

Histopathological Changes Induced by Malassezin: A Novel Natural Microbiome Indole for Treatment of Facial Hyperpigmentation

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ABSTRACT

Background: Malassezin is a natural indole compound produced by the fungus *Malassezia furfur* and preclinical investigations have demonstrated an ability to suppress melanogenesis.

Objective: To investigate the histopathological effects of malassezin for treatment of facial hyperpigmentation.

Methods: In this proof-of-concept study, seven subjects with facial hyperpigmentation caused by melasma or photodamage applied topical malassezin twice daily for 14 weeks, followed by eight weeks of observation. At baseline, 2 mm punch biopsies were taken from hyperpigmented areas and adjacent uninvolved skin. Skin biopsies from hyperpigmented areas were repeated at 8, 14, and 22 weeks. Paraffin-embedded sections were cut and stained with H&E, Fontana Masson, and MART 1 and assessed for histopathological changes.

Results: Increased epidermal melanin and dermal melanophages were observed in all biopsies at baseline in the hyperpigmented compared to uninvolved skin of all subjects. Eight and 14 week biopsies of involved skin revealed decreased epidermal melanin in all subjects treated with malassezin. Melanocytes appeared less dendritic compared to baseline, and numbers were slightly reduced at eight weeks. Biopsies at 22 weeks showed no significant difference in epidermal melanin levels compared to baseline hyperpigmented skin, and melanocytes were comparable in number and dendricity to baseline. There was no evidence of melanocyte atypia in any of the biopsies. These features were similar in melasma and photo-damaged skin.

Conclusion: This study documents the histopathological features and ability of malassezin, a novel agent unique to the skin microbiome, to decrease epidermal pigmentation and the temporary and revisable nature of the process.

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INTRODUCTION

Common acquired disorders of hyperpigmentation include melasma, post-inflammatory hyperpigmentation, the dyschromia of photoaging, and solar lentigines.^{1,2} Melasma and post-inflammatory hyperpigmentation are the most common conditions in subjects with skin of color affecting more than five million people globally.³⁻⁵ These conditions are often disfiguring, have a profoundly negative impact on quality of life, and can be therapeutically challenging.^{6,7}

Treatment approaches currently involve a multimodality protocol

incorporating photoprotective agents, antioxidant treatments, skin lighteners, exfoliants, and resurfacing procedures.^{6,7} Commonly used skin lighteners include hydroquinone, kojic acid, azelaic acid, vitamin C, and retinoids. However, these agents are not effective in many patients and there remains an unmet need for new efficacious and safe topical products to treat facial hyperpigmentation.

Malassezia species comprise a genus of lipophilic fungi and represent the predominant single eukaryotic commensal

organisms accounting for 50% to 80% of the total skin microbiome.⁸ The genus comprises 17 species, including *Malassezia furfur*, *M. glabosa*, *M. restricta*, and *M. sympodialis*. Colonization of the skin with malassezia begins immediately after birth and increases until six to 12 months of age. Colonization levels are low and stable until puberty, at which time levels rise when sebaceous gland activation occurs.⁹ Recent studies have described the capacity of malassezia species to produce indoles that inhibit commensal and opportunistic fungi.¹⁰ These observations support a protective effect of the organisms to prevent skin colonization by more pathogenic species.⁸

Malassezia species have been implicated in tinea versicolor, seborrheic dermatitis, atopic dermatitis, and psoriasis. However, tinea versicolor represents the primary skin disorder associated with the species.¹¹⁻¹⁴ Malassezin, a natural indole compound, was initially isolated from ethyl acetate extracts of *M. furfur* cultures and was reported to induce melanocyte apoptosis and dose-dependent induction of apoptotic markers after the cultivation of melanocytes with malassezin.^{15,16} It was also observed that the biosynthesis of melanin was reduced in a similar dose-dependent manner.¹⁶

Our preliminary in vitro and ex vivo investigations have documented the ability of malassezin to suppress melanogenesis and decrease skin pigmentation, and therefore, support the efficacy and safety of malassezin as a novel modality for skin lightening.^{17,18} In a randomized dose-ranging, double-blind proof-of-concept study assessing the lightening effects for facial hyperpigmentation, malassezin produced substantial lightening of the skin at 14 weeks and was well tolerated (doi:https://doi.org/10.1016/j.jaad.2021.10.008).

