Autologous non-cultured melanocyte–keratinocyte transplantation in the treatment of vitiligo: patient selection and perspectives

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Abstract: Autologous non-cultured melanocyte–keratinocyte transplantation procedure (MKTP) is one of the simplest cellular grafting techniques. Various modifications were done over the years to make the technique easier and more economical which led to its great popularity among dermatologists. Proper patient selection and good technical skills are essential for achieving success with this technique. In this review, different patient-related and procedure-related factors that affect the outcome are discussed. This review may guide dermatologists to select suitable candidates, and explains what to expect in each case and indicates different techniques which can be used. The expected complications and stability of acquired pigmentation, which are an essential part of the pretreatment patient counseling, are also discussed.

Keywords: cellular grafting, vitiligo surgery, patient variables, procedure variables, repigmentation

Introduction

Surgical treatment of vitiligo is the final resort to regain the pigmentation in lesions failing to repigment despite various medical and light therapies. Multiple cellular and tissue graft techniques are used successfully to introduce melanocytes and/or their stem cells to vitiligo lesions devoid of them.1

Autologous non-cultured melanocyte–keratinocyte transplantation procedure (MKTP) is one of the simplest cellular grafting techniques and is currently the most popular among dermatologists. It offers 50%–100% repigmentation rates with 1:3 up to 1:10 donor-to-recipient ratio and very good color matching in most of the treated cases.2–6 Since its first description by Gauthier and Surleve-Bazeille in 1992,7 the technique of cellular grafting evolved over the years with various modifications simplifying it and improving the results.

The response to MKTP in general is affected by several factors. As with other surgical techniques, proper patient selection is a crucial point as well as good technical skills. Different patient-related and procedure-related factors that affect the outcome are discussed in this review. This review may guide dermatologists to select suitable candidates, and explains what to expect in each case and indicates different techniques which can be used. The expected complications and stability of acquired pigmentation, which are an essential part of the pretreatment patient counseling, are also discussed.

Technique evolution

In 1992, Gauthier and Surleve-Bazeille7 described the MKTP as a 2-day procedure. On the first day, a shave biopsy was harvested from the scalp and incubated overnight.
in 0.25% trypsin at 4°C. The recipient site was also prepared by liquid nitrogen. On the second day, the trypsinized epidermis was placed in EDTA for 15 minutes, after which it was transferred to a calculated volume of saline. The basal layer was rubbed, the skin was agitated to dislodge the cells and finally the suspension was aspirated by an insulin syringe and injected into cryoubes.

Several modifications aiming at simplifying the technique and improving the outcome were done over the years. Tissue was harvested from the gluteal area which allowed for harvesting a larger area and was also less vascular. Shave biopsy is simple and fast but may lead to development of textural change or scar in the donor site which prompted the biopsy is simple and fast but may lead to development of textural change or scar in the donor site which prompted the researchers to search for a solution. In vivo preparation of epidermal cell suspension was introduced by Gupta et al with excellent response in five treated cases. MKTP was performed entirely in sterile blisters on the patient’s body with no donor site scarring and very high cell viability (99%). Roofs of suction blisters were used in another study for suspension preparation; however, no clear data were given in the study about repigmentation rate. Nevertheless, suction blister formation is a time-consuming process.

Since the hair follicles are the main reservoir and the source of melanocytes that repopulate the epidermis in non-glabrous skin, it was only a matter of time before dermatologists thought of harvesting this rich source of melanocytes for cellular transplantation. In 2009, Vanscheidt and Hunziker used plucked hair follicles for preparation of cell suspension which produced >90% repigmentation in 3/5 cases of vitiligo. Mohanty et al used follicular unit extraction technique instead of plucking to harvest anagen hair follicles which was a more tedious process but provided a significantly higher number of stem cells, as well as ten times more cell yield per hair follicle. Although this suspension was rich in highly proliferative melanocytes and stem cells, it lacked an abundance of healthy keratinocytes which are essential for supplying melanocytes with growth factors needed for their proliferation. In a trial to get the best of both worlds, recent publications used a mixture of epidermal and follicular suspensions with better results than epidermal suspension alone.

Preparation of the suspension underwent major changes too. Olsson and Juholm incubated the skin at 37°C for 50 minutes in a CO2 incubator, used trypsin inhibitor to stop tissue digestion, centrifuged the cells to obtain a cell pellet and added supplements such as antimicrobials and growth factors to the suspension medium. These additional steps increased the cost of the procedure. Mulekar made the procedure easier and more economical by using an ordinary incubator and DMEM/F12 without any additives for suspending the cell pellet. He later replaced trypsin inhibitor by washing the epidermis several times in DMEM/F12 before separating it from the dermis. Later, PBS was used during suspension preparation to further cut the cost. Kumar et al introduced a four-compartment technique in 2014 in which pipettes, autoclaved tips, centrifuge tube and centrifuge machine were no longer needed. The dermatologists estimated and used the exact amount of PBS to prepare the suspension according to the size of the lesion to be treated which was then aspirated by a syringe and spread evenly onto the denuded recipient surface.

Finally, in order to complete the procedure in 1 day, preparation of the recipient area using dermabrasion or laser resurfacing as opposed to cryotherapy was done. Not only did this save time, but it produced better cosmetic outcome as the cells were evenly spread over the whole lesion. Less invasive methods such as dermaroller or fractional CO2 were recently used to introduce the cells into the skin to minimize the downtime. To improve cell handling, collagen sheets or hyaluronic acid (HA) was used to create a paste. This allowed using MKTP even on curved surfaces with ease. Details of these changes over the years are given in Table 1. The effect of these changes on the response is discussed in detail later in this review.

**Effect of patient-related factors on response to MKTP**

**Duration of disease stability**

A strict selection of patients with stable vitiligo is the most important factor for successful outcome. Disease activity is defined as the appearance of new lesions or enlargement of old ones observed in the past year and/or the presence of Koebner phenomenon. Our only available activity score, the vitiligo index of disease activity score, depends on clinical history given by the patient. However, certain clinical features can help the dermatologist to identify disease activity, including hypomelanotic color of vitiligo lesions and poorly defined borders as opposed to amelanotic lesions with sharply demarcated borders which denote stability. Other signs of activity include confetti lesions and trichrome vitiligo (Figure 1).

