Melasma and reflectance confocal microscopy: from baseline to treatment monitoring

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Abstract:

Melasma is a frequent condition worldwide and it represents one of the most challenging disorders to treat in cosmetic dermatology. One of the key factors for treatment prognosis is the assessment of depth and distribution of pigment within the hyperpigmented area. Nowadays, non-invasive skin imaging techniques, such as reflectance confocal microscopy (RCM), have been used to estimate pigment distribution and depth within different skin layers.

This article aims to provide a systematic review on RCM applications on melasma, providing terminology and investigating characteristics of melasma at baseline and after medical and laser treatment.

Our results support the recognition of two main types of melasma, epidermal and mixed type, thanks to the role of RCM in highlighting the precise pigment depth location in the skin, non-invasively. RCM treatment monitoring enables to objectify pigment variations after treatment, as well as to identify prognostic factors for different treatment modalities. After the era of application of RCM as a technique applied strictly to skin cancers, additional cosmetic applications are emerging, such as the application to melasma treatment monitoring.

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Introduction

Melasma is an acquired disorder characterized by light-to-dark-brown hyperpigmentation. This condition affects millions of people worldwide. Mostly, melasma occurs in women with Fitzpatrick skin phototypes III to VI, and it is located on the face; occasionally it can be observed on other sun-exposed areas, such as neck and forearms [1,2].

Many predisposing factors have been described such as genetic predisposition, hormones, pregnancy as well as exposure to ultraviolet radiation and drug assumption[3]. Melasma is one of the most challenging disorders to treat in cosmetology and a multimodal approach is often required[4]. According to current evidence, the first line of treatment is considered topical treatment, including hydroquinone-based products (such as triple combination topical hydroquinone, steroid and tretinoin). Nevertheless, short-term treatments with hydroquinone should be taken into account to avoid exogenous ochronosis. Second-line treatments include peelings while lasers and lights are considered as a third-line therapy[5]. Treatment plan should encompass many factors among which the most crucial is assessment of the distribution and depth of melanin deposition[6].

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Melasma has been classified according to pigment depth location in epidermal, dermal and mixed type, being characterized, respectively, by epidermal, dermal or combined involvement of layers. However, more recently, the existence of a pure dermal type has been argued[7,8]. Wood’s lamp has been widely employed to estimate pigment depth, as well as dermoscopy, however these two practical and easy to use tools did not enable an assessment of pigment localization correlating with histology[9,10].

As a matter of fact, RCM has been proven as an excellent add-on tool for diagnostic purposes in Dermatology as well as for the analysis of healthy skin, since it provides an optical ‘‘histological’’ biopsy of the living tissue in a totally non-invasive manner, therefore avoiding scars, which is pivotal for aesthetic areas such as the face[11-14]. Specifically, RCM has been proven a good correlation with histology for the analysis of pigment location[15,16], therefore representing an interesting technique to obtain information about baseline characteristics of melasma and treatment monitoring.[17]

The aim of this systematic review is to summarize current evidence of RCM application to melasma for studying baseline characteristics as well as for analyzing variations related to both topical and laser treatment.

Materials and methods

Studies regarding melasma and RCM were screened on electronic databases including MEDLINE (PubMed), Web of Science and Cochrane library. Our search was performed using keywords as follows: "melasma" AND “reflectance confocal microscopy". Our search included studies from inception to March 2023.

Abstracts were screened for inclusion and exclusion criteria independently (SG and RR). Eventual discordance leads to a third opinion (APB).

Exclusion criteria:

- language other than English
- in vitro or animal studies
- reviews, case reports
- not involving melasma

Information extracted after screening were: first author, year of publication, number of patients involved, age, skin type/ethnicity, study type, eventual treatment, RCM criteria at baseline and variations post-treatment, follow up timing, efficacy and safety, other notes such as wide-probe or handheld RCM.

Results

Eighty-six papers were screened after duplicate removal. Based on title and abstract screening, 59 studies were excluded. Therefore, twenty-seven full texts were assessed for eligibility and 13 papers were included in the qualitative synthesis, Figure 1.

According to baseline or post-treatment RCM evaluation of melasma, 3 types of papers were identified: baseline RCM features (n=5)[12,13,18-20], post-chemical treatment (n=5) [21-26], post-laser treatment (n=2) [27,28].

RCM features described in melasma are reported in Table 1; a summary of study findings is presented in Tables 2-4.

RCM studies on melasma confirmed the observation that two main categories of melasma exist: epidermal and mixed type [6,8].

Most of the papers employed the wide probe to reveal RCM features of melasma. This tool enables the evaluation of an area of 4x4 mm which is useful for description of overall pigment depth within a lesion[11].