The primary objective of this study was to investigate the histopathological features of malassezin treatment in a subgroup of subjects with facial hyperpigmentation included in proof-of-concept investigation.

MATERIALS AND METHODS

Study Design

A 22-week proof-of-concept study was conducted from April 2019 to January 2020 at the Vitiligo and Pigmentation Institute of Southern California. The study was conducted in accordance with the Declaration of Helsinki and International Conference on Harmonization Guidelines for Good Clinical Practice. Subjects provided written, informed consent before study procedures were performed. The protocol, informed consent form, and patient information were approved by the Institutional Review Board Company, INC, Buena Park, California.

Subjects, Treatment, and Histopathological Assessments

The study enrolled 20 subjects with facial hyperpigmentation secondary to photodamage or melasma who underwent

treatment for 14 weeks with an additional eight weeks of follow-up. Key inclusion criteria were age 30 to 65 years; good general health; Fitzpatrick Skin Types 2 through 5, with mild, moderate, or severe facial hyperpigmentation due to melasma or photodamage. Subjects who used hydroquinone, retinoids, glycolic acid, or any agents that impacted pigmentation for at least six weeks prior to study enrollment were excluded. Subjects with Fitzpatrick Skin Types 1 and 6 and pregnant females were also excluded.

Topical natural malassezin was synthesized by Versicolor Technologies LLC, in an oil in water emulsion (0.1%, 0.5%, and 1%). In the interventional study, subjects were randomized (1:1:1:1) for twice-daily topical facial treatment with one of the concentrations of malassezin or control. The morning application was followed by SPF 50 Sheer Zinc Sunscreen, and all subjects used Cetaphil® cleanser (Galderma Laboratories, L.P., United States) twice daily.

Two-millimeter punch biopsies of the involved hyperpigmented skin, and adjacent uninvolved facial skin, were taken at baseline from seven malassezin-treated subjects (melasma n=3, photodamage n=4); week 8 (melasma n=2, photodamage n=2); week 14 (melasma n=2, photodamage n=1); and week 22 (melasma n=3, photodamage n=4). Paraffin-embedded specimens (4µm sections) were stained with hematoxylin and eosin (H&E) to assess tissue morphology; Fontana-Masson to visualize and quantitate melanin; and immunohistochemistry was performed with melanoma antigen recognized by T cells (MART-1) to evaluate and quantitate melanocytes. Epidermal melanin was evaluated using image analysis on digitized images of the interfollicular epidermis at 20x magnification. Melanocytes were counted along the entire length of the epidermis in MART-1-stained sections at 20x magnification.

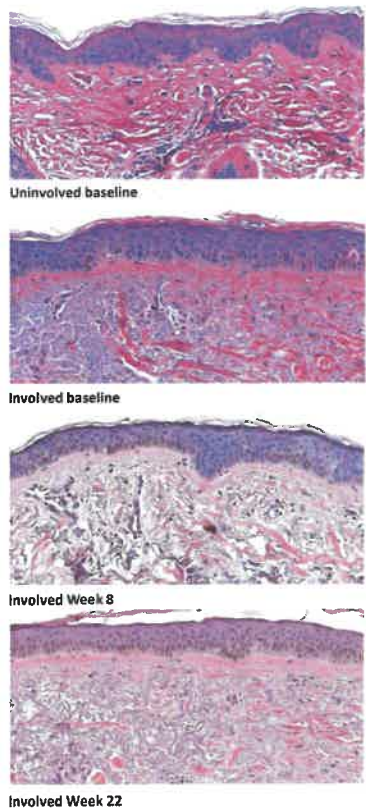
RESULTS

The option to undergo a skin biopsy was offered to all patients randomized to the active arm in the proof-of-concept study, and of the 20 subjects enrolled, seven malassezin-treated and

TABLE 1.

