

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

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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 cryoblebs.

Several modifications aiming at simplifying the technique and improving the outcome were done over the years. Tissue was harvested from the gluteal area^{8,9} 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 researchers to search for a solution. In vivo preparation of epidermal cell suspension was introduced by Gupta et al¹⁰ 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.¹¹ 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 Hunziker¹² used plucked hair follicles for preparation of cell suspension which produced >90% repigmentation in 3/5 cases of vitiligo. Mohanty et al¹³ 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.¹⁴ 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.^{15,16}

Preparation of the suspension underwent major changes too. Olsson and Juhlin⁸ 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.³ He later replaced trypsin inhibitor by washing the epidermis several times in DMEM/F12 before separating it from the dermis.¹⁷ Later, PBS was used during suspension preparation to further cut the cost.¹⁸ Kumar et al¹⁹ 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 dermabrasion⁸ or laser resurfacing⁹ as opposed to cryotherapy⁷ 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 dermaroller²⁰ or fractional CO₂²¹ were recently used to introduce the cells into the skin to minimize the downtime. To improve cell handling, collagen sheets⁸ or hyaluronic acid (HA) was used to create a paste.⁹ 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.⁵ Our only available activity score, the vitiligo index of disease activity score,²² 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.²³ Other signs of activity include confetti lesions and trichrome vitiligo (Figure 1).²⁴

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,^{2,4,17,25,26} while others required 1 year of disease stability.^{8,9} In the early reports by Mulekar,^{2,4} 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),

Table 1 Different modifications in autologous non-cultured melanocyte–keratinocyte transplantation technique

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 cm ² 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 cm ² pieces and incubated at 37°C in 5% CO ₂ 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	Suspension covered by a thin collagen film, M2-moistened gauze and Tegaderm	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		Supplemented melanocytic medium: M199 medium supplemented with insulin, hydrocortisone, cholera toxin, penicillin/streptomycin and transferrin/triiodothyronine	CO ₂ laser resurfacing	0.5–1 mL of hyaluronic acid added to suspension to increase viscosity	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 In 2009, trypsin inhibitor step replaced by washing tissues	No supplements: DMEM/F12 only	High-speed dermabrader fitted with a diamond fraise wheel	Suspension covered by collagen film, DMEM/F12-moistened gauze and Tegaderm	Left immediately	Mulekar (2003) ² Mulekar (2004) ³ Mulekar (2005) ⁴ 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 viscid 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	Suspension spread and covered with a collagen dressing of fish origin	Not stated	Mohanty et al (2011) ¹³

(Continued)

Table 1 (Continued)

Number of days	Donor tissue	Trypsinization technique	Medium used for suspension	Recipient site preparation	Application of suspension	Patient immobilization	Reference
One	Suction at 380 mmHg in 5 mL syringe(s) applied to thigh to create suction blister(s). Each blister: 0.785 cm ² . Total donor 1/10 of recipient. Blister fluid aspirated and roofs removed	Blister roof incubated at 37°C in trypsin until a cloud of cells separated. Aspirated blister fluid added to act as trypsin inhibitor. Epidermal undersurface scraped and stratum corneum discarded	Ringer lactate added to the cell suspension and centrifuged at 2,000 rpm for 5 minutes. Cell pellet resuspended in 0.5–1 mL of Ringer lactate	CO ₂ or Er:YAG laser resurfacing	Suspension spread and covered with collagen dressing	Not stated	Jeong et al (2016) ¹¹
One	Combined epidermal suspension and ORSHFS. NCES: 1:10 ratio taken from thigh. ORSHFS: 15–25 HFUs extracted from the scalp	NCES: CO ₂ incubator used for 1 hour, followed by rinsing in PBS and cell separation. ORSHFS: as in Mohanty et al (2011) ¹³	NCES and ORSHFS centrifuged at 78 × g for 5 minutes and cell pellet resuspended in PBS and mixed in a ratio of 1:5	Manual dermabrasion extending 5 mm beyond lesion borders	Suspension spread and covered with collagen dressing	Not stated	Razmi et al (2017) ¹⁵
One	Superficial skin samples from the occipital scalp	Cold trypsinization followed by addition of patient's serum for trypsin inhibition	Suspension centrifuged and cell pellet resuspended in patient's serum	Suspension applied to intact skin followed by dermaroller	A dermaroller with 0.2 mm needles repeatedly passed over lesion for 10 minutes	Not stated	Benzekri and Gauthier (2017) ²⁰

Abbreviations: FBS, fetal bovine serum; HFUs, hair follicle units; NCES, non-cultured epidermal suspension; ORSHFS, outer root sheath hair follicle suspension.

