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Treatment Strategies for Hypopigmentation in the Context of Burn Hypertrophic Scars

Bonnie C. Carney, BS*†
Jacqueline P. McKesey, MD†
Dean S. Rosenthal, PhD*
Jeffrey W. Shupp, MD†‡

Dyspigmentation in burn scars can contribute to the development of psychosocial complications after injury and can be detrimental to social reintegration and quality of life for burn survivors. Although treatments for skin lightening to treat hyperpigmentation have been well reviewed in the literature, skin-darkening strategies to treat hypopigmentation have not. The following potential treatment options in the context of burn hypertrophic scar will be discussed: use of the melanocyte-keratinocyte transplantation procedure, use of ectopic synthetic analogues of alpha-melanocyte stimulating hormone to initiate melanogenesis, and use of FK506 to induce melanogenesis. A proposed future direction of research in laser-assisted drug delivery of inducers of local melanin production, with the hope of developing a targeted, effective approach to dyspigmentation in hypertrophic scar is also discussed. *(Plast Reconstr Surg Glob Open 2018;6:e1642; doi: 10.1097/GOX.0000000000001642; Published online 18 January 2018.)*

INTRODUCTION

Due to advancements in infection control, fluid resuscitation methods, skin grafting techniques, and technology, the survivability of large total body surface area (TBSA) burn injuries has increased over several decades. Today, outside of patients at the extremes of age, burn patients are expected to survive their injury, and the 50% mortality rating only occurs at approximately 81% TBSA or greater.[1]

Although this trend toward decreased mortality is promising for burn patients, there is an associated increased population of patients who have survived large TBSA burns who are now plagued with hypertrophic scars (HTSs).[2] HTS is the most common complication that occurs after burn injury, with a prevalence of 30–70% and is most commonly observed in patients with higher Fitzpatrick skin types (Type IV–VI). Further, dyspigmentation is often evident when excision and grafting are delayed, allowing partial or full reepithelialization of the skin between grafting procedures (Fig. 1).[3] The mechanism of hypopigmentation in burn scars may be related to loss of melanocytes or damage to these cells causing a decrease in their function. Both of these mechanisms have potential treatment options, although they are understudied in patients with HTS. Dyspigmentation is an even more prevalent concern in developing countries, where limited resources delay the timing of excision and grafting.

HTSs are characteristically red, raised, contracted, pruritic, and dyspigmented.[4] In addition to the functionally debilitating effects of scars, burn survivors experience significant psychosocial impairment due to the disfiguring nature of their injuries. Without the potential for the treatment of dyspigmentation within scars, burn survivors will have a constant reminder of the traumatic event surrounding their injury, and will be limited in their ability to improve their quality of life and social reintegration.[5] There are treatment options that have been shown to be effective in alleviating symptoms of scar, including pressure therapy and scar massage to reduce scar height, contracture, and pruritus.[6,7] However, there are no definitive treatments for dyspigmentation within burn scars. The only treatment available is excision of the scar or treatment with laser therapy. The mechanism of action of laser therapy is not fully known and its use in the treatment of scar warrants a separate review. It is likely that the use of laser therapy, in combination with the other therapies discussed in this review may be an option for intervention, as discussed below.[8,9]

The normal mechanism that modulates skin pigmentation changes following exposure to ultraviolet light is well established in the literature (Fig. 2).[10–12] Pigmentation involves 2 cell types in the epidermal layer of the skin: melanocytes and keratinocytes. Pigmentation is orchestrated through