Baseline Demographics and Clinical Characteristics	
Subjects	Malassezin (n=7)
Female gender, n (%)	6 (86%)
Mean age, years (range)	51.64 (39-60)
Skin type, n (%)	
Fitzpatrick Skin Type 3	4 (57%)
Fitzpatrick Skin Type 4	2 (29%)
Fitzpatrick Skin Type 5	1 (14%)
Diagnosis, n (%)	
Melasma	3 (43%)
Photodamage	4 (57%)

FIGURE 1. H&E staining of biopsies of twice-daily malassezin-treated facial skin from uninvolved areas at baseline and involved areas of hyperpigmentation secondary to photodamage at baseline, weeks 8 and 22 in a 57-year-old male with Fitzpatrick Skin Type 3.



no control subjects were included in the histopathological investigation. Most of the subjects were female (86%) with a mean age of 51.64 years; 57% were Fitzpatrick Skin Type 3; 29% were Type 4; and 14% were Type 5 (Table 1).

The H&E staining of hyperpigmented (involved) skin revealed an unremarkable epidermis with increased epidermal pigmentation in lesional skin as compared to uninvolved skin at baseline. While pigmentation appeared to be decreased at week 8, this returned to baseline levels by week 22 (Figure 1). These findings were confirmed by Fontana-Masson staining as described below. There was no evidence of inflammatory infiltrates, or keratinocyte or melanocyte atypia at any time point following malassezin treatment, and observations were similar in subjects with melasma and photodamage.

Table 2 and Figures 2 and 3 describe the histological outcomes in malassezin-treated subjects. Fontana-Masson staining revealed an increase in epidermal melanin in all biopsies at baseline in the involved hyperpigmented compared to uninvolved areas of skin in all subjects assessed. A reduction in epidermal melanin was evident at weeks 8 and 14, with a gradual return to baseline levels evident in the week 22 biopsies (Table 2 and Figures 2A and 3A). MART-1 staining of melanocytes from biopsies of involved skin appeared to be less dendritic compared to baseline at weeks 8 and 14 with a gradual return to baseline by week 22 (Figures 2B and 3B). Overall, the number of melanocytes did not change at weeks 8 and 14 but were slightly fewer in three specimens at week 8. Of the seven subjects who had week 22 biopsies, the number of melanocytes were comparable to baseline number quantifications (Table 2). There was no evidence of melanocyte atypia in any of the biopsies.

TABLE 2.

Histology Outcomes in Malassezin Treated Subjects

Parameters	Uninvolved	Involved			
		Baseline	Week 8	Week 14	Week 22
Subjects with melasma, n	3	3	2	2	3
Epidermal thickness, μm	43.6	58.0	52.5	59.7	55.0
Melanocytes, n	12.3	11.0	8.5	9.0	13.0
Dermal melanophages, n	1.75	1.5	1.0	1.0	1.5
Epidermal melanin, %	5.5	16.4	14.5	5.4	11.8
Subjects with photodamage, n	4	4	2	1	4
Epidermal thickness, μm	44.7	52.7	49.3	51.0	50.5
Melanocytes, n	12	9.2	7.0	10.5	11.5
Dermal melanophages, n	1	1.5	1.0	2.0	1.0
Epidermal melanin, %	12.7	21.2	8.3	17.3	19.5

FIGURE 2. Fontana-Masson staining of melanin (A) and MART-1 staining of melanocytes (B) in biopsies of twice-daily malassezin-treated facial skin from uninvolved areas at baseline and involved areas of hyperpigmentation secondary to photodamage at baseline, weeks 8 and 22 in a 57-year-old male with Fitzpatrick Skin Type 3.