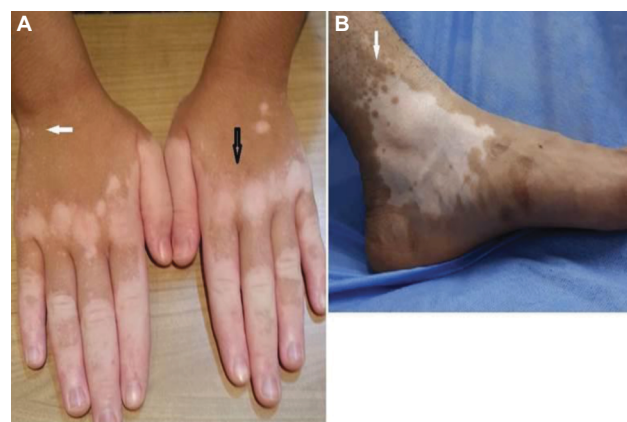


Figure 1 Clinical signs of disease activity.

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).

respectively. However, a high relapse rate was noted in 15 of those responders. Huggins et al²⁶ 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

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 ($\geq 95\%$ 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 study⁶ 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^{12,3,8,26,28,43} 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.^{2,5,6,8,17,25,27,30,31,34,36,38,41,43,46} Two studies focused solely on the treatment outcome in this age group. Mulekar et al²⁵ treated 25 children and adolescents using general anesthesia with $\geq 95\%$ 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.¹⁸ 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 two^{6,41} 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.^{6,7,26,37,46} 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 $\geq 95\%$ repigmentation.^{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 $\geq 95\%$ 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 al³⁵ 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 less^{5,18,29,30,38,46} of the lesions over the hands and feet showing $\geq 95\%$ 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 presternal lesions and those on the trunk and

extremities.⁶ On the contrary, some authors reported an excellent response of lesions overlying joints with $\geq 95\%$ 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.⁴⁶ Holla et al³⁵ 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 ($\geq 75\%$ repigmentation), color matching and side-effect profile. However more cases achieved excellent repigmentation ($\geq 90\%$) in the NCES group ($P=0.002$). Patient satisfaction and dermatological quality-of-life score reduction were also significantly higher in the NCES group.³⁴ 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 found^{29,36} despite the significantly higher total cell count yielded by NCES.²⁹ 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.

Anatomical site used

The density of melanocytes varies at different body sites from around 900 melanocytes/mm² on the back to around 1,500 melanocytes/mm² in the genital region.⁵⁰ The scalp was originally chosen for skin harvesting.⁷ This site has pros and cons. Being covered by hair, textural or color changes that may occur are unapparent, and being rich in hair follicles, the upper part of the outer root sheath is probably included in the sample. However, harvesting of skin necessitates shaving part of the scalp which may be inconvenient to the patient; also, the procedure is a bit more difficult as the scalp is curved and more vascular. The gluteal region^{3,4,8,9} or thigh^{18,35} is usually chosen as the donor area in MKTP. We found no significant difference in melanocytic cell count between both sites (unpublished data, Bassiouny et al, 2017). However, it is better to avoid the front of the thigh as a scar, color or textural change may occur.