*From the *Department of Biochemistry and Molecular and Cellular Biology, Georgetown University Medical Center, Washington, D.C.; †Firefighters' Burn and Surgical Research Laboratory, MedStar Health Research Institute, Washington, D.C.; and ‡The Burn Center, MedStar Washington Hospital Center, Washington, D.C.*
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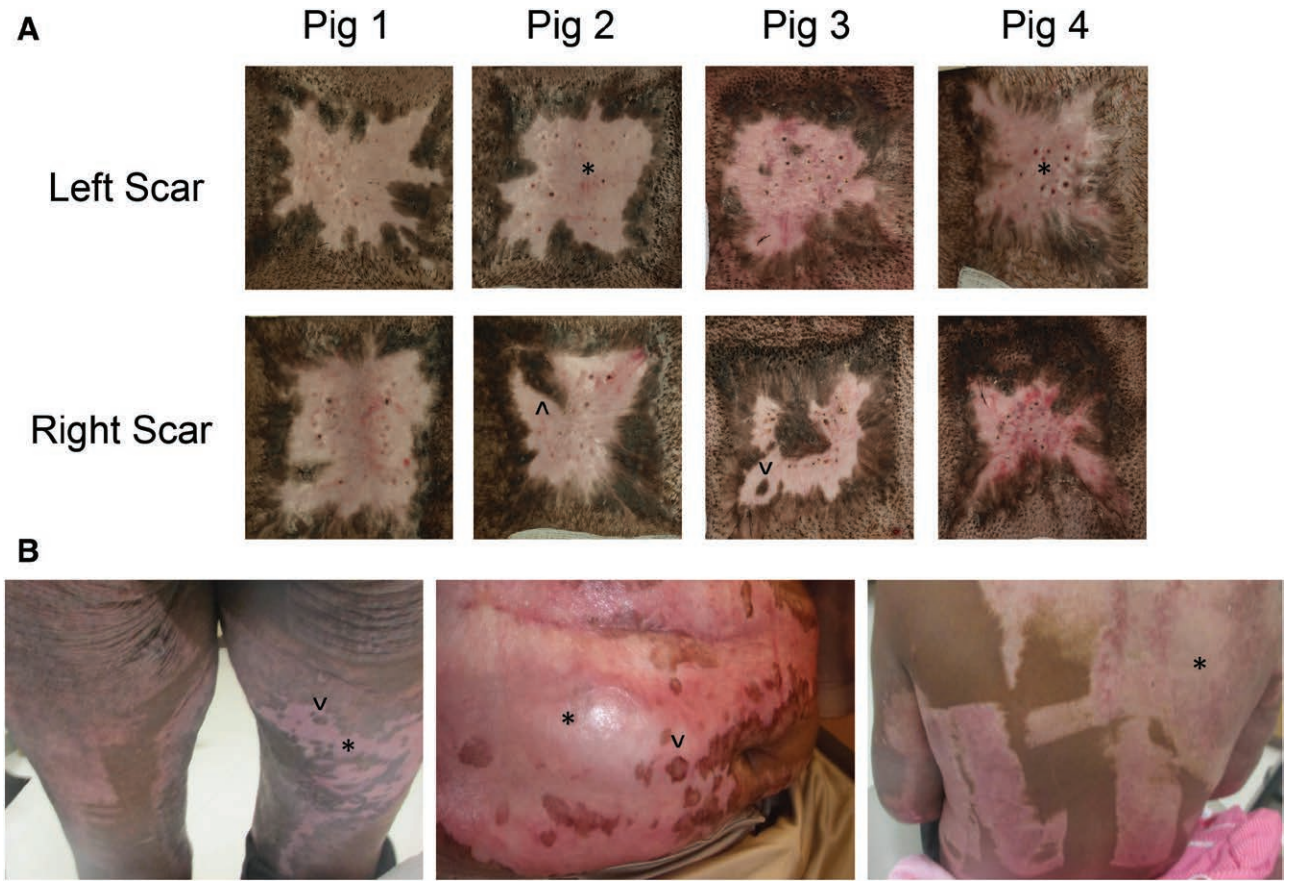

Fig. 1. Hypertrophic scars often contain regions of heterogeneous hyperpigmentation and hypopigmentation. Four different duroc pigs, each with bilateral scars on their flanks, healed with dyspigmented hypertrophic scars 135 days postwounding (A). The same dyspigmented scar phenomenon is observed in a patient 8 months postinjury on the right lower extremity (left), abdomen (center), and back after donor-site healing (right) (B) (*indicate areas of hypopigmentation, ^indicate islands of hyperpigmentation).

keratinocyte synthesis, proteolytic processing, and secretion of alpha-melanocyte–stimulating hormone (α-MSH) in response to UV light-induced DNA damage. The released α-MSH in turn binds with high specificity to the melanocortin receptor (MC1R) expressed on nearby melanocytes. Binding of α-MSH to MC1R initiates a cascade resulting in the synthesis of melanin in a process termed melanogenesis. The rate-limiting enzyme for melanogenesis is tyrosinase (Fig. 3).

Since these mechanisms are well established, there is an opportunity to target different steps within the signaling cascade to treat regions of hyperpigmentation and hypopigmentation in scars. Dyspigmented scars are frequently heterogeneous, containing regions of both hyperpigmentation and hypopigmentation, necessitating treatment of both pigmentary aberrations separately. The best treatment strategy for dyspigmentation in burn HTS has not been extensively discussed in the literature, and there are no current options for treating hypopigmentation that do not involve the transfer of cells from an unaffected area. However, there are many treatments for skin darkening in the context of cosmetic pigmentation or for other dermatologic disorders that could be repurposed for treating hypopigmentation in burn scars. The evidence reviewed is meant to provide a background for thinking about treatments that are already available that could be slightly altered and used in burn care. Because treatment strategies for hyperpigmentation are relatively well defined[13,14]; this review will focus on hypopigmentation. Additional strategies that could be considered in the future, but that are not reviewed here, include grafting with thin epidermal sheets,[15,16] initiating melanogenesis using natural extracts,[17,18] and initiating melanogenesis using small molecule modulators.[19,20]

Strategy 1: Transplant Skin Cells to the Affected Area using the Melanocyte-Keratinocyte Transplantation Procedure