On reviewing the literature, earlier studies using MKTP in the treatment of vitiligo chose different durations of disease stability as an inclusion criterion. Some authors considered 6 months of disease stability to be sufficient, while others required 1 year of disease stability. In the early reports by Mulekar, in which cases with a minimum of 6 months of disease stability were included, ≥95% repigmentation occurred in 65/122, 13/19 and 36/43 cases of generalized vitiligo (GV), focal vitiligo and segmental vitiligo (SV), respectively.
| Number of days | Donor tissue | Trypsinization technique | Medium used for suspension | Recipient site preparation | Application of suspension | Patient immobilization | Reference |
|----------------|--------------|--------------------------|-----------------------------|---------------------------|--------------------------|------------------------|-----------|
| Two            | Day 1: superficial skin samples from the occipital scalp using a dermatome with a razor blade | Day 1: cold trypsinization. Skin incubated for 18 hours at 4°C in 0.25% trypsin; Day 2: skin incubated in EDTA for 15 minutes at room temperature | Saline (1 mL/1 cm² skin) | Day 1: liquid nitrogen induced blisters 1 and 2 cm apart; Day 2: partial aspiration of blister fluid and injection of 0.1 mL of cellular suspension/blister by a 25-gauge insulin syringe | 20 minutes | Gauthier and Surleve-Bazeille (1992)⁷ |
| One            | Superficial skin sample with a 1:4–1:10 donor-to-recipient area ratio taken with a Goulian biopsy knife | Warm trypsinization. Skin sample torn into 2 cm² pieces and incubated at 37°C in 5% CO₂ for 50 minutes in trypsin/EDTA solution. Trypsin inhibitor added following incubation | Supplemented melanocytic medium: M2 medium supplemented with basic fibroblast growth factor, penicillin and streptomycin | A high-speed dermabrader, fitted with a diamond wheel | 4–5 hours | Olsson and Juhlin (1998)⁸ |
| One            | A shave biopsy (1/2–1/4 of recipient area) from the gluteal region using a hand dermatome | Warm trypsinization. Skin sample torn into 2 cm² pieces and incubated at 37°C in 5% CO₂ for 50 minutes in trypsin/EDTA solution. Trypsin inhibitor added following incubation | Supplemented melanocytic medium: M199 medium supplemented with insulin, hydrocortisone, cholera toxin, penicillin/streptomycin and transferrin/riboflavin | CO₂ laser resurfacing | 6 hours | van Geel et al (2001)⁹ |
| One            | A shave biopsy (1/3–1/10 of recipient area) from the gluteal region by a silver skin grafting knife | Warm trypsinization. Ordinary incubator used followed by addition of trypsin inhibitor step replaced by washing tissues | No supplements: DMEM/F12 only | High-speed dermabrader fitted with a diamond fraise wheel | Left immediately | Mulekar (2003)⁰ Mulekar (2004)¹ Mulekar (2005)² Mulekar et al (2009)¹⁷ Mulekar et al (2010)²⁵ |
| Two            | Day 2: superficial skin samples from the gluteal region (1/10 of recipient area) | Day 2: skin incubated for 40 minutes at 37°C in an ordinary incubator followed by 1% FBS addition to neutralize trypsin | Supplemented melanocytic medium: Ham F12 supplemented with L-glutamine, penicillin–streptomycin, hydrocortisone, basic fibroblast growth factor, isobutylmethylxanthine and 3′,5′-cAMP | Day 1: liquid nitrogen induced blisters 1 and 2 cm apart; Day 2: hyaluronic acid added to suspension in 1:6 ratio to form an injectable viscous suspension | 2 hours | El-Zawahry et al (2011)³⁰ |
| One            | Anagen HFUs from the occipital region (15–25) to produce ORSHFS | HFUs incubated at 37°C for three 30-minute cycles. HFUs placed in a new tube of trypsin–EDTA and trypsin inhibitor added to previous tube each time | Suspensions of all three tubes combined in a single tube and filtered through a 70 µm cell strainer, centrifuged and suspended in DMEM | A motorized dermabrader fitted with diamond fraises used till pinpoint bleeding appeared | Not stated | Mohanty et al (2011)³¹ |

(Continued)
respectively. However, a high relapse rate was noted in 15 of those responders. Huggins et al.26 achieved ≥95% repigmentation in only 4/23 treated cases with 6-month duration of stability. In a retrospective long-term follow-up study of cases treated over the past 6 years, 6 months of stability was sufficient as an inclusion criterion. More than 75% repigmentation was noted in 71% and 54% of SV and non-segmental vitiligo (NSV) cases, respectively.27 On the contrary, a higher percentage of repigmentation was noted in one study where all three SV and 13/17 NSV cases with 1-year stability showed 95%–100% repigmentation8 and in another study where all four cases achieved 84%–100% repigmentation.9

In a retrospective study by Olsson and Juhlin,28 cases that showed stable improvement of their vitiligo had a 78% repigmentation in response to different melanocyte transplantation techniques including MKTP after 4.8 years compared to cases with unstable vitiligo that showed a 33% repigmentation after 6.5 years. The authors recommended that patients with extensive GV and those who have not had completely stable, non-progressive vitiligo for at least 2 years should not be chosen for transplantation.

Active disease and the presence of Koebner phenomenon were found to negatively influence treatment results.5,13 Activity resulted in failure of MKTP suspension in one study5 with a median area percentage of repigmentation of zero compared to 93% in stable cases at 12-month posttreatment follow-up. Similarly, 79% repigmentation occurred in cases with ≥1-year stability vs only 18% in cases with <1-year duration of stability ($P=0.02$) in another study.13 A significant positive correlation between duration of stability and percentage repigmentation of the lesions was also found in a recent study.29

### Table 1 (Continued)

| Number of days | Donor tissue |
|----------------|--------------|
| One            | Combined epidermal suspension and ORSHFS: FBS (0.1), NH1 (0.1), NH2 (0.1), NH3 (0.1), ORSHFS: 15–25 HFUs extracted from the scalp |
| One            | Superficial skin samples from the occipital scalp |
| One            | Sections of tissue, NCS: CO$_2$ incubator used for 1 hour, followed by rinsing in PBS and cell separation ORSHFS: as in Mohanty et al (2011) |
| One            | Cold trypsinization followed by addition of patient’s serum for trypsin inhibition |

### Figure 1 Clinical signs of disease activity.

Notes: (A) Active disease is characterized by ill-defined borders and hypopigmented lesions (black arrow). Other signs of activity include Koebner phenomenon and confetti-like lesions (white arrow). (B) Stable disease shows milky white lesions with well-defined borders. Note perifollicular repigmentation at the borders of the lesion (white arrow). (A) Courtesy of Marwa A Abdallah, Professor of Dermatology and Venereology, Ain Shams University, Cairo, Egypt.)

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**Abbreviations:** FBS, fetal bovine serum; HFUs, hair follicle units; NCS, non-cultured epidermal suspension; ORSHFS, outer root sheath hair follicle suspension.
Based on the above data, since 2004 the majority of authors including Mulekar³ consider 1 year as the minimum duration of stability needed for a favorable outcome of surgery.³⁰-⁴¹

When uncertain about stability, a longer pretreatment observation documented by photography or a minigrafting test⁴² may be indicated to avoid unfavorable outcome of surgery. Disease stability should be considered in both SV and NSV. SV can respond to medical treatment during the first 6 months.

Type of vitiligo
When it was first described by Gauthier and Surleve-Bazeille in 1992,⁷ MKTP was used in the treatment of localized vitiligo areas of ≤50 cm² in three SV and eight focal vitiligo cases. The response was better in SV cases with an average repigmentation rate of 92% vs 41% in focal vitiligo cases with 4/7 cases failing to repigment. However, comparable excellent response (≥95% repigmentation) was found in a series of 25 children and adolescents with SV and focal vitiligo.²⁵

SV cases showed a significantly better response than NSV with 85% vs 70% repigmentation (P=0.011) in one study⁴ and >50% repigmentation in 88% vs 71% of cases in another study (P=0.007).⁴⁰ Other studies reported a better response in SV cases, although statistical analysis was not performed.²,3,5,8,26,28,4² or was not significant.¹³,³⁸ Immunological disturbances probably interfere with the outcome of transplantation in GV.²⁸

No difference in improvement according to the type of vitiligo was noted in a recent study using outer root sheath hair follicle suspension (ORSHFS) in the treatment of 25 cases of stable vitiligo (nine SV, eleven acrofacial vitiligo, five GV).⁴⁴

Mixed vitiligo cases responded less favorably than SV and GV cases to MKTP.³⁸,⁴⁰

Acrofacial type in general is less responsive to surgical therapy. Lesions on the fingertips were even considered an exclusion criterion by some authors.²,²⁷ Interestingly, the presence of vitiligo on lips and fingertips (lip-tip type) was associated with a poor response, even when MKTP was performed at other sites in the same patient.²⁶