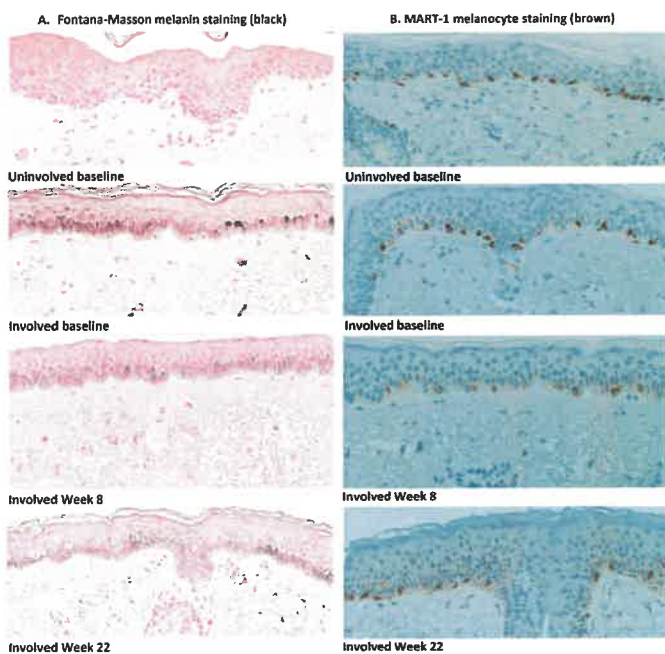
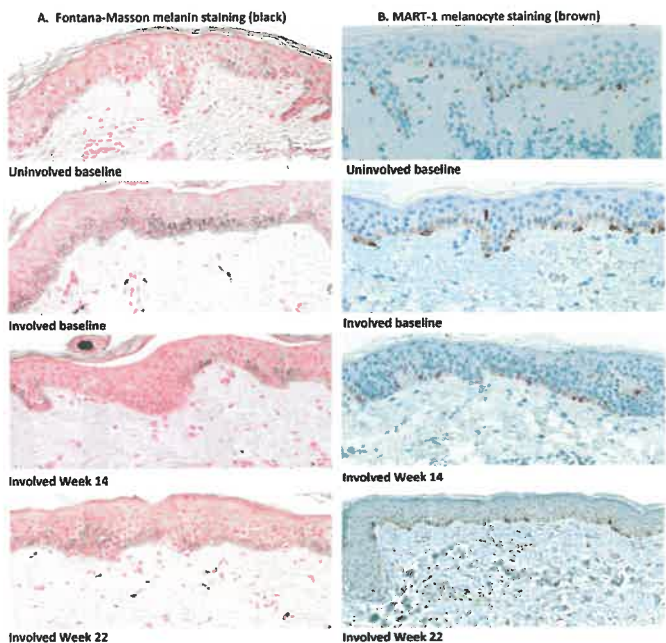


FIGURE 3. Fontana-Masson staining of melanin (A) and MART-1 staining of melanocytes (B) in biopsies of twice-daily malassezin-treated facial skin from uninvolved areas at baseline and involved areas of hyperpigmentation (melasma) at baseline, weeks 14 and 22 in a 39-year-old female with Fitzpatrick Skin Type 4.



DISCUSSION

To our knowledge, this is the first seminal proof-of-concept study to evaluate the histopathological features of facial hyperpigmentation treated with malassezin. This novel study documented the capacity of malassezin to decrease epidermal melanin, as assessed by Fontana Masson and MART-1 staining, for subjects with a broad range of skin types and facial hyperpigmentation. Histopathology results from this study also document the temporary and reversible nature of the pigment reduction.

We recognize that the limitations of the investigation include the small sample size. However, this study aimed to investigate the role of malassezin as a pigment lightening agent and, despite the limited number of subjects, we were able to detect reductions in epidermal melanin. Malassezin was well tolerated, and the histopathological changes described here corroborate our clinical observations which demonstrated that malassezin significantly increased lightening compared to placebo at week 14, with significance evident by week 4 (data submitted for publication elsewhere).