Donor-to-recipient area ratio

Despite the fact that NCES can be prepared using donor tissue which is one-tenth the recipient area treated, a closer look at literature reveals that a donor area of one-third to one-fifth the recipient site was used in the majority of cases, with 1:10 ratio reserved for cases with a relatively large recipient area.^{3,4,8,9,17,18,33,40,45}

The ratio of donor to recipient areas will be reflected on the cell count and number of transplanted cells/cm². Olsson and Jhulin⁸ suggested that a melanocytes count of 190/mm² was the lower limit capable of producing repigmentation. In an interesting study of effect of donor-to-recipient area ratio on repigmentation, a significant difference in total and melanocytes cell counts as well as extent of repigmentation was

found between cases with a 1:3 and those with a 1:5 donor-to-recipient ratio. The authors concluded that the minimum number of melanocytes in epidermal cell suspension required to produce satisfactory repigmentation (>75%) was probably in the range of 210–250 cells/mm².⁴⁷

Cell viability is another important factor. As expected, a positive correlation was found between the percentage of repigmentation and the total number of all viable cells and viable melanocytes transplanted.⁴⁵

Regarding ORSHFS, a lower mean number of melanocytes of 119 cells/mm² was associated with optimum pigmentation which may be due to the different morphology and ultrastructural characteristics of hair follicle melanocytes. A count of 76 cells/mm² CD200+ stem cells was present in cases achieving >75% repigmentation with a significant positive correlation between repigmentation rate at 6 months and both melanocyte and hair follicle stem cell counts.³⁸ No similar correlation between percentage of CD200+ cells and clinical repigmentation was found in a more recent study.⁴⁴

Recipient site preparation

Recipient vitiligo skin may be prepared by different methods including cryoblebbing,^{7,29,30,47} dermabrasion^{2–4,8,15–18,25–28,33–38,41,43,44,46,51} 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,²¹ 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 Gauthier²⁰ 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. Intraleisional 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.²¹ 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.⁷ Over the years, several studies used post-transplantation phototherapy^{5,20,29,30,40,44} or sun exposure^{18,43} 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 CO₂ 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 μm vs 70% of lesions at 209 μm 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%^{3–5,15,18,26,33,34,43} to $>50\%$ 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 improving⁶ or worsening² 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

Paul³³ 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 al¹⁸ 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%–25% of cases in several studies.^{3,27,33,41} Extending the dermabrasion 2–3 mm into normal skin decreased the occurrence of this complication.³³

Scars or textural change

Textural changes or scar may occur at the donor site,^{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.^{3,6} Repigmentation was maintained in 19/23 (83%) treated cases after 5 years of follow-up in one study.⁴⁰ 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%,⁴ 16%,⁶ 11%⁴⁰ and 21% of cases²⁷ 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.⁶ More pigmentation was reported in 67% and 62% of treated areas in SV and NSV cases, respectively, during 12–24 months of follow-up.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% repigmentation of hair occurred in 58% (10/17), 28% (2/7) and 12% (2/16) of lesions over the trunk, scalp and face, respectively, after MKTP.⁵⁵ 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.⁵⁶ Similar improvement was reported by other authors.^{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 periangular 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% 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/mm² 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, Bassiouny 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.