Extent of vitiligo and size of treated lesion
Surgical therapy in general is indicated in stable cases with limited areas of vitiligo which are nonresponsive to medical therapy. Several authors excluded cases with widespread vitiligo involving >30% of the body surface area.²,³⁷,⁴³ The probability of a successful transplantation outcome to non-cultured epidermal suspension (NCES), ultrathin sheet transplantation and cultured epidermal suspension (CES) was found to be 20 times higher (OR) in patients with <100 cm² white areas, three times higher in the 101–500 cm² group and two times higher in the 501–1,500 cm² group, compared with patients with >1,500 cm² white area.²⁸ Similarly, a negative correlation was found between Vitiligo Area Scoring Index, and Vitiligo European Task Force area and stage scores and percentage repigmentation in a more recent study.²⁸

A significant negative relation was found by the authors of a study between the total treated surface area and the treatment outcome (P=0.0086).⁶ No similar correlation was found by other authors.²⁹ The majority of authors used MKTP in treatment of lesions <100 cm² with a favorable outcome.⁵,⁷,¹³,¹⁵,³¹,³⁴,³⁶,³⁸,⁴¹,⁴⁵ A few achieved favorable outcome for lesions up to 250 cm².³,⁸,⁹,1⁸,2⁵,2⁶,2⁹,3⁰,3³

Skin type
Most of the reports do not comment on the skin type of cases treated; however, when analyzed statistically, similar repigmentation rates were reported in different ethnic groups²⁶ and in different skin phototypes.⁶,²⁷

Age
Age did not affect the percentage of repigmentation in several studies.⁶,²⁷ Many studies included children and adolescents in the treated cases.²,⁵,⁶,⁸,¹⁶,¹⁷,²⁵,²⁷,³⁰,³¹,³⁴,³⁶,³⁸,⁴¹,⁴³,⁴⁶ Two studies focused solely on the treatment outcome in this age group. Mulekar et al¹² treated 25 children and adolescents using general anesthesia with ≥95% repigmentation in 8/13 SV and 7/12 focal vitiligo cases. New lesions developed during follow-up in 5/12 focal vitiligo cases which could be attributed to the short duration of disease stability (6 months) applied in this study. The second study involved 13 cases of vitiligo (six SV, 1 focal vitiligo and six GV) with 1-year disease stability. Topical anesthesia followed by local infiltration was applied in 15/19 lesions achieving >90% repigmentation.¹⁹ In both studies, the procedure was well tolerated and accepted by both children and their parents. The main concern in children was pain intolerability and fidgeting during the procedure. Increasing the concentration of topically applied creams can be a good option in cooperative children, but general anesthesia may be still needed in selected cases.

Gender
No significant difference was found in repigmentation between males and females.⁶,²⁷,²⁸,⁴¹

Disease duration
The effect of this variable was assessed in a few studies with no correlation found in two⁶,⁴¹ and a negative correlation where patients with shorter disease duration got better treatment results in another.²⁷
Site of lesion

On reviewing the literature, the head and neck lesions usually showed the best response, lesions on the limbs (excluding the elbows, knees and ankles) and trunk showed an intermediate response and lesions over the joints and acral skin tended to respond less favorably.6,7,26,46 Two exceptions were the study by Olsson and Juhlin28 where the neck was found to show the poorest response to transplantation and the study by Mulekar4 where the response over the face was worse than other sites and was explained by the traumatizing action of UV sunrays. Other authors did not find a significant effect of the site of treated lesion on the rate of repigmentation.27,34,36,38,40,41,44

Over the head and neck, response rates ranged from 70% to 100% with over half of the lesions achieving ≥95% repigmentation.2,7,9,18,25,40,46

Acral lesions are usually resistant to medical therapy, and hence, MKTP may be one of the few available effective modalities of therapy. It is important to note that lesions over the dorsum of the hands and feet respond better than those over the fingers or toes. Some authors do not recommend treating fingertips (distal fingers) owing to the poor response.2,27 Mulekar4 reported an excellent response in 62.5% of lesions over the dorsum of hands and feet and 66% of those over the fingers in a series of 142 cases of NSV. Fingers and toes also responded well with 42% of lesions achieving ≥95% repigmentation in another report by the same authors.17 However, it was not clear in both studies if any of those lesions were over the fingertips (distal fingers). Holla et al15 achieved >75% repigmentation in 78% of lesions over the dorsum of hands and feet and 42% of lesions over fingers and toes with only 6/80 lesions over acral skin showing <50% response (two over the ankle and four in distal fingers). Fingertips were found to show full repigmentation in one case in which cryoblebbing was used for recipient site preparation29 raising the possibility that perhaps poor response was due to the difficulty in performing dermabrasion or laser resurfacing at this site. Cryoblebs produce separation at the dermoepidermal junction regardless of the skin thickness.

In a recent study, 35% of acral lesions (excluding those on fingertips and toes) demonstrated excellent repigmentation with no difference in outcome found between difficult-to-treat sites and other sites.27 Others, however, showed less favorable outcome with 25%,13 15%,26,28 or less5,18,29,30,38,46 of the lesions over the hands and feet showing ≥95% repigmentation.

Similarly, lesions over the joints including elbows, knees and ankles tend to respond less favorably with <30% of the lesions showing excellent repigmentation.5,7,26,28,30,43 Lesions over the joints showed a significantly lower response compared with prexsternal lesions and those on the trunk and extremities.6 On the contrary, some authors reported an excellent response of lesions overlying joints with ≥95% repigmentation achieved in 54% of lesions in one study4 and in 100% of lesions in others.18,27 Inadequate depth of dermabrasion due to heavily cornified skin as well as the high mobility at these sites may explain the poor response.6,46 Holla et al15 used strict immobilization up to the extent of using plaster casts when needed and achieved >75% repigmentation in 21/33 (64%) treated lesions. In another study, a diamond fraise wheel at a high speed of 12,000–15,000 rpm was used to assure proper dermabrasion of the thick cornified skin with an excellent response achieved in 15/43 (35%) lesions over joints without applying strict immobilization.17

Certain sites are considered difficult to manage due to the delicacy of the skin and/or the difficulty in immobilization such as the eyelids, nipples and genital skin. Manual dermabrasion or diamond fraise wheel at a low speed of 5,000 rpm was used with an excellent response achieved in 67% of eyelid lesions and 25% of genital lesions.17

Effect of procedure-related factors on response to MKTP

Donor tissue

Type of tissue used

Currently, there are two types of suspension used in MKTP, NCES8 where the epidermis is the source of cells and ORSHFS13 where the anagen hair follicle is the source.