Two papers explored the correlation with histopathology, providing a good correlation between RCM findings and histopathology[15,16]. Additionally, Kang et al in 2009 proved the correlation of RCM hyperrefractive pattern at basal cell layer of epidermis and epidermal pigmentation at histology and plump bright cells in the papillary dermis identified with RCM and melanophages in histology. Interestingly, bright dendritic cells at the DEJ level corresponded to activated melanocytes with immunohistochemistry, even though dendritic cells at spinous layer with RCM cannot be routinely identified as either Langerhans cells or dendritic melanocytes[29].

The hallmark of melasma is confirmed as the presence of pigmentation located at epidermal level, being named hyperrefractive keratinocytes or cobblestone pattern but that should be more properly called as mottled pigmentation[27] when clustered bright keratinocytes are observed Table 1. At dermal-epidermal junction, strongly visible papillary rings around the dermal papillae[13] can be observed while at dermal level, pigment corresponds to melanophages [18,19].

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Interestingly, the presence of melanophages and other alterations confined at dermal level such as well-defined oval and banana-shaped non-reflective material are a peculiar finding of exogenous ochronosis, enabling a differentiation from melasma.

Additional information came from the comparison of melasma lesional skin and perilesional normal skin[13,19], showing more hyperrefractive epidermis and an increased solar elastosis and vessels in lesional skin, therefore revealing an associated photodamage.

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Overall, 8 papers[21-28] focused on RCM treatment monitoring of melasma, of which 6 prospective and 2 retrospective studies. Additionally, 6 studies were related to topical treatments[21-26] and 2 to lasers[27,28], Tables 3,4.

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Treatments described in studies included in the current systematic review were:

- topical treatments: hydroquinone, trenexamic acid, phytic acid, glycolic acid, lactic acid, octinoxate, glabridin, andrographolide, hydroxyphenoxy proprionic acid, 4-n-butylresorcinol; pyruvic acid [21-26]
- laser treatment: 1064 Nd:YAG Q-switched laser or 755 nm picosecond laser (PSL) alexandrite[27,28]

Topical treatments, including both creams and peelings, proved to be effective on epidermal component of melasma (an example of practical application of RCM in melasma management is represented in Figure 2) but failed to prove substantial effectiveness at dermal level[21-23]. Additionally, studies employing laser therapy, QS and picosecond laser, did not prove the efficacy on dermal melasma component either, for two reasons including recruitment of patients with epidermal melasma only [27] and short (24-hour) follow up[28].

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However, RCM seems to provide interesting information concerning the dynamic of melasma treatment monitoring, offering prognostic perspectives. Specifically, Jo et al performed a prospective split face study involving 8 patients treated with either PSL or Q-switched laser for melasma, showing an increase of activated melanocytes at basal layer of epidermis or an increased amount of melanophages at upper dermis 24 hours after treatment[28].

Interestingly, Longo et al described the presence of activated melanocytes in perifollicular areas in patient showing an early relapse (3 months) of melasma after laser treatment, suggesting their influence in the therapeutic outcomes[27]. Finally, Tsilika et al in 2011 supported that one of the factors influencing treatment outcome is the presence of dermal involvement, which may impair treatment efficacy[23]; therefore, to identify pigment location prior to treatment with RCM should be considered an advantage.

Discussion

Melasma represents one of the most challenging conditions to treat in dermatology, also due to the high rate of recurrence. Despite its cosmetic relevance, this disorder may affect the quality of life of patients affected[30]. Accordingly, social life, emotional well-being, and physical health, as well as social life, have been shown to be compromised in melasma patients.[30]

One of the factors impairing melasma treatment outcome is considered the depth of pigmentation. Traditional diagnostic techniques that can be used to evaluate depth of pigment location are Wood's lamp and dermoscopy. However, these techniques have been shown limitations in pigment depth evaluation, due to patients' selection or lack of comparison with histology [8,31]

By offering non-invasive, real-time evaluation of skin changes, RCM can assist clinicians in customizing treatments to meet the individual needs of patients, optimizing treatment outcomes[17]. Most of the papers included in this systematic review employed RCM wide-probe to examine melasma. The wide probe enables the exploration of a wider area of the skin, usually 4 mm (up to 8 mm), as per standardized protocol[11,13]; however, with hand-held RCM it is

