases in melanocyte dendriticities 1 hour after laser treatment. However, there were no clinical changes in hyperpigmentation. Yamashita et al. [24] reported melanocyte activation detected on RCM imaging in all nine subjects after 5 days of IPL treatment; however, there was no post-inflammatory hyperpigmentation. Therefore, RCM imaging may be a useful tool to observe hyper-activated melanocytes before and after treatment. However, further studies are needed to determine whether melanocyte dendriticities reflecting hyper-activation after treatment can be a morphologic marker to monitor the therapeutic and adverse effects of laser treatments.

We compared the therapeutic efficacy of PAL and QS Nd:YAG lasers for hyperpigmentation in a split-face study in one subject. We adjusted the laser intensity so that the two lasers introduced comparable treatments. However, it was still difficult to arrange for perfect theoretical comparison conditions, because the two lasers had different pulse durations. A patient's response to lasers varies depending on the individual's Fitzpatrick's skin type, melanin amount and distribution, skin thickness and dermal density. Therefore, although we ensured that the laser treatments were performed at the same intensities, the energy that is ultimately concentrated in melanin likely differs. To set an appropriate standard intensity, after controlling for the spot size and repetitive time, the energy density was adjusted based on the energy needed to whiten the hair to the point where the pain remained tolerable for each patient. The laser energy density was 1.6 J/cm² in the PAL and 1.8 J/cm² in the QS Nd:YAG. Nonetheless, the PAL group had a slightly more frequent tingling reaction and a stronger thermal response than did those in the QS Nd:YAG group. There was also a better treatment response in the PAL group than in the QS Nd:YAG group.

In this study, we observed the perifollicular reflectance accentuation that is a phenomenon that hyperpigmentation around the hair follicles aggravate after laser treatment. It was observed in 37.5% of patients after PAL and QS Nd:YAG treatments. We divided the cases of follicular and interfollicular epithelial pigmentation and analyzed the results. In three of eight patients who received QS Nd:YAG treatment and two of eight patients who received PAL treatment, melanocyte activation was observed near the follicular epithelium. Yamashita et al. [24] also reported a black pigmented ring around the hair follicle after IPL treatment. The characteristics of the follicular epithelium may be different from those of the interfollicular epithelium, and the depth of the follicular epithelium may be deeper than that of the interfollicular epithelium. Therefore, energy of laser treatment can not

directly reach the follicular epithelium. However, the thermal energy may be more concentrated in the follicular epithelium if laser thermal energy is delivered through follicular unit. And follicular epithelium can be affected by the surrounding dermal microenvironments. These RCM findings suggest that mottled hypopigmentation reported after repetitive laser treatments may occur due to concentrated thermal energy around the melanin of hair follicular epithelium.

The limitation of our study is its small number of subjects. And we studied activated melanocytes for 24 hours after laser treatment. However, long-term follow-up is needed to determine that the activated melanocytes can predict post-inflammatory hyperpigmentation. In addition, although we observed one session of laser treatment, melasma generally requires repeated treatments to improve these conditions.

CONCLUSION

This study described the distribution of melanin to be considered in laser treatment in melasma lesion using RCM. Images of RCM can be used to follow-up and monitor the response to therapy and to screen subjects who may have adverse reactions to laser treatments. Further studies are necessary to clarify the utility of RCM in characterization and monitoring of melasma and other pigmented skin disorders.