Two studies compared cellular grafting in the form of NCES to tissue grafting in the form of suction blister epidermal grafting (SBEG). The first involved two groups of cases with comparable results regarding very good repigmentation (≥75% repigmentation), color matching and side-effect profile. However more cases achieved excellent repigmentation (≥90%) in the NCES group (P=0.002). Patient satisfaction and dermatological quality-of-life score reduction were also significantly higher in the NCES group.14 On the contrary, NCES, CES and SBEG were compared in the same patient in another study with significantly better response in SBEG lesions while NCES and CES lesions were comparable.39

In another study, no significant difference was found between NCES and CES with >70% response achieved in 62% and 52% of cases, respectively. Although CES could cover large areas using a small donor sample, it required expensive equipment and reagents in addition to highly trained personnel.37

A few studies compared NCES and ORSHFS with no significant difference found29,36 despite the significantly higher total cell count yielded by NCES.39 This may be due to the higher variety of cell populations including melanocyte stem cells in ORSHFS.49,50 On the other hand, inferior results
were found in ORSHFS group compared to NCES group (43% vs 90% of cases with >75% repigmentation) in another study.41 The authors explained these poor results in ORSHFS group by the higher number of elderly people and the lack of proper surgical skills in follicle unit extraction which led to the use of insufficient numbers and transected hair follicles for preparation of the cellular suspension. These comments highlight the importance of proper choice of patients with abundant dark anagen hairs as well as higher level of experience required for performing this technique. It should be noted that long-term stability of pigmentation from hair follicle-derived melanocytes has not been established yet. Hair graying is known to occur with aging.48

Finally, combined NCES and ORSHFS produced superior repigmentation when compared to NCES in lesions over the joints and acral skin,15,16 while similar repigmentation rates were seen over the face15 in two recent studies.

**Recipient site preparation**

Recipient vitiligo skin may be prepared by different methods including cryoblebbing,7,29,30,47 dermabrasion7,14,15,18,25–28,33,38,41,44,46,51 or laser resurfacing.5,9,29,40,45,52,53 The ideal method should be simple to perform, safe with minimal side effects and efficient reaching the dermoepidermal junction to avoid scarring or loss of the grafted melanocytes. Dermabrasion is more economical and relatively safe but requires technical skills. Pinpoint bleeding denotes reaching the ideal level. CO₂ laser resurfacing produces uniform resurfacing but is more expensive. However, pinpoint bleeding does not appear. Two studies were done comparing different ablative CO₂ laser settings with similar repigmentation rates achieved using less invasive resurfacing. A depth of 209 vs 300 µm was used in one study,52 while 144 vs 209 µm was used in the second.53 As expected, less invasive resurfacing resulted in faster healing and less persistent erythema at 6-month follow-up.53 In the same study, fractional laser resurfacing failed to produce an efficient response when used for recipient site preparation.53 In a pilot study, dermabrasion using a high-speed dermabrader fitted with a diamond fraise wheel produced better repigmentation than fractional CO₂ laser resurfacing,51 but the latter was faster and simpler to perform. In our experience, laser resurfacing surpassed manual dermabrasion in improving repigmentation following MKTP in acral and non-acral lesions (unpublished data, Esmat et al, 2014).

Cryoblebbing must be done 24 hours before MKTP, and therefore, the procedure is done over 2 days. It also
requires longer healing time; however, it may have a role in certain sites like the fingertips.\(^{30}\) Only one study compared cryoblebbing to laser resurfacing. Cryoblebbing produced \(\geq 75\%\) repigmentation in significantly more lesions (38 vs 10 lesions) \((P=0.001)\) mainly due to excellent response achieved over the distal fingers.\(^{29}\)

Less invasive methods of cellular suspension delivery were recently described. Benzekri and Gauthier\(^{20}\) delivered cellular suspension using a dermaroller equipped with 0.2 mm microneedles with >75% repigmentation in 3/5 cases with lesions over the face. Successful migration of viable melanocytes to the basal epidermal layer was demonstrated using this minimally invasive technique. However, larger case series are needed to assess this innovative method. Intralesional injection of NCES was also attempted in a recent study involving a large number of cases (300) where >50% repigmentation was obtained in 32.2% of treated patches (1,060) 9 months after therapy.\(^{54}\)

**Postoperative dressing and wound care**
The type of dressing used postoperatively was found to affect the outcome of repigmentation at 12 months. In one study, 83% of cases where collagen dressing was used vs 63% of those where HA was used achieved \(\geq 50\%\) response \((P=0.017)\).\(^{40}\) Collagen dressing was compared to petrolatum-impregnated gauze in the same patient in a pilot study with no significant difference in repigmentation. However, the gauze was more difficult to remove after 1 week.\(^{31}\) This encouraging finding is useful when collagen sheet is unavailable or too expensive to use in certain developing countries.

**Factors enhancing repigmentation after MKTP**
A few solar exposures lead to coalescence of pigmented areas in the earliest description of MKTP.\(^{2}\) Over the years, several studies used post-transplantation phototherapy\(^{5,20,29,30,48,44}\) or sun exposure\(^{16,43}\) to enhance repigmentation. No comparative studies were done to confirm this enhancing role; in fact, targeted phototherapy (UVB + UVA) post-grafting did not significantly improve the rate or the final repigmentation outcome at 12-month follow-up when compared to cases where it was not used.\(^{40}\) Interestingly, hyperpigmentation was linked to sun exposure recommended by the dermatologist postoperatively in one study in an Indian population,\(^{2}\) while sun exposure had a significant beneficial effect on color mismatch in another study performed in Belgium.\(^{6}\) This is probably related to skin type as darker skin types have a higher tendency of tanning. More studies are needed to explore the impact of post-MKTP phototherapy in different skin types.

**Complications**
MKTP is a safe technique with minimal complications. These include short-term complications in the form of infection or erythema and long-term complications, the most important of which are scars and color mismatch.

**Short-term complications**
***Infection***
A low incidence of infection in 5%–16% of cases was reported by some authors.\(^{18,31,37,44,46}\) A slightly higher incidence of 29% was reported in cases where cryoblebbing was used at the recipient site. This was attributed to the moist nature of the lesion and longer healing time.\(^{29}\) In all cases, infection was well controlled by broad-spectrum systemic antibiotics.

***Persistent erythema***
A bright pink color or mild erythema which lasts for a few weeks is expected after MKTP.\(^{2,5}\) Sometimes, erythema persisted for a few months, especially in cases where CO\(_2\) laser resurfacing was used for recipient site preparation. This was reported less frequently when more superficial full ablation was performed (50% of lesions at 144 \(\mu\)m vs 70% of lesions at 209 \(\mu\)m depth of ablation).\(^{53b}\)

**Long-term complications**
***Color mismatch***
This is probably the commonest long-term complication. Treated lesions may appear slightly darker or slightly lighter than the surrounding skin. It was reported in several studies in varying percentages ranging from 5%–20%\(^{5,15,18,26,33,34,43}\) to \(\geq 50\%\) of cases treated.\(^{2,6,9,27,36,40,47}\)

This mismatch improved after 6–8 months in some studies.\(^{2,5,9}\) A degree of mismatch persisted in 64% of lesions (36% darker, 28% lighter) 16.5 months after MKTP in one study, but this did not bother most of the cases (79%).\(^{6}\) Sun exposure can have an effect on improving\(^{6}\) or worsening\(^{6}\) the color mismatch as mentioned earlier. In another study, hyperpigmentation was more frequent over the joints which led the authors to suggest it may be due to frictional melanosis.\(^{35}\)

Paul\(^{35}\) linked color mismatch to donor-to-recipient tissue ratio. He noticed that hyperpigmentation occurred in cases where a larger donor area (<1:5 ratio) was harvested while hypopigmentation affected cases in which the donor-to-
recipient ratio was more than 1:10. A similar observation was reported by Sahni et al\textsuperscript{18} where hypopigmentation was found in 1/13 treated cases in whom a large donor-to-recipient ratio of 1:10 was used.

**Hypopigmented halo**

A rim of hypopigmentation at the edges of the lesion was reported in 6\textperthousand{}–25\textperthousand{} of cases in several studies.\textsuperscript{3,27,33,41} Extending the dermabrasion 2–3 mm into normal skin decreased the occurrence of this complication.\textsuperscript{33}

**Scars or textural change**

Textural changes or scar may occur at the donor site,\textsuperscript{6,29,45,52} and therefore, tissue should be harvested from a relatively concealed area over the thigh or buttocks.