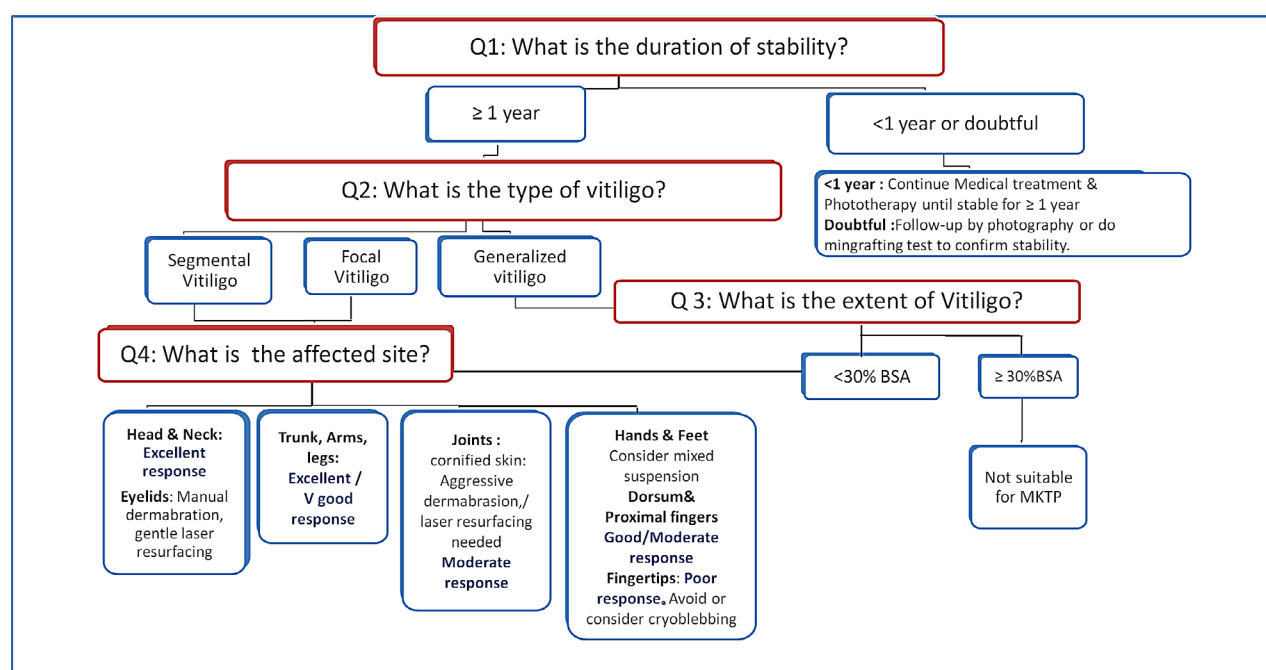


Figure 2 How to proceed in a case of vitiligo resistant to medical therapy in whom MKTP is considered?
Abbreviations: BSA, body surface area; MKTP, melanocyte–keratinocyte transplantation procedure.

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/ no. of lesions	Vitiligo type	Skin type	Age	Technique			Area (cm ²)
					D:R ratio	R	Suspension	
Gauthier and Surleve-Bazeille ¹ Prospective	12	SV: 3 NSV, all focal: 8	Not stated	20–65	D: ≥2 cm ² R: ≤50 cm ²	Cryo	Cold trypsin Saline	4–50
Olsson and Juhlin ² Prospective	20	SV: 3 NSV: 17	Not stated	13–52	1:4–10	Derma	Mel medium Collagen film	7–194
van Geel et al ³ Prospective pilot study	4	SV: 2 NSV: 2	III: 2 IV: 2	30–52	1:2–4	CO ₂ laser	Hyal A Mel medium	36–110
Olsson and Juhlin ⁴ Retrospective	132 CES: 5 EpS: 1 NCES: 8	SV: 15 GV: 107 Focal: 2	Not stated	12–61	CES: 8 cm ² NCES: 10 cm ²	Derma	Collagen dressing or silicone netting	CES: 60–500 EpS and NCES: not stated
Muleker ⁵ Prospective	184	SV: 43 GV: 122 Focal: 19	Not stated	12–70	1:10	Derma	Ordinary incubator Collagen film	Not stated
van Geel et al ⁶ Prospective double blinded placebo controlled	28/66	NSV (19 stable and 9 active)	II–IV: 25	15–65	1:1	CO ₂ laser	Hyal A Mel medium	0.2–8.9
Mulekar ⁷ Prospective	64	SV: 49 Focal: 15	Not stated	>12	1:3–10	Derma	DMEM/F12 medium Collagen film	1–120
Mulekar ⁸ Prospective	142	GV	Not stated	18–70	1:10	Derma	DMEM/F12 medium Collagen sheet	2–298
Pandya et al ⁹ Prospective controlled	27	SV: 2 NSV: 25	Not stated	>8	1:10	Derma	Supplemented medium/collagen dressing	Not stated
Tegta et al ¹⁰ Prospective comparative	20 Two different cellular dilutions	SV: 4 GV: 11 Focal: 5	Not stated	10–54	1:3 vs 1:5	Blister (suction, liquid N ₂ or UVA)	Injection into blister from floor	6–24
Mulekar et al ¹¹ Prospective	49	SV: 9 NSV: 40	Not stated	7–65	1:3–10	Derma	DMEM/F12 medium Collagen sheet	Not stated