Hypopigmented burn scars are often compared with depigmented lesions in vitiligo vulgaris, a condition in which an autoimmune response causes cellular stress to local melanocytes, resulting in progressive skin depigmentation. If hypopigmented lesions within burn scars do not have melanocytes, or have nonfunctional melanocytes, then the treatment of hypopigmented scar may be similar to treatment for patients with vitiligo. Based on work completed by our laboratory, there appears to be a ubiquitous presence of melanocytes in hyperpigmented and hypopigmented scar regions as determined by positive immunostaining for the melanocyte marker S100β. Potential

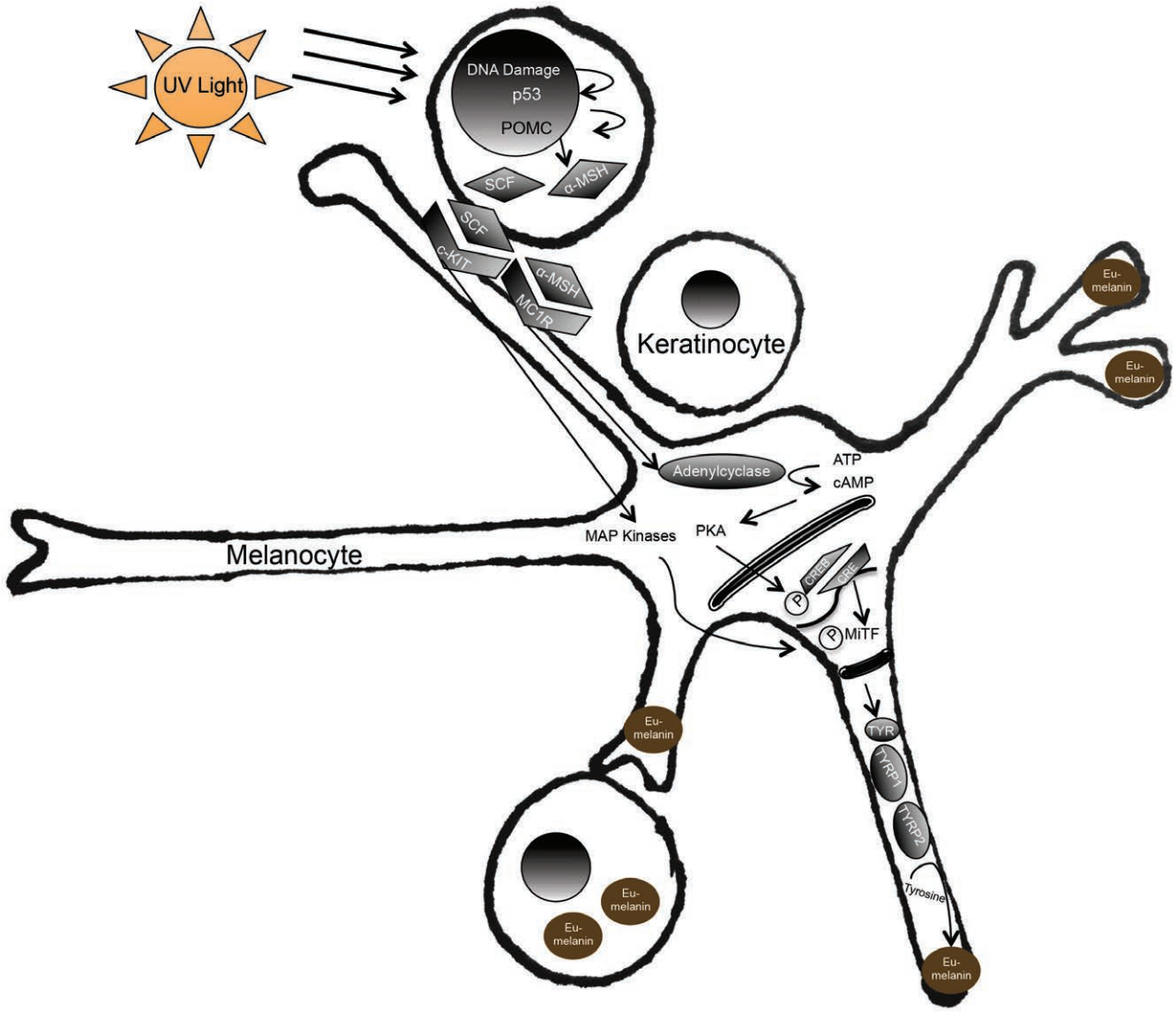

Fig. 2. Skin pigmentation in response to UV light. When UV light causes damage to the DNA within keratinocytes, tumor protein 53 (p53) transcription is increased within the cell. p53 then acts as a transcription factor for the gene proopiomelanocortin (POMC) within keratinocytes, increasing its transcription and hence, protein expression. POMC is then proteolytically cleaved to its products: adrenocorticotropic hormone (ACTH) or α-MSH, and these molecules get secreted from the keratinocytes. These signaling molecules bind to the g-protein coupled receptor on melanocyte membranes called the MC1R. The binding of ACTH or α-MSH to MC1R then activates the secondary messengers of MC1R, specifically adenylcyclase, which converts adenosine triphosphate to cyclic adenosine monophosphate. cAMP can then activate protein kinase A (PKA) by binding to its catalytic region. PKA goes on to phosphorylate cAMP response element binding protein (CREB). Phosphorylated CREB can then bind to the promoter region in the microphthalmia transcription factor (MiTF) gene and increase its transcription. Damaged keratinocytes also signal to melanocytes through a secondary mechanism. Stem cell factor gets secreted and binds to a receptor tyrosine kinase on melanocyte membranes, the stem cell growth factor receptor. The binding of the ligand to the receptor dimerizes and activates the receptor, and this dimerization activates the mitogen-activated protein (MAP) kinase pathway within melanocytes. These MAP kinases ultimately go on to phosphorylate the MiTF that was transcribed in the initial mechanism. p-MiTF can then bind to the M-Box promoter region of the 3 genes that are essential to produce eu-melanin, specifically, tyrosinase (TYR), tyrosinase-related protein 1 (TYRP1), and TYRP2, also referred to as dopachrome tautomerase. Tyrosinase is the rate-limiting enzyme for melanogenesis during the conversion of tyrosine to eu-melanin. Once eu-melanin is produced, it is then packaged into lysosome-like organelles called melanosomes, and melanosomes are transferred back to keratinocytes through the protease-activated receptor. The newly synthesized melanin then acts as protection for DNA within keratinocytes by absorbing the UV light that would otherwise cause damage to the DNA.

treatments are vastly different based on the presence of these cells and will be discussed further below.[21]

The notion of processing tissues into single cell suspensions to achieve larger expansion ratios than traditional grafting techniques has been in use for multiple decades in the treatment of vitiligo.[22] The general concept of epidermal grafting is older still and has been thought of as a treatment for dyschromic regions of skin since the 1970s.[23] Surgical techniques for treating vitiligo involve the transfer of melanocytes and keratinocytes from uninvolved sites