**Stability of acquired pigmentation**

A few long-term follow-up studies have emerged which gave a clear idea about stability of repigmentation achieved following MKTP. Excellent retention of acquired repigmentation was found in cases of SV on long-term follow-up.\textsuperscript{3,6} Repigmentation was maintained in 19/23 (83\textperthousand{}) of treated cases after 5 years of follow-up in one study.\textsuperscript{49} In GV, the majority of cases retain acquired repigmentation. However, some GV cases may lose some of this repigmentation especially if disease activity occurs. This was reported in 4\textperthousand{},\textsuperscript{4} 16\textperthousand{},\textsuperscript{6} 11\textperthousand{},\textsuperscript{40} and 21\textperthousand{} of cases\textsuperscript{27} in long-term follow-up studies. It is therefore essential to inform these patients clearly that MKTP is not a cure for vitiligo and does not prevent new lesions from appearing in the future in order to avoid patient frustration.

Improvement of repigmentation continued for a mean period of 10 months in one study.\textsuperscript{6} More pigmentation was reported in 67\textperthousand{} and 62\textperthousand{} of treated areas in SV and NSV cases, respectively, during 12–24 months of follow-up.\textsuperscript{27} This increase was significant in SV cases. These data are very useful since patients sometimes require a second session of MKTP to achieve full repigmentation. It would be therefore wise to wait for several months for full response to be judged.

Finally, patients are sometimes concerned about associated leucotrichia. An interesting retrospective study found that >90\textperthousand{} repigmentation of hair occurred in 58\textperthousand{} (10/17), 28\textperthousand{} (2/7) and 12\textperthousand{} (2/16) of lesions over the trunk, scalp and face, respectively, after MKTP.\textsuperscript{25} The authors noticed that the skin repigmented first followed by hair repigmentation after a lag period which was attributed to retrograde migration of melanocytes from the repigmented epidermis.\textsuperscript{56} Similar improvement was reported by other authors.\textsuperscript{18,27}

Details about patients’ data and technique used in cited papers are included in Table S1.

**Recommendation for a successful MKTP**

Success in MKTP is achieved by fulfilling certain criteria with 12-month stability being its cornerstone. In our opinion, a minigrafting test is essential to predict the response to MKTP and should be routinely done in all cases because a clear-cut objective method of ensuring disease stability is still unavailable. The site and size of the lesions are influential factors too. Some sites such as the face, trunk and limbs are easier to resurface with ability to secure the dressings used postoperatively. Technique adjustments are needed at other sites such as delicate skin of eyelids or genitals where gentle resurfacing is needed as opposed to thick acral skin or skin overlying the joints where aggressive resurfacing is mandatory. Lesions over the fingers remain a challenge; the authors are dedicating research focusing on the best option especially in periangual lesions; cryoblebbing seems to give hope and splints using a tongue depressor are a simple way of limiting movement. The procedure is more suited to patients with limited extent of vitiligo, while cases with extensive lesions involving >30\textperthousand{} of the body surface area are less likely to respond.

The tissue harvested for suspension preparation is another variable to consider. Keeping the ratio of donor to recipient areas around 1:3 or 1:5 when feasible increases the cell count/mm\textsuperscript{2} improving the response. Exposing the donor area to ultraviolet rays a few weeks before grafting was found to significantly increase the melanocytic count in NSV cases (unpublished data, Bassiouney et al, 2017) which can further boost the response. Finally, the use of mixed suspension (NCES and ORSHFS) may be beneficial in acral skin. Postoperative wound dressing and phototherapy are also important and are still fresh fields for exploring.

Most of the SV cases achieve an excellent response to MKTP because they possess many good prognostic factors, namely long periods of disease stability and small-sized lesions located over the face. Paying special attention to details of the technique can improve the response in NSV even in difficult-to-treat areas. Knowing what to predict and how to handle each case allows for successful outcome and realistic expectations (Figure 2). MKTP is an effective method of treatment in stable cases of vitiligo which produces long-lasting repigmentation with very good color matching offering a beam of hope for vitiligo patients.
Disclosure
The authors report no conflicts of interest in this work.

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## Supplementary material

### Table S1 Details of prospective and retrospective MKTP studies

| Authors              | Study design       | No. of cases | Vitiligo type | Skin type | Age       | Technique                          | Area (cm²)         |
|----------------------|--------------------|--------------|---------------|-----------|-----------|------------------------------------|--------------------|
| Gauthier and Surleve-Bazeille¹ | Prospective       | 12           | SV: 3         | Not stated | 20–65     | D: ≥2 cm² R: ≤50 cm²              | Cryo               |
| Olsson and Juhlin²   | Prospective       | 20           | SV: 3 NSV, all focal: 8 | Not stated | 13–52     | 1:4–10                | DermA              |
| van Geel et al³      | Prospective pilot study | 4           | SV: 2 III: 2 IV: 2 | Not stated | 30–52     | 1:2–4                 | CO₂ laser          |
| Olsson and Juhlin⁴   | Retrospective     | 132          | SV: 15 GV: 107 Focal: 2 | Not stated | 12–61     | CES: 8 cm² NCES: 10 cm² | Collagen dressing or silicone netting |
| Mulekar⁵             | Prospective       | 184          | SV: 43 IV: 122 Stable and 9 active | Not stated | 12–70     | 1:10                 | Collagen film      |
| van Geel et al⁶      | Prospective double blinded placebo controlled | 28/66 | NSV (19 stable and 9 active) II–IV: 25 | Not stated | 15–65     | 1:1                  | CO₂ laser          |
| Mulekar⁷             | Prospective       | 64           | SV: 49 Focal: 15 | Not stated | >12       | 1:3–10               | DermA              |
| Mulekar⁸             | Prospective       | 142          | GV            | Not stated | 18–70     | 1:10                 | DMEM/F12 medium Collagen film |
| Pandya et al⁹        | Prospective controlled | 27          | SV: 2 NSV: 25 | Not stated | >8        | 1:10                 | Supplemented medium/collagen dressing |
| Tegta et al¹⁰        | Prospective comparative | 20          | SV: 4 Two different cellular dilutions | Not stated | 10–54     | 1:3 vs 1:5           | Injection into blister from floor |
| Mulekar et al¹¹      | Prospective       | 49           | SV: 9 NSV: 40 | Not stated | 7–65      | 1:3–10               | DMEM/F12 medium Collagen sheet |

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*Note: SV = Solar Veil, NSV = Normal Solar Veil, Gv = Gem, Focal = Focal Lesion, D: Dose, R: Reactivity, NSW = Not Stated, NCeS = Not Complete Superficial, epS = Epidermal Superficial, CeS = Complete Epidermal, R: Reactivity, D: Dose, %: Percent, v: vehicle, Mel medium = Melanin medium, Saline = Saline, Suspension = Suspension, CO₂ laser = Carbon Dioxide laser, Hyal A = Hyaluronic acid, Mel medium = Melanin medium, DermA = Dermatological assistance, Cold trypsin = Cold trypsin, Saline = Saline,*