Stability	Average % repigmentation	Response according to site No. of lesions with $\geq 95\%$ response/total no. (% pigmentation)	Follow-up (months)	Complications *Remarks
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 Except 2 cases	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 *Patient rested 4–5 hours after the procedure
>1 year	SV: 90% NSV: 88%	Face: 1/2 (92) Presternal: 0/2 (88)	6–20	Color mismatch (disappeared in 5 months)
Not stated	SV: $\geq 95\%$ 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 Pieb: 5 All $\geq 95\%$ response
6 months	SV: 92% GV: 57% Focal: 68%	Not stated	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)	Joints: 5/18 (70) Extremity: 1/20 (88) Trunk: 4/12 (72) H&F: 0/10 (37) Fingers: 0/6 (0)	3–12	Color mismatch (darker): 5/66 lesions improved in 6 months *Combined by NB-UVB or PUVA
1 year	SV: $\geq 95\%$ in 41/49 Focal: $\geq 95\%$ in 11/15 cases	H&N: 26/34 Joints: 1/1 Extremity: 6/7 Trunk: 13/14 H&F: 3/3	12–60	Color mismatch (lighter in 8 cases, darker in 1 case) P halo in 4 cases New lesions in 2 cases
6 months	$\geq 95\%$ in 80 cases; $\geq 65\%$ in 15	H&N: 19/49 Joints: 64/119 Extremity: 54/84 Trunk: 19/35 H&F: 40/64 Fingers: 12/18	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	H&N: 2/2 Joints: 1/2 Extremity: 6/17 Trunk: 1 H&F: 4/28	6	Infection: 7% of donor, 11% of recipient sites Koebner donor 1 *CES: 4 cases (cells $< 1,000/\text{mm}^2$)
1 year	>75% response 5/10 cases of 1:3 ratio 0/10 cases of 1:5 ratio	Not stated	3	Color mismatch: darker in 3 cases, lighter in 8 cases *Vitiligo >10% BSA excluded
6 months	SV: $\geq 95\%$ in 3/9 lesions NSV: $\geq 95\%$ 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)

Table S1 (Continued)

Authors Study design	No. of cases/ no. of lesions	Vitiligo type	Skin type	Age	Technique			Area (cm ²)
					D:R ratio	R	Suspension	
Mulekar et al ¹² Retrospective	25	SV: 13 Focal: 12	Not stated	4–16	1:3–10	DermaA	DMEM/F12 medium Collagen sheet	4–123
van Geel et al ¹³ Retrospective	87	SV: 33 NSV: 33 Mixed: 6 Others: 15	II, III: 77 IV–VI: 10	9–65		CO ₂ laser	Hyal A Mel medium	Not stated
El-Zawahry et al ¹⁴ Prospective	25	SV: 2 NSV: 23 (2 focal)	Not stated	8–45	1:10	Cryo	Mel medium Hyal A	Not stated
Toossi et al ¹⁵ Prospective controlled	8/14	NSV	Not stated	13–43	1:5–10	DermaA	DMEM/F12 Collagen sheet	1–7
Sahni et al ¹⁶ Prospective	13/19	SV: 6 GV: 6 Focal: 1	Not stated	8–17	1:2–10	DermaA	PBS Cold trypsin	3–200
Vasquez-Martinez et al ³² Prospective comparative	11	Not stated	Not stated	35–48	1:10	DermaA	DMEM/F12 Collagen dressing	Not stated
Paul ¹⁸ Retrospective	49	Not stated	Not stated	Not stated	1:5–1:10	DermaA Er:YAG on eyelids	DMEM/F12 Collagen sheets	2–230
Mohanty et al ¹⁹ Prospective	14	SV: 3 GV: 8 ACF: 3	Not stated	17–32	15–25 HF	DermaA	DMEM/F12 Collagen dressing	4–96
Huggins et al ²⁰ Prospective	23/29	SV: 2 GV: 15 Focal: 6	II–III: 12 IV–VI: 11	18–60	1:10	DermaA	DMEM/F12 Collagen dressing	1–116
Budania et al ²¹ Prospective comparative	41/54 NCES (21/28) vs SBEG (20/26)	SV: 16 GV: 15 Focal: 10	Not stated	12–40	NCES 1:10	DermaA	CO ₂ incubator PBS Collagen dressing	3–35
Holla et al ²² Retrospective	36/80	GV: 33 Focal: 3	Not stated	16–47	1:10	DermaA	Cold trypsin ±Mel medium, serum	Not stated