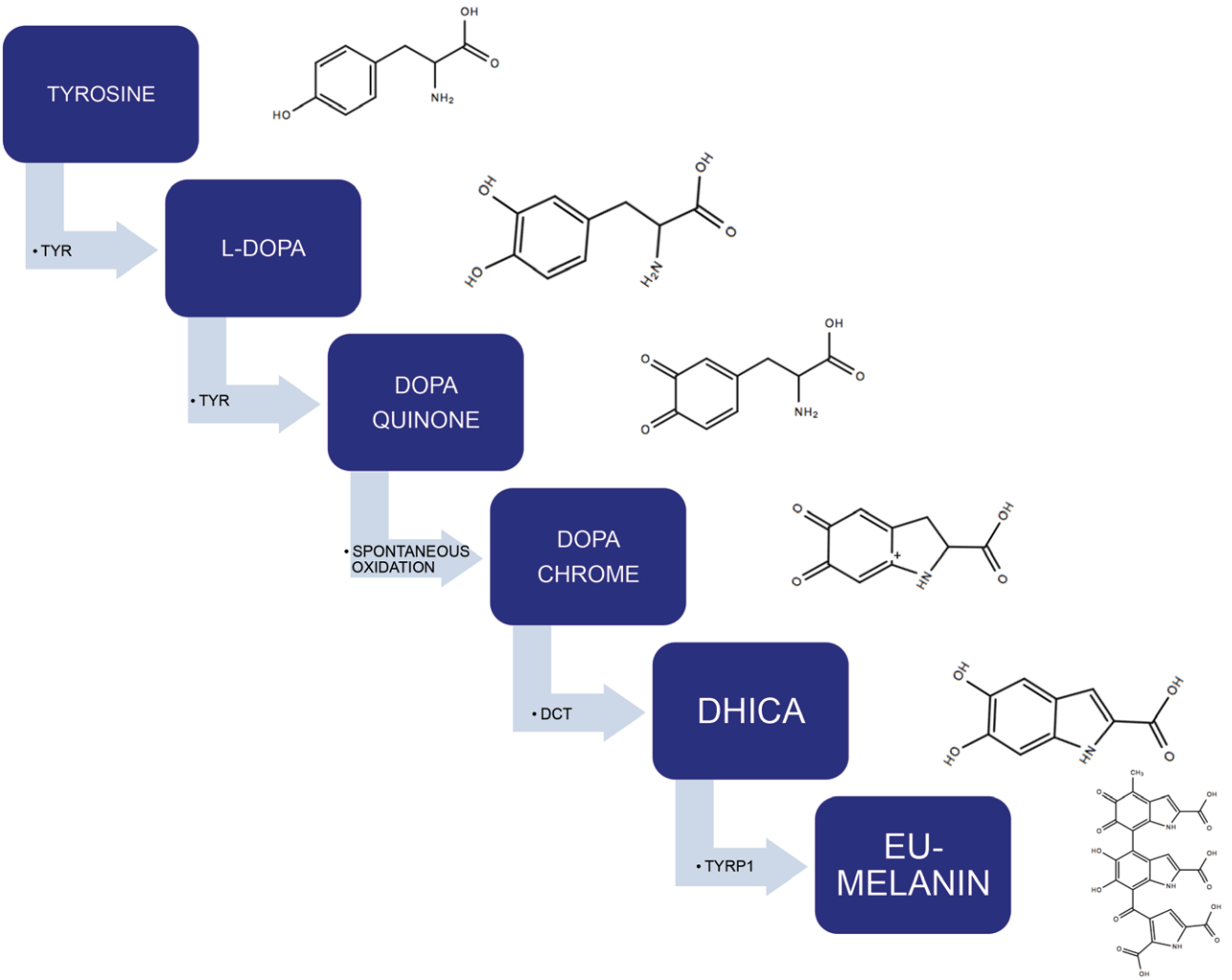

Fig. 3. Tyrosine is converted to eu-melanin. Tyrosinase (TYR) hydroxylates tyrosine to L-3, 4-dihydroxyphenylalanine (L-DOPA) and further oxidizes L-DOPA to DOPAquinone. DOPAquinone then undergoes spontaneous oxidation to form DOPAchrome. TYRP2/dopachrome tautomerase serves as a dopachrome tautomerase and catalyzes the reaction of DOPAchrome to 5,6-dihydroxyindole-2-carboxylic acid (DHICA). Last, TYRP1 catalyzes the reaction of DHICA to the formation of eu-melanin.

to the affected area and can involve long-term culturing of these cells or culture-free conditions. A promising surgical technique that can be repurposed for burn patients is the use of the melanocyte-keratinocyte transplantation procedure (MKTP). MKTP involves the harvesting of donor site skin, enzymatic digestion of this skin with trypsin, creation of a cell suspension from the epidermal pieces, and finally application of the cell suspension to the affected area after microdermabrasion to the epidermal/dermal junction.

MKTP was first used by Olsson et al.[24,25] in the 1990s and was further refined by Mulekar[26] in the early 2000s. It served as an attractive treatment because of the short procedure length and the potential for treating patients in an outpatient setting.[27,28] In 2005, Dr. Mulekar's group reported results from a 6-year follow-up of vitiligo patients treated with MKTP, with 56% of patients experiencing an excellent response (95–100% repigmentation) at the time of follow-up.[29] These results were confirmed and extended in a 2017 publication associated with Dr. Mulekar's group where 100 patients with 236 anatomically based lesions of vitiligo or leukoderma were treated with MKTP, and 53%, 64%, and 53% of lesions maintained > 75% repigmentation at 24, 48, and 72 months, respectively.[30] Although these are promising results, these large cohort studies are limited by their retrospective nature and relatively large number of patients lost to follow-up.