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*Remark: 7–194: 75% response of 1:10 ratio, 95% in 80 cases; 95 in 11/15 cases, ≥95% in 14/15 cases (5 CeS, 1 Gv); 95% in 41/49 NSv and CeS cases (not stated); 95% in 3/9 lesions; 95 in 0/10 cases of 1:5 ratio; 95% in 10/72 lesions; 95% in 29/72 lesions.*
| Stability          | Average % repigmentation                                                                 | Response according to site No. of lesions with ≥95% response/total no. (% pigmentation) | Follow-up (months) | Complications |
|--------------------|------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|-------------------|---------------|
| Not stated         | SV: 92% NSV: 41%                                                                        | H&N: 2/4 (90) Joints: 0/4 (40) Extremity: 1/2 (50) Hands: 0/2 (35)                     | 3                 | *1 case of nevus depigmentosus showed 80% repigmentation |
| >1 year            | SV: 100% NSV: 78.5%                                                                   | H&N: 7/12 (80) Joints: 5/5 (100) Extremity: 9/12 (82) Trunk: 4/5 (97) H&F: 2/5 (72) | 6–12              | None mentioned |
| Except 2 cases     |                                                                                    |                                                                                        |                   | *Patient rested 4–5 hours after the procedure |
| >1 year            | SV: 90% NSV: 88%                                                                        | Face: 1/2 (92) Preternal: 0/2 (88)                                                     | 6–20              | Color mismatch (disappeared in 5 months) |
| Not stated         | SV: ≥95% in 14/15 cases (5 CES, 1 EpS, 8 NCES) GV: 42% CES, 59% EpS, 49% NCES Focal: 100% in 2 (NCES) | H&N: 13/65 Joints: 10/78 Extremity: 33/108 Trunk: 11/26 H&F: 14/92                       | 12–84             | *Halo nevus: 3 | All ≥95% response |
| 6 months           | SV: 92% GV: 57% Focal: 68%                                                            |                                                                                         | 12                | Color mismatch (darker) disappeared in 6–8 months Relapse in 15 cases (12 NSv, 3 SV) |
| >1 year vs active  | Stable: 77 Active: 0 (placebo-treated lesions: 20% response in 3 cases)               |                                                                                         | 3–12              | Color mismatch (darker): 5/66 lesions improved in 6 months *Combined by NB-UVB or PUVA |
| 1 year             | SV: ≥95 in 41/49 Focal: ≥95 in 11/15 cases                                             |                                                                                         | 12–60             | Color mismatch (lighter in 8 cases, darker in 1 case) P halo in 4 cases New lesions in 2 cases |
| 6 months           | ≥95 in 80 cases; ≥65 in 15                                                             |                                                                                         | 12–72             | Relapse in 15 cases Color mismatch in 11 cases P halo in 6 cases |
| 2 years            | Excellent in 52% NCES vs 50% in 4 CES cases Control patch in 20 cases, no pigmentation |                                                                                         | 6                 | Infection: 7% of donor, 11% of recipient sites Koebner donor 1 CES: 4 cases (cells <1,000/mm²) |
| 1 year             | >75% response 5/10 cases of 1:3 ratio 0/10 cases of 1:5 ratio                         |                                                                                         | 3                 | Color mismatch: darker in 3 cases, lighter in 8 cases *Vitiligo >10% BSA excluded |
| 6 months           | SV: ≥95% in 3/9 lesions NSV: ≥95% in 29/72 lesions                                      | Eyelids: 6/9 Joints: 15/43 Areola: 5/6 Fingers and toes: 8/19 Genital: 1/4              | 6–12              | *No trypsin inhibitor. No immobilization of joints |

(Continued)
| Authors                  | Study design | No. of cases | Vitiligo type | Skin type | Age | Technique | D:R ratio | R          | Suspension | Area (cm²) |
|-------------------------|-------------|--------------|---------------|-----------|-----|-----------|------------|------------|------------|------------|
| Mulekar et al<sup>12</sup> | Retrospective | 25           | SV: 13        | Not stated | 4–16 | 1:3–10    | DermA      | DMEM/F12 medium Collagen sheet | 4–123      |
| van Geel et al<sup>13</sup> | Retrospective | 87           | SV: 33        | II, III: 77 | 9–65 | CO₂ laser | Hyal A     | Mel medium | Not stated |            |
| El-Zawahry et al<sup>14</sup> | Prospective  | 25           | SV: 2         | Not stated | 8–45 | 1:10      | Cryo       | Mel medium | Not stated |            |
| Toossi et al<sup>15</sup> | Prospective controlled | 8/14        | NSV: 23       | Not stated | 13–43 | 1:5–10    | DermA      | DMEM/F12 Collagen sheet | 1–7        |
| Sahni et al<sup>16</sup> | Prospective  | 13/19        | SV: 6         | Not stated | 8–17 | 1:2–10    | DermA      | PBS Cold trypsin | 3–200      |
| Vasquez-Martinez et al<sup>17</sup> | Prospective comparative | 11          | Not stated    | Not stated | 35–48 | 1:10      | DermA      | DMEM/F12 Collagen dressing | Not stated |
| Mohanty et al<sup>19</sup> | Prospective  | 14           | SV: 3         | Not stated | 17–32 | 15–25 HF  | DermA      | DMEM/F12 Collagen dressing | 4–96       |
| Huggins et al<sup>20</sup> | Prospective  | 23/29        | SV: 2         | II–III: 12 | 18–60 | 1:10      | DermA      | DMEM/F12 Collagen dressing | 1–116      |
| Budania et al<sup>21</sup> | Prospective comparative | 41/54       | SV: 16        | Not stated | 12–40 | NCES 1:10 | DermA      | CO₂ incubator PBS Collagen dressing | 3–35       |
| Holla et al<sup>22</sup> | Retrospective | 36/80        | GV: 33        | Not stated | 16–47 | 1:10      | DermA      | Cold trypsin Mel medium, serum | Not stated |
| Stability | Average % repigmentation | Response according to site No. of lesions with ≥95% response/total no. (%) pigmentation | Follow-up (months) | Complications *Remarks |
|-----------|--------------------------|--------------------------------------------------------------------------------------|-------------------|------------------------|
| 6 months  | SV: 79% Focal: 85%       | H&N: 12/20 (78) Joints: 1/1 (100) Extremity: 1/1 (98) Trunk: 2/2 (98) H&F: 3/5 (93) | 9–54              | New lesions in 5/12 NSV cases *General anesthesia and O₂ by face mask |
| No       | SV: 75%                  | Genital > extremity, trunk > dorsum, H&F > H&N > joints 7.9 patient satisfaction score | 11–92             | Color mismatch: 80% of cases (improved by sun exposure) Loss of color: 3/45 NSV cases (activity) |
| Koebnerization | SV: 85% NSV: 70% Mixed: 37% Stable (89%) vs active (48%) | 6 lesions DermA only: no response | 4–6               | Infection 1 case |
| >1 year  | SV: 60% NSV: 50%         | H&N: 1/4 (46) Joints: 2/22 (47) Extremity: 3/5 (87) Trunk: 1/9 (43) H&F: 3/33 (42) Fingers: 10/10 (100) | 6–17              | *Distal fingers showed 100% repigmentation in 1 case |
| >1 year  | >90% response            | >90% response | 12 | Infection: 1 case Color mismatch: 3 lesions *Combined with sun exposure |
| SV: in all cases | GV: in 8/12 lesions Focal: in 1 case | H&N: 7/8 Joints: 4/4 Extremity: 1/1 Trunk: 3/3 H&F: 0/2 | 12 | *Image analysis for % repigmentation |
| >1 year  | No significant difference in area of repigmentation DermA + NCES vs DermA | Trunk: 64% Extremity: 27% Face: 9% | 24 | Color mismatch: 10 cases (6 darker), donor darker in 5 Relapse in 8 cases (3 lost grafted pigmentation) P halo: 12 |
| 1 year   | 32 cases: >90% 9 cases: 70%–89% 4 cases: 30%–69% 4 cases: <30% | Response according to site not stated | 24 | Color mismatch: 10 cases (6 darker), donor darker in 5 Relapse in 8 cases (3 lost grafted pigmentation) P halo: 12 |
| ≥1 year: 11 cases | SV: 87% GV: 53% ACF: 80% (79% stable vs 18% active) | H&N: 0/2 (70) Joints: 1/1 (95) Trunk: 0/6 (70) Extremity: 1/2 (93) H&F: 1/4 (38) | 1–15              | Color mismatch lighter: 5 |
| <1 year: 3 cases | SV: 1/2≥95%, GV: 21≥95% Focal: 1/6≥95% | H&N: 3/12 Joints: 1/6 Extremity: 1/9 Trunk: 1/5 H&F: 1/6 | 3–6               | Color mismatch darker: 7 lesions (4 NCES, 3 SBEG) lighter: 7 lesions (2 NCES, 5 SBEG) *Strict immobilization (sometimes plaster casts) |
| 1 year   | ≥90% response NCES: 20/28 SBEG: 7/26 lesions | Not stated but no significant effect of site on response NCES higher satisfaction & DLQI reduction | 4 | Color mismatch darker: 7 lesions (4 NCES, 3 SBEG) lighter: 7 lesions (2 NCES, 5 SBEG) *Strict immobilization (sometimes plaster casts) |
| >1 year  | >75% in 51/80 50–75 in 23/80 <50 in 6 (2 ankles, 4 distal fingers) | >75% response | 6–18 | Color mismatch |