Tinea versicolor is a common, benign, superficial fungal skin infection characterized by hyperpigmented or depigmented/

hypopigmented scaly lesions occurring particularly frequently in seborrheic areas of the trunk, neck, and proximal extremities.¹⁹ Studies examining the histological alterations of the depigmented/hypopigmented areas have reported a spectrum of changes, including sparse or absent inflammatory infiltrates and equal numbers of melanocytes in the involved compared to normal skin. Change in the shape of melanocytes, and decreased numbers of melanosomes in keratinocytes, was also observed.¹⁹⁻²¹ Ultrastructural changes included mitochondrial and cytoplasmic vacuoles of varying intensity, visible cytoskeleton changes, and decreased melanin production.¹⁹⁻²¹

Previous reports by Nazzaro-Poro, suggested that the cause of depigmentation/hypopigmentation in tinea versicolor was azelaic acid, a dicarboxylic acid produced by *Pityrosporum*, via oxidation of oleic acid to azelaic acid.^{22,23} While azelaic acid is a competitive inhibitor of tyrosinase, given its lack of effect in normal skin, it is not considered the putative cause of depigmentation in tinea versicolor.

Malassezin was first isolated from ethyl acetate extracts of *M. furfur* cultures.¹⁵ The compound undergoes intracellular transformation to indole (3,2,-b)carbazole, a highly active agonist

of the aryl hydrocarbon receptor with essential homeostasis functions for regulating metabolism, immune signaling, cell cycle, wound healing, stem cell maintenance, and cellular differentiation. Kramer et al reported on the ability of malassezin to induce apoptosis in human melanocyte cultures and dose-dependent induction of apoptosis markers.¹⁶ However, in our subjects, there was no clinical evidence of localized or distal depigmentation. Moreover, at week 22, epidermal melanocytes and melanin were approximating baseline measurements. Given the role of malassezin as the active compound inducing hypopigmentation in tinea versicolor, we are aware of no reported cases of permanent depigmentation for this condition.

Preclinical investigations, including a detailed series of in vitro and ex vivo experiments, were performed to evaluate the safety and ability of malassezin to decrease melanin. Chemically synthesized malassezin tested negative in both genotoxicity and phototoxicity assessments. Studies of malassezin in MelanoDerm™ models demonstrated reductions in melanin which compared favorably to kojic acid (5-hydroxy-2-hydroxymethyl-4-pyrone), a naturally occurring hydrophilic fungal product. Ex vivo studies showed that malassezin was not a tyrosinase inhibitor and differential gene expression confirmed the novel mechanism of action of malassezin.¹⁸

CONCLUSION

Despite the substantial negative impact on quality of life, hyperpigmentation continues to present clinical management challenges for dermatologists, and the availability of safe and effective treatment options represents an ongoing unmet need. This proof-of-concept study documents the histopathological features and ability of malassezin to decrease epidermal melanin and the temporary and reversible nature of the process. The encouraging results from twice-daily application of this highly novel agent of the skin microbiome, with its potentially new mechanism of action corroborated by detailed histopathological assessments, support the need for more extensive clinical studies. Malassezin potentially represents a simple, effective, and well-tolerated treatment for facial hyperpigmentation.

DISCLOSURES

PG is Consultant, Investigator, and Shareholder of Versicolor Technologies LLC, and Consultant and Investigator for Incyte, L'Oreal, Arcutis, and Sun Pharmaceutical. JB is Investigator for Versicolor Technologies LLC. MDH is Employee and Stockholder at DermTech, and Consultant Advisor and Stockholder of Versicolor Technologies LLC. SD is Consultant for Versicolor Technologies LLC. EC is Consultant and Stockholder for Versicolor Technologies LLC, and Employee for Skin Science Advisors LLC. ME is Founder of Versicolor Technologies LLC, and Stockholder and Intellectual Property Rights. AMS is Founder of Versicolor Technologies LLC, and Stockholder and Intellectual Property Rights. AY is Consultant for Versicolor Technologies

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