Stability	Average % repigmentation	Response according to site No. of lesions with $\geq 95\%$ 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 O ₂ by face mask
No Koebnerization	77% SV: 85% NSV: 70% Mixed: 37% Stable (89%) vs active (48%)	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)
>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	58% (≥ 95 in 4 lesions, ≥ 65 in 1 lesion) 6 lesions Derma only: no response	Joints: 1/3 Extremity: 1/2 Trunk: 1/1 H&F: 1/2	4–6	Infection 1 case
>1 year	>90% response ^a SV: in all cases GV: in 8/12 lesions Focal: in 1 case	>90% response H&N: 7/8 Joints: 4/4 Extremity: 1/1 Trunk: 3/3 H&F: 0/2	12	Infection: 1 case Color mismatch: 3 lesions *Combined with sun exposure
>1 year	No significant difference in area of repigmentation Derma + NCES vs Derma	Trunk: 64% Extremity: 27% Face: 9%	12	*Image analysis for % repigmentation
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 <1 year: 3 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	
6 months	SV: 1/2 $\geq 95\%$, GV: 2/15 $\geq 95\%$ Focal: 1/6 $\geq 95\%$	H&N: 3/12 Joints: 1/6 Extremity: 1/9 Trunk: 1/5 H&F: 1/6	3–6	Color mismatch lighter: 5
1 year	$\geq 90\%$ 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)
>1 year	>75 in 51/80 50–75 in 23/80 <50 in 6 (2 ankles, 4 distal fingers)	>75% response Joints: 21/33 H&F: 22/28 Fingers/toes: 8/19	6–18	Color mismatch *Strict immobilization (sometimes plaster casts)

(Continued)

Table S1 (Continued)

Authors Study design	No. of cases/ no. of lesions	Vitiligo type	Skin type	Age	Technique			Area (cm ²)
					D:R ratio	R	Suspension	
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	DermaA	CO ₂ 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	DermaA Manual	Cold trypsin ± Mel medium, serum	Not stated
Verma et al ²⁵ Prospective comparative	25 CES (6) vs NCES (19)	SV: 2 NSV: 20 Focal: 3	Not stated	Not stated	NCES 1: 10 CES 1: 100	DermaA	Collagen dressing	Not stated
Vinay et al ²⁶ Prospective	30/60	SV: 2 GV: 11 Focal: 17	Not stated	8–38	ORSHFS	DermaA	Collagen sheet	<100 cm ²
Komen et al ²⁷ Prospective RCT	10	SV: 5 Pieb: 5	Not stated	34	1:5	CO ₂ 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	CO ₂ 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	CO ₂ 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	DermaA	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	DermaA	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	DermaA (CO ₂ 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/cm ²	CO ₂ laser vs cryo No significant difference (P=0.3)	NCES vs ORSHFS No significant difference (P=0.6)	5–160