(Continued)
### Table S1 (Continued)

| Authors | Study design | No. of cases/ no. of lesions | Vitiligo type | Skin type | Age | Technique | Area (cm²) |
|---------|--------------|------------------------------|---------------|-----------|------|-----------|------------|
| Singh et al 23 | Prospective comparative | 30/54 NCeS (15/24) vs ORSHFS (15/23) | SV: 11; GV: 15; Focal: 4 | Not stated | 13–35 | NCeS 1: 10 ORSHFS: 15–25 HF | DermA | CO₂ incubator PBS Collagen dressing | 4–48 |
| Holla et al 24 | Retrospective | 31/42 | SV: 16; GV: 12; Focal: 3 | Not stated | 10–38 | 1:10 | DermA Manual | Cold trypsin ± Mel medium, serum | Not stated |
| Verma et al 25 | Prospective comparative | 25 | SV: 2; NSV: 20; Focal: 3 | Not stated | Not stated | NCeS 1: 10 Ces 1: 100 | DermA | Collagen dressing | Not stated |
| Vinay et al 26 | Prospective | 30/60 | SV: 2; GV: 11; Focal: 17 | Not stated | 8–38 | ORSHFS | DermA | Collagen sheet | <100 cm² |
| Komen et al 27 | Prospective RCT | 10 | SV: 5; Pieb: 5 | Not stated | 34 | 1:5 | CO₂ laser | ReCell Kit | 27 |
| Bao et al 28 | Prospective comparative | 83 | SV: 40; NSV: 43; CES vs NCeS vs SBEG | Not stated | 25 | CES 1:20 NCeS 1:5 using suction blister roof SBEG 1:1 | CO₂ laser | Vaseline gauze and F12-soaked gauze CES: 40–80 NCeS: 20–40 SBEG: 2–10 |
| Gan et al 29 | Retrospective | 177 | SV: 77; NSV: 98; Mixed: 2 | III: 2; IV: 152; V: 23 | 34±15 | 1:5 | CO₂ laser | Collagen sheet vs Hyal A | Not stated |
| Donaparthi and Chopra 30 | Prospective comparative | 11 | SV: 1; NSV: 9; Focal: 1 NCeS: 6; ORSHFS: 5 | Not stated | 12–42 | NCeS: 1:10 ORSHFS: 15–25 HF | DermA | Collagen sheet | 1–64 |
| Shah et al 31 | Prospective | 20 | SV: 3; GV: 8; ACF: 6; Focal: 3 | Not stated | 18–43 | 20–25 HF | DermA | Collagen dressing | Mean: 37 |
| Silpa-Archa et al 32 | Retrospective | 83/200 | SV: 43; NSV: 40 | I–II: 25; III–IV: 29; V–VI: 29 | 9–60 | 1:10 | DermA (CO₂ laser in 5 cases with large or delicate sites) | Collagen sheet | 2–250 |
| El-Zawahry et al 33 | Prospective comparative | 37/174 NCeS vs ORSHFS | NSV | Not stated | 13–58 | NCeS 1:5 ORSHFS: 1 HF/cm² | CO₂ laser vs cryo | No significant difference (P=0.3) | NCeS vs ORSHFS | 5–160 |
| Stability          | Average % repigmentation | Response according to site No. of lesions with ≥95% response/total no. (% pigmentation) | Follow-up (months) | Complications *Remarks |
|--------------------|--------------------------|----------------------------------------------------------------------------------------|-------------------|-------------------------|
| I year             | NCES: ≥90 in 20/24 lesions (83%) ORSHFS: ≥90 in 15/23 lesions (65%) | Not stated but no significant effect of site on response NCES higher satisfaction, similar DLQI reduction | 4                 | Color mismatch: darker 11 lesions (6 NCES, 3 ORSHFS), lighter 6 lesions (3 in each group) |
|                    | Not stated                | Leucotrichia improved >90% Scalp: 2/7 lesions Face: 2/16 lesions Body: 10/17 lesions | 6–12              | 20 min/day sun Methylcobalamin |
| I year ≤30% BSA    | >70% response             | Face, lips, trunk and legs better response than acral and joint lesions                | 6                 | Infection: 1 NCES, 5 CES Relapse: 2 cases CES: 100–200/cm² | |
|                    | NCES: 62% CES: 52%        |                                                                                       |                   | 1:5 PUVA sol            |
| I year             | >90% response             | >90% response H&N: 2/8 Trunk: 0/3 Extremity: 7/31 H&F: 1/18                             | 6                 | Color mismatch: 23/60 darker (38%), 2/60 lighter (3%) |
|                    | SV: 1/3 lesions           |                                                                                       |                   |                         |
|                    | GV: 1/25 lesions          |                                                                                       |                   |                         |
|                    | Focal: 8/32 lesions       |                                                                                       |                   |                         |
| Not stated         | Median response: 78% in NCES 0% in CO₂ only 0% in control | Not stated                                                                             | 6                 | Mild textural change in 2 donor sites |
| I year             | ≥90% response             | ≥90% response CES, NCES, SBEG H&N: 22, 24, 30/33 Extremity: 6, 6, 12/24 Trunk: 16, 16, 21/26 | 12                | Color mismatch (darker 22 SBEG) *SBEG significantly better than NCES and CES (both comparable) |
|                    | CES: 55% NCES: 53% SBEG: 76% of cases |                                                                                       |                   |                         |
| I year             | SV: 88% of cases ≥50% NSV: 70% of cases ≥50% Mixed: 33% of cases ≥50% SV > NSV Stable > active Collagen > Hyal A | ≥50% response H&N: 67/85 Extremity: 15/17 Trunk: 17/24 H&F: 9/14 | 12–60             | Color mismatch in 56% of cases Donor darker 14, HTS 8 Loss of pigmentation: 11.5% (activity) *Plus targeted phototherapy (45% of cases) |
|                    | Site did not affect response 30 lesions/group Most of lesions over extremities: 70% in NCES vs 93% ORSHFS |                                                                                       |                   |                         |
| I year             | Overall: 80% SV: 88% NSV: 80% (>90% in 12/20) | Sites treated: LL > H&N > trunk > hands > UL Response according to site not stated | 6                 | Color mismatch: darker in 75% of cases |
|                    | >90% response             | >90% response H&N: 27/57 Joints: 15/39 Extremity: 10/17 Trunk: 5/14 | 12–72             | Color mismatch 6% of cases P halo in 18% of NSV *67% (SV), 62% (NSV) continued improvement 12–24 months |
| 6 months           | SV and Focal: 58% NSV: 36% of cases |                                                                                       |                   |                         |
| >1 year            | Overall ≥90%: 6 cases 75%–50%/16 cases <50/: 15 cases | ≥90% response H&N: 2/6 Joints: 3/39 Extremity: 3/5 Trunk: 1/11 H&F: 5/88 Fingers: 10/24 | 18                | NCES: donor site scar in 12/31 cases Cryo: infection in 6/21 and longer healing time High patient satisfaction in 8/37 cases |