Studies by Huggins et al.[27] and Silpa-Archa et al.[30] have demonstrated an improvement in the vitiligo area-scoring index with MKTP treatment, regardless of race or vitiligo type. These data are promising in the treatment of a racially diverse burn patient population; however, future prospective studies that are more statistically rigorous are required to support its use as a definitive treatment. Additionally, there may be suboptimal treatment responses to MKTP, which include complete failure of procedure and the need for multiple procedures, among others.[31]

MKTP has also been used to treat postburn hypopigmented scars.[32] A case report of 10 patients who underwent MKTP was reported in 2010. Of the 7 patients who remained in the study throughout the full time-course,

there was a range of 80–100% repigmentation with good color matching. Further, some groups have attempted to alter MKTP cell suspension solutions to improve repigmentation rates.[33]

If it is assumed that hypopigmented burn scars do not contain melanocytes, then ectopic melanocyte transfer to the affected area may be one of the only ways to regain pigmentation (strategy 1). This approach assumes that it is not the scar environment that led to the absence of melanocytes in the first place, and that upon ectopic replacement, these cells would be able to grow, proliferate, and undergo melanogenesis. On the other hand, if melanocytes are present in the scar, there may be simpler, less invasive methods to stimulate pigmentation in cells that are already present (strategies 2 and 3).