Stability	Average % repigmentation	Response according to site No. of lesions with ≥95% response/total no. (% pigmentation)	Follow-up (months)	Complications *Remarks
1 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	Not stated improvement according to vitiligo type	Leucotrichia improved >90% Scalp: 2/7 lesions Face: 2/16 lesions Body: 10/17 lesions	6–12	*20 min/day sun Methylcobalamine
1 year ≤30% BSA	>70% response NCES: 62% CES: 52%	Face, lips, trunk and legs better response than acral and joint lesions	6	Infection: 1 NCES, 5 CES Relapse: 2 cases CES: 100–200/cm ² *Plus PUVA sol
1 year	>90% response SV: 1/3 lesions GV: 1/25 lesions Focal: 8/32 lesions	>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%)
Not stated	Median response: 78% in NCES 0% in CO ₂ only 0% in control	Not stated	6	Mild textural change in 2 donor sites
1 year	≥90% response CES: 55% NCES: 53% SBEG: 76% of cases	≥90% response CES, NCES, SBEG H&N: 24, 22, 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)
1 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)
1 year	>75% repigmentation in 90% of NCES vs 43% in ORSHFS	Site did not affect response 30 lesions/group Most of lesions over extremities: 70% in NCES vs 93% ORSHFS	6	Color mismatch in ORSHFS P halo in NCES
1 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
6 months	>90% response SV and Focal: 58% NSV: 36% of cases	>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
>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/ no. of lesions	Vitiligo type	Skin type	Age	Technique			Area (cm ²)
					D:R ratio	R	Suspension	
Benzekri and Gauthier ³⁴ Prospective pilot study	5	SV: 4 Post halo nevus: 1	Not stated	20	1:1	Derma roller (0.2 mm)	Cold trypsin Patient plasma	4–10
Razmi et al ⁴⁰ Prospective comparative	5/12	GV: 3 Focal: 1 ACF: 1	Not stated	21–33	ORSHFS and NCES mixed in a ratio of 1:5 vs NCES	Derma	Collagen sheets	6–40
Ramos et al ³⁵ Prospective	20	SV: 12 GV: 7 ACF: 1	II, III: 14 IV: 6	10–50	Up to 1:10	Derma	Collagen sheet	
Lommerts et al ³⁶ Prospective RCT	10 Full (2 levels) vs FrCO ₂	SV: 3 Pieb: 7	Not stated	18–62	1:4	CO ₂ laser	ReCell Kit	16
Silpa-Archa et al ³⁷ Comparative inpatient	6/35	SV: 2 GV: 4	I, II: 2 IV, V: 4	20–65	1:10	Derma vs FrCO ₂	Collagen dressing vs PG gauze	21–204
Kumar et al ³⁸ Prospective	25/54	SV: 8 GV: 5 ACF: 12	Not stated	18–36	50 HF	Derma	DMEM + antibiotic, antifungal Collagen dressing	Not stated
Orouji et al ³⁹ Prospective	300/1,060	SV: 10 GV: 231 Focal: 59	Not stated	12–71	1:3–1:10	Intralesional 0.05–0.1 mL, 0.5 cm apart	Overnight cold incubation dispase II second day trypsin EDTA Cells in saline +10% own serum	Mean 86

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; EpS, epidermal suspension; FrCO₂, fractional carbon dioxide; GV, generalized vitiligo; HF, hair follicle; H&F, hands and feet; H&N, head and neck; Hyal A, hyaluronic acid; LL, lower limb; MKTP, 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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Stability	Average % repigmentation	Response according to site No. of lesions with $\geq 95\%$ response/total no. (% pigmentation)	Follow-up (months)	Complications *Remarks
1 year	>75% in 3 cases 26%–50% in 2 cases Control patch dermaroller only: 2 cases poor response	Face all cases	6	*Plus NB-UVB
1 year	Combined: 94% (7/6 >90%) NCES: 78% (1/5 >90%)	Face: 0/2 Extremity: 1/2 Trunk: 1/2 lesions H&F: 3/6 lesions	4	Color mismatch: darker in 1 case only NCES
1 year	SV: 72% (4/12 cases $\geq 90\%$) NSV: 52% (1/8 cases $\geq 90\%$)	H&N: 2/8 (80) Joints: 0/4 (50) Extremity: 0/2 (80) Trunk: 0/5 (53) H&F: 0/2 (60) Genitals: 0/1 (0)	3–12	Color mismatch: 3 cases (2 darker) Koebner donor 1 * >30% BSA excluded, plus sun exposure
1 year	Full CO ₂ at 209 μm : 69% Full CO ₂ at 144 μm : 58% FrCO ₂ and control: zero%	Extremity: 6 cases Trunk: 2 cases Joints: 2 cases Response according to site not stated	6	Color match: 10% darker Scar 2 donor sites Mild erythema: 70% of 209 vs 50% 144
1 year	>90% response SV: 2 Derma, 1 FrCO ₂ GV: 3 Derma, 1 FrCO ₂	>90% response H&N: 3/6 Joints: 2/6 Extremity: 1/1 H&F: 4/8	3–11	Color mismatch: darker (35% FrCO ₂ , 18% derma) P halo: 12% Derma Scar: 1 case *Derma >FrCO ₂ Dressings similar
1.5 years	SV: 41% GV: 69% ACF: 45%	H&N: 49% better than bony parts 39% but not significant ($P=0.6$)	6	Color mismatch: 11/25 (darker) Infection: 4/25 *Plus phototherapy
1 year	>50% response at 12 months in 35% of lesions GV better than SV	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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