(Continued)
Table S1 (Continued)

| Authors                                      | Study design                      | No. of cases/| Vitiligo type | Skin type | Age | Technique | D:R ratio | R | Suspension | Area (cm²) |
|----------------------------------------------|-----------------------------------|--------------|---------------|-----------|-----|-----------|-----------|---|------------|------------|
| Benzeki and Gauthier                        | Prospective pilot study          | 5            | SV: 4         | Post halo nevus: I | Not stated | 20 | 1:1 | Dermroller (0.2 mm) | Cold trypsin | Patient plasma | 4–10       |
| Razmi et al                                 | Prospective comparative          | 5/12         | GV: 3         | Focal: I       | Not stated | 21–33 | ORSHFS and NCES mixed in a ratio of 1:5 vs NCES | DermA | Collagen sheets | 6–40       |
| Ramos et al                                 | Prospective                      | 20           | SV: 12        | II, III: 14   | 10–50 | Up to 1:10 | DermA | Collagen sheet |            |
| Lommerts et al                              | Prospective RCT                  | 10           | SV: 3         | Pieb: 7       | Not stated | 18–62 | 1:4 | CO₂ laser | ReCell Kit | 16          |
| Silpa-Archa et al                           | Comparative intrapatient         | 6/35         | SV: 2         | I, II: 2      | 20–65 | 1:10 | DermA vs FrCO₂ | Collagen dressing vs PG gauze | 21–204     |
| Kumar et al                                 | Prospective                      | 25/54        | SV: 8         | ACF: 12       | Not stated | 18–36 | 50 HF | DermA | DMEM + antibiotic, antifungal | Collagen dressing | Overnight cold incubation dispase II | Mean 86 |
| Orooji et al                                | Prospective                      | 300/1,060    | SV: 10        | ACF: 50       | Not stated | 12–71 | 1:3–1:10 | Intrallesional 0.05–0.1 mL, 0.5 cm apart | EDTA | Cells in saline +10% own serum |            |

Note: >95% not stated in manuscript.

Abbreviations: ACF, acrofacial vitiligo; BSA, body surface area; CES, cultured epidermal suspension; D, donor; DermA, dermabrasion; DLQI, Dermatology Life Quality Index; Ep5, epidermal suspension; FrCO₂, fractional carbon dioxide; GV, generalized vitiligo; HF, hair follicle; H&N, head and neck; Hyal A, hyaluronic acid; LL, lower limb; MKTF, melanocyte-keratinocyte transplantation procedure; NB-UVB, narrow-band ultraviolet B; NCeS, non-cultured epidermal suspension; NSV, non-segmental vitiligo; ORSHFS, outer root sheath hair follicle suspension; Pieb, piebaldism; P halo, perilesional hypopigmented halo; PUVA, psoralen and ultraviolet A; R, recipient; RCT, randomized controlled trial; SBEG, suction blister epidermal grafting; SV, segmental vitiligo; UL, upper limb; Mel, melanocytes; PUVA sol, Psoralen plus sun exposure; PG, petrolatum gauze; HTS, hypertrophic scar; Sol, solar light.

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### Table 1: Autologous non-cultured MKTP in the treatment of vitiligo

| Stability | Average % repigmentation | Response according to site | No. of lesions with ≥95% response/total no. (%) pigmentation | Follow-up (months) | Complications |
|----------|--------------------------|-----------------------------|--------------------------------------|------------------|---------------|
| I year   | >75% in 3 cases          | Face                        | 100% response total no. (%) pigmentation | 6                | *Plus NB-UVB  |
|          | 26–50% in 2 cases        |                             |                                      |                  |               |
|          | Control patch dermaroller only: 2 cases poor response |                             |                                      |                  |               |
| I year   | Combined: 94% (7/6 >90%) | Face: 0/2                   |                                      | 4                | Color mismatch: darker in 1 case only NCeS |
|          | NCeS: 78% (1/5 >90%)     | Extremity: 1/2               |                                      |                  |               |
|          |                           | Trunk: 1/2 lesions           |                                      |                  |               |
|          |                           | H&F: 3/6 lesions             |                                      |                  |               |
| I year   | SV: 72% (4/12 cases ≥90%)| H&N: 2/8 (80)               |                                      | 3–12             | Color mismatch: 3 cases (2 darker) Koebner donor 1 |
|          | NSv: 52% (1/8 cases ≥90%)| Joints: 0/4 (50)            |                                      |                  |               |
|          |                           | Extremity: 0/2 (80)         |                                      |                  |               |
|          |                           | Trunk: 0/5 (53)             |                                      |                  |               |
|          |                           | H&F: 0/2 (60)               |                                      |                  |               |
|          |                           | Genitals: 0/1 (0)           |                                      |                  |               |
| I year   | Full CO₂ at 209 μm: 69%  | Extremity: 6 cases          |                                      | 6                | Color match: 10% darker Scar 2 donor sites Mild erythema: 70% of 209 vs 50% 144 |
|          | Full CO₂ at 144 μm: 58% | Trunk: 2 cases              |                                      |                  |               |
|          | FrCO₂ and control: zero% | Joints: 2 cases             |                                      |                  |               |
|          |                           | Response according to site not stated |                  |                  |               |
| I year   | >90% response            | >90% response               |                                      | 3–11             | Color mismatch: darker (35%) FrCO₂, 18% dermA |
|          | SV: 2 DermA, 1 FrCO₂     | H&N: 3/6                    |                                      |                  | P halo: 12% DermA |
|          | GV: 3 DermA, 1 FrCO₂     | Joints: 2/6                 |                                      |                  | Scar: 1 case |
|          |                           | Extremity: 1/1              |                                      |                  | *Derma > FrCO₂ |
|          |                           | H&F: 4/8                    |                                      |                  | Dressings similar |
| 1.5 years| SV: 41%                  | H&N: 49% better than bony parts | 39% but not significant (P<0.6) | 6 | Color mismatch: 11/25 (darker) Infection: 4/25 |
|          | GV: 69%                  |                             |                                      |                  | *Plus phototherapy |
|          | ACF: 45%                 |                             |                                      |                  |               |
| I year   | >50% response at 12 months in 35% of lesions | H&N and trunk significantly better than extremities | 6–30 | Pigment loss in 20% of lesions mainly during the first year Recipient: ecchymosis Donor: 11% hyperpigmentation Keloid: 2 cases Koebner: 6 cases |

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