Strategy 2: Initiate Melanogenesis through the Use of Ectopic Synthetic Analogues of α-MSH

There is a paucity of data to support the presence or absence of melanocytes in hypopigmented scar. Using a red duroc pig model of excisional wounds that form dyspigmented HTSs, our laboratory has shown that S100β-positive melanocytes are present in the basal layer of the epidermis in regions of both hyperpigmentation and hypopigmentation.[21] Additional experiments are needed to confirm these findings. If present, then melanocyte stimulation could be an effective means to return pigment, which can be achieved with synthetic analogues of α-MSH to initiate signaling. This initiation would bypass the need for stimulation with UV light, which would otherwise cause damage to this subset of vulnerable keratinocytes that are not protected by melanin (Fig. 2).

One such synthetic analogue of α-MSH is Nle^4DPhe^7 α-MSH (NDP α-MSH). It has been used as a cosmetic skin darkener for many years. The amino acid substitutions in NDP α-MSH contribute to its ability to resist enzymatic digestion, resulting in its long half-life in vivo.[34] In 1994, Hunt et al.[35] demonstrated the ability of NDP α-MSH to stimulate melanogenesis in cultured human melanocytes, showing the dose-dependency of this "drug."

In 1991, a clinical trial used subcutaneous NDP α-MSH in healthy Caucasian men to induce tanning.[36] This study was the first to show significant skin darkening following administration of NDP α-MSH with few harmful side effects. NDP α-MSH was referred to as Melanotan-I (MTI). Future clinical trials evaluated the ability of MTI to confer increased UV protection.[37,38] The results were promising, as MTI increased melanin content over a 3-month period. Barnetson et al.[38] also exposed patients to 3X their original baseline minimal erythema dose (MED) before and after 90 days of treatment with MTI. There was a lower number of sunburned cells and thymine dimers after exposure to 3X MED at day 90 compared with day 0 in treated skin, suggesting a UV-protective effect of MTI. This piece of data is particularly relevant to burn patients because they are often counseled to stay out of the sun entirely after their injury, although this is a detriment to social reintegration and quality of life.[39]

Following these clinical trials, MTI was marketed as afamelanotide and was used to treat Erythropoietic Protoporphyria, a genetic disorder that leads to dermal pain upon exposure to UV light.[40] Eu-melanin is protective in these patients. Afamelanotide was administered as a slow-release subcutaneous implant (marketed as SCENESSE, Clinuvel Pharmaceuticals) and resulted in an increased melanin level, subsequently decreasing symptoms of Erythropoietic Protoporphyria. Importantly, clinical trials demonstrated the long-term safety and efficacy of afamelanotide in increasing melanin density, making it a promising treatment for hypopigmentation.[41–43]

Afamelanotide has also been used in clinical trials to treat vitiligo. A 2013 study by Grimes et al.[44] reported the results of 4 patients who were treated with slow-release implants of afamelanotide and showed higher repigmentation rates compared with patients receiving UV therapy alone. The small case report was expanded upon in 2015 with a multicenter randomized clinical trial by Lim et al.,[45] which reported similar findings.

The use of NDP α-MSH to treat hypopigmented burn scar is possible if there are functional melanocytes within these regions. The long history of the peptide's use, grounded in basic science evaluation of the peptide and its efficacy in clinical trials make it a unique treatment option. However, its use in the heterogeneous burn scar will need to be optimized. The use of a slow-release dermal implant for the delivery of this compound is not appropriate, as it would lead to systemic administration of the drug instead of a scar-targeted, regional approach. CO_2 fractional ablative LASER drug delivery may be one such scar-targeted delivery approach, as many burn scars are already being treated with LASER therapy in an attempt to alleviate additional symptoms of scar such as height and contracture.[46] The LASER generates pores through which the drug can travel and be directed to the epidermal/dermal junction using specific LASER settings that would need to be optimized before treatment (Fig. 4).[47–49] These pores are known to reepithelialize and reestablish barrier function within 48 hours of LASER therapy.[50] As such, the scar would be treated with LASER therapy, and then a topical application of NDP α-MSH would be applied directly following the treatment. Although LASER drug delivery is most likely a good option, it is only one drug delivery technique, and others, such as drug delivery after microdermabrasion or scar-targeted injections could be considered.

Strategy 3: Initiate Melanogenesis with Topical Tacrolimus (FK506)

Tacrolimus (FK506) is a nonsteroidal anti-inflammatory drug, commonly used as a systemic anti-rejection drug in transplant patients, was first used as a topical therapy in the 1990s to treat atopic dermatitis.[51] Furthermore, a 2003 case series of vitiligo patients showed complete repigmentation following twice-daily topical treatment with 0.1% tacrolimus ointment. The safety and efficacy of this treatment has been demonstrated in many clinical trials. The mechanism of tacrolimus' effect on the pigmentation pathway in vitiligo patients was investigated by Lan et al.[52,53] in 2012. Keratinocytes were treated with increasing concentrations of FK506, and supernatants were collected to treat melanocytes or melanoblasts. They showed