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REFERENCES

- Gupta AK, Gover MD, Nouri K, Taylor S. Treatment of melasma: A review of clinical trial. *J Am Acad Dermatol* 2006;55:1048–1065.
- Grimes PE. Melasma: Etiologic and therapeutic considerations. *Arch Dermatol* 1995;131:1453–1457.
- Cestari TF, Hassun K, Sittart A, de Lourdes Viegas M. A comparison of triple combination cream and hydroquinone 4% cream for the treatment of moderate to severe facial melasma. *J Cosmet Dermatol* 2007;6:36–39.
- Niwa Massaki AB, Eimpunth S, Fabi SG, Guiha I, Groff W, Fitzpatrick R. Treatment of melasma with the 1,927-nm fractional thulium fiber laser: A retrospective analysis of 20 cases with long-term follow-up. *Lasers Surg Med* 2013;45:95–101.
- Zaleski L, Fabi SG, Goldman MP. Treatment of melasma and the use of intense pulsed light: A review. *J Drugs Dermatol* 2012;11:1316–1320.
- Anderson RR, Parrish JA. Selective photothermolysis: Precise microsurgery by selective absorption of pulsed radiation. *Science* 1983;220:524–527.
- Wang HW, Liu KY. Efficacy and safety of low-energy QS Nd:YAG and QS-alexandrite laser for melasma. *Zhongguo Yi Xue Ke Xue Yuan Xue Bao* 2009;31:45–47.
- Newcomer VD, Lindbert MC, Stenbert TH. A melanosis of the face. *Arch Dermatol* 1961;83:84–97.
- Lee YJ, Shin HJ, Noh TK, Choi KH, Chang SE. Treatment of melasma and post-inflammatory hyperpigmentation by a picosecond 755-nm alexandrite laser in Asian patients. *Ann Dermatol* 2017;29:779–781.
- Fuchs CSK, Andersen AJB, Ardigo M, Philipsen PA, Haedersdal M, Mogensen M. Acne vulgaris severity graded by *in vivo* reflectance confocal microscopy and optical coherence tomography. *Lasers Surg Med* 2018;2:e23008.
- Rajadhyaksha M, Marghoob A, Rossi A, Halpern AC, Nehal KS. Reflectance confocal microscopy of skin *in vivo*: From bench to bedside. *Lasers Surg Med* 2017;49:7–19.
- Banzhaf CA, Lin LL, Dang N, Freeman M, Haedersdal M, Prow TW. The fractional laser-induced coagulation zone characterized over time by laser scanning confocal microscopy—A proof of concept study. *Lasers Surg Med* 2018;50:70–77.
- Rajadhyaksha M, Grossman M, Esterowitz D, Webb RH, Anderson RR. *In vivo* confocal scanning laser microscopy of human skin: Melanin provides strong contrast. *J Invest Dermatol* 1995;104:946–952.
- Chalermchai T, Rummaneeethorn P. Effects of a fractional picosecond 1,064 nm laser for the treatment of dermal and mixed type melasma. *J Cosmet Laser Ther* 2017;11:1–6.
- Choi YJ, Nam JH, Kim JY, et al. Efficacy and safety of a novel picosecond laser using combination of 1064 and 595 nm on patients with melasma: A prospective, randomized, multicenter, split-face, 2% hydroquinone cream-controlled clinical trial. *Lasers Surg Med* 2017;49:899–907.
- Grimes PE, Yamada N, Bhawan J. Light microscopic, immunohistochemical, and ultrastructural alterations in patients with melasma. *Am J Dermatopathol* 2005;27:96–101.
- Kang WH, Yoon KH, Lee ES, et al. Melasma: Histopathological characteristics in 56 Korean patients. *Br J Dermatol* 2002;146:228–237.
- Kang HY, Bahadoran P, Suzuki I, et al. *In vivo* reflectance confocal microscopy detects pigmentary changes in melasma at a cellular level resolution. *Exp Dermatol* 2010;19:e228–e233.
- Kang HY, Bahadoran P. Application of *in vivo* reflectance confocal microscopy in melasma classification. *J Am Acad Dermatol* 2012;67:157.
- Sarvjit V, Sharma S, Mishra S, Singh A. Melasma: A clinicopathological study of 43 cases. *Indian J Pathol Microbiol* 2009;52:357–359.
- Liu H, Lin Y, Nie X, et al. Histological classification of melasma with reflectance confocal microscopy: A pilot study in Chinese patients. *Skin Res Technol* 2011;17:398–403.
- Lee DJ, Park KC, Ortonne JP, Kang HY. Pendulous melanocytes: A characteristic feature of melasma and how it may occur. *Br J Dermatol* 2012;166:684–686.
- Richtig E, Hofmann-Wellenhof R, Kopera D, El-Shabrawi-Caelen L, Ahlgrimm-Siess V. *In vivo* analysis of solar lentigines by reflectance confocal microscopy before and after Q-switched ruby laser treatment. *Acta Derm Venereol* 2011;91:164–168.
- Yamashita T, Negishi K, Hariya T, et al. Intense pulsed light therapy for superficial pigmented lesions evaluated by reflectance-mode confocal microscopy and optical coherence tomography. *J Invest Dermatol* 2006;126:2281–2286.