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Over the past decades, an increased number of non-invasive treatments for melasma became available[30]. Among these, a plethora of topical combinations of acids and depigmenting agents, such as the traditional hydroquinone and the innovative tranexamic acid, have been used. In detail, hydroquinone-based treatments have been described as the most favorable option for both efficacy and safety.[31] Nevertheless, short-term treatments should be taken into account to avoid exogenous ochronosis, appearing as a gray pigmentation, corresponding to alterations confined at dermal level consisting in well-defined oval and banana-shaped non-reflective material and melanophages.[32]¶
Additionally lines of treatment encompass peelings and laser therapy, according to patients' requests[33-36]. ¶

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possible to evaluate difficult "curved" areas of the face such as the nose and offer the possibility to have a real-time idea of pigment depth location *in vivo*.[32]

According to our results, the application of RCM to melasma supports the observation that epidermal pigmentation is the hallmark of melasma. Additionally, a mixed-type can be observed and it is characterized by the presence of melanophages or vessels at dermal level[16,18,19].

Treatment monitoring of melasma with RCM revealed interesting results in terms of efficacy and safety of both medical and laser therapies. Specifically, topical treatments have proven to be effective on the superficial component of melasma while no results on the dermal component were observed. This result is in line with the mechanisms of action of topical treatments and the depth they can actually reach.

Additionally, the effectiveness of laser treatments on dermal component could not be estimated due to lack of mixed-type melasma patients enrollment and short time follow-up. However, a previous study showed that laser treatments can also act on the dermal component of melasma.[33] Indeed, one month after multiple sessions of Q-switched Nd-YAG laser for mixed melasma, there was a reduction in MART-1 positive cells at the level of the epidermis (melanocytes) and the dermis (melanophages), observed through histopathology and immunohistochemistry on skin biopsies. [33]

Nonetheless, RCM laser monitoring revealed interesting RCM findings that can justify a poor response to lasers, which are the presence of activated melanocytes at basal layer of epidermis, close to the hair follicle and melanophages at dermal level[22,23].

Taken together, our results show that RCM seems to offer a reliable "mapping" of melanin distribution in melasma, being an excellent guide not just for selection, but also for treatment monitoring, since RCM is a highly sensitive instrument for detecting even clinically undetectable changes during melasma treatment.

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In conclusion, screening of melasma with RCM, when feasible, can reveal important details concerning the classification of melasma as well as the presence of characteristics which may impair the treatment efficacy. Further studies are needed to assess the effectiveness of different treatment modalities on the dermal component of melasma.

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Tables

Table 1. Description of RCM features

Epidermis	
Regular honeycombed	Polygonal keratinocytes with homogeneous size and shape; cell border is well outlined and preserved
Mottled pigmentation	Clustered bright keratinocytes in context of honeycombed pattern
Extracellular deposits of melanin	bright round-to-polygonal areas and aggregated granules observed throughout the epidermis
Dendritic cells	Bright dendritic cells
Dermo-epidermal junction	
Polycyclic papillary contours	Bulbous projections and cords, with variably convoluted arrangement
Edged dermal papillae – papillary rings	Dark round-to-oval structures surrounded by a rim of bright monomorphic cells
Flattening of rete ridges	Abrupt transition from stratum spinosum to papillary dermis
Dermis	
Melanophages	Bright particles at upper dermis with ill-defined borders
Elastosis	Highly refractive thick and short undulated fibers, sometimes forming compact masses when severe solar elastosis is present
Vessels	Dark round to tubular structures within the upper dermis

Attached on separate files:
Supplementary Table 1. Summary of studies relative to baseline characteristics of melasma, RCM treatment monitoring of chemical and laser treatments for melasma

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Figure legend.

Figure 1. PRISMA flow diagram

Figure 2. Pictures of a 35-year-old woman showing melasma on the frontal area before (A-C) and 3-month after one session of peeling followed by one month of home-based therapy with depigmenting agents (D-E).
(A) Clinical appearance showing slight visibility of hyperpigmented area; area in the black circle analyzed with reflectance confocal microscopy (RCM), (B) mottled pigmentation at epidermal level (arrows), (C) increased brightness of papillary rings at dermoepidermal (DEJ) (asterisks).
Conclusion: epidermal melasma.
(D) regular honeycombed pattern, disappearance of mottled pigmentation at epidermal level (E) reduction but focal persistence of bright papillary rings at DEJ (stars).
Conclusion: efficacy of the treatment on the epidermal layer but focal persistence of hyperpigmentation at DEJ.

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Figure 2. Reflectance confocal microscopy (RCM) pictures of a melasma patient (A) before and (B) 3-month after 3 sessions of 1.064 Nd:YAG picosecond laser treatment. Specifically, pictures are taken at dermal-epidermal junction and (A) shows diffused bright papillary rings while (B) shows focal persistence of brightness after treatment (white arrow). Scale bar 100 µm¶