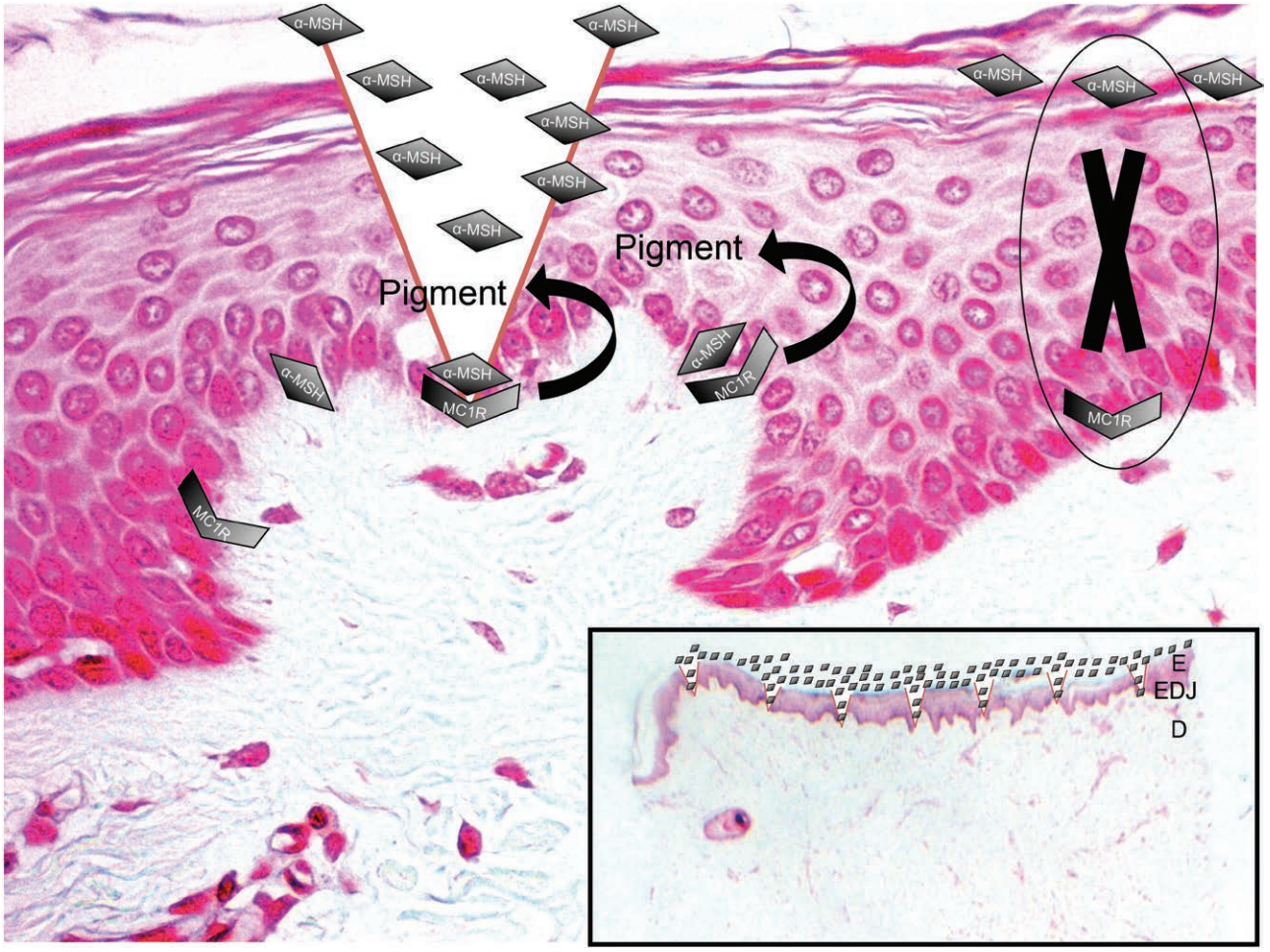

Fig. 4. CO_2 fractional ablative drug delivery of NDP α-MSH. CO_2 fractional ablative drug delivery generates many shallow channels (red triangles) in the scar (inset) that will allow NDP α-MSH (grey diamonds) to penetrate the epidermis to reach the MC1R on melanocytes. NDP α-MSH can be applied topically following laser therapy. Binding of NDP α-MSH to this receptor will then lead to pigment synthesis and secretion of the pigment to the surrounding keratinocytes. In scar that does not have channels (circled), NDP α-MSH has minimal ability to penetrate the epidermis. D, dermis; E, epidermis; EDJ, epidermal-dermal junction.

an increase in melanocyte growth and proliferation with treatment. There was also a significantly elevated keratinocyte release of stem cell factor into the culture supernatant after treatment with FK506. These findings suggest a duel mechanism of this compound in promoting melanocyte proliferation as well as melanogenesis.

FK506's dual role in melanogenesis and melanocyte proliferation was further studied by Kang and Choi[54] in 2006. When human melanocytes were treated with FK506, both melanin content and tyrosinase activity increased. The group also used a scratch assay to evaluate melanocyte migration, demonstrating that the rate of gap closure in the scratch was significantly higher when cells were treated with FK506 compared with controls, supporting tacrolimus' ability to stimulate melanocyte migration. FK506 may therefore be useful in burn hypopigmentation since it can stimulate melanocytes that are already present within the lesion, as well as promote migration of neighboring melanocytes.

Cavalié et al.[55] focused on the ability of tacrolimus to maintain repigmentation of the skin over a long-term follow-up period. This study showed that after repigmentation and 6 months of twice-weekly topical application of 0.1% tacrolimus, only 27% of treated patients regressed to depigmented skin, whereas 48% of patients regressed in the control group. FK506's effect on human melanocytes was further investigated, demonstrating that total melanin levels increased when stimulated with FK506.[56,57] Jung and Oh[57] also demonstrated that FK506 promotes melanocyte maturation, increases melanin content and tyrosinase levels within melanocytes, and enhances keratinocyte uptake of melanosomes. Jung and Oh[57] used sucrose fractionation and pH sensitive dyes to separate and evaluate melanosomes by maturity to evaluate these end points.

Unfortunately, large-scale studies using tacrolimus as a monotherapy to achieve repigmentation are lacking. Additionally, when treating hypopigmentation with tacrolimus, significant repigmentation usually requires months of treatment, with a recent 2017 study demonstrating only significant repigmentation in vitiligo patients after 6 months of twice daily 0.1% tacrolimus ointment.[58,59] FK506 must therefore be applied for long periods, typically twice

daily until repigmentation, and then twice weekly to prevent pigmentation regression. Ultimately, the repigmentation response and necessary length of treatment would have to be tested and optimized in hypopigmented HTS and may be an option for patients if more invasive therapy such as NDP α-MSH or MKTP was not desired.

Proposal for Future Studies

Based on the evidence reviewed, we propose the best treatment strategy for hypopigmented regions of burn scar is to use ectopic treatment with synthetic analogues of α-MSH using fractional ablative laser-assisted drug delivery to stimulate melanocytes to produce melanin (Fig. 4). Although most studies have evaluated the safety and efficacy of NDP α-MSH as a sustained-release implant in humans, we propose laser-assisted drug delivery as a vehicle for this peptide, enabling a targeted approach to hypopigmented regions of HTSs. Fractional ablative laser-assisted drug delivery may not be the only delivery method for the compound. MKTP has successfully used microdermabrasion to deliver cells to the epidermal/dermal junction as well. NDP α-MSH can be used to initiate melanogenesis over long periods of time, favoring this treatment as a more definitive option for hypopigmented scars. Furthermore, this melanocyte-stimulating treatment is supported by the fact that our laboratory has demonstrated the presence of melanocytes in hypopigmented scars. The use of this compound is feasible in models of burn HTS to evaluate its efficacy.

For the aforementioned treatment modalities to become options for practical and immediate clinical use, translational research on this topic must be first accomplished. Fortunately, there are animal models of hypopigmented scar that closely resemble human scar dyspigmentation (Fig. 1). Red duroc pig models of HTS are most similar to human HTS at the gross, cellular, and molecular levels.[60] Excisional wounds created in duroc pigs result in dyspigmented scars that are ideal for experiments moving forward. The presence of melanocytes within regions of hypopigmentation should be confirmed through the use of multiple melanocyte markers as well as the use of primary cell culture to visualize and manipulate cells in vitro. Characteristic dendritic cells must be grown in culture, and their response to α-MSH and NDP α-MSH or similar pigmentation inducers should be confirmed. The heterogeneous nature of pigmentation within burn scars is one the challenging aspects of their treatment. In treating hypopigmentation with inducers of melanogenesis, there is the risk that the scar may then become hyperpigmented. Appropriate NDP α-MSH concentration and dosing must be tested to alleviate this risk. These experiments will be the first step in identifying a treatment strategy specific to hypopigmented burn scars and are currently underway in our laboratory. One promising aspect of the use of the above treatment is that it can be carried out by burn surgeons, plastic surgeon, or dermatologists. This treatment paradigm can be similar to the framework established by fractional ablative therapy in that the LASER was invented and tested by a dermatologist and is now being used by dermatologists, plastic surgeons, and burn surgeons alike.

CONCLUSIONS

The treatment of dyspigmented burn scars is a significant challenge to burn providers that has not been thoroughly addressed in the literature. Hypopigmented lesions can be treated surgically with MKTP, less invasively with LASER-assisted drug delivery of synthetic inducers of pigmentation, such as NDP α-MSH, or noninvasively with topical application of tacrolimus.

Jeffrey W. Shupp, MD
The Burn Center
110 Irving Street, NW
Suite 3B-55
Washington, DC 20010
E-mail: jeffrey.w.shupp@medstar.net

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