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ORIGINAL STUDY

Treatment of Postburn Hypopigmentation with Non-cultured Epidermal Grafting

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Abstract

Objectives: **To evaluate the applicability of cell therapy using non-cultured epidermal transplantation regarding the aesthetic outcome, and possible complications in the treatment of postburn hypopigmentation.**

Background: **New non-cultured autologous epidermal techniques have been developed with similar results to the cultured melanocytes techniques, but are simpler, less expensive, and less time-consuming.**

Methods: **This prospective study was conducted on 35 postburn hypopigmentation patients presented at the Plastic and Reconstructive Surgery Department in Menoufia University Hospitals between April 2020 and April 2023.**

Result: **We found that the repigmentation ranged between 20 and 95 %, and the color matching ranged between 30 and 80 %. Most cases had good repigmentation, 60 %, while 22.9 % had fair repigmentation. Excellent repigmentation was detected in 14.3 %, while 2.9 % had poor repigmentation. There is a significant association between the development of complications and the burn site (more in the face and neck), the presence of obstacles, and the severity of the burn (more in full thickness than partial thickness burn). Also, there is a statistically significant association between the development of complications and poor outcomes in the studied cases. There is no statistically significant association between obstacles and poor outcomes in the studied patients. There is no significant correlation between the repigmentation% and the clinical data of the studied population.**

Conclusions: **Cell therapy using non-cultured epidermal transplantation effectively treats postburn hypopigmentation.**

Keywords: Burn, Cell therapy, Epidermal grafting, Hypopigmentation, Transplantation

1. Introduction

Surgical methods in managing leucoderma (both vitiliginous and nonvitiliginous) have a well-documented and established role in managing resistant cases. The basic principle of all surgical methods is the transfer of melanocytes from uninvolved skin into a stable leucoderma lesion, where they grow into and function as effective epidermal-melanin units. Although noncellular grafts such as split thickness, suction blister, and punch grafts are the mainstay of surgical management, several cellular methods have become increasingly popular in recent times [1,2].

Surgical techniques have proven to be effective in stable cases, and melanocyte transfer may be performed by punch mini grafting, split-thickness skin grafting, hair follicle transplantation, suction blisters, epidermal curettage techniques, and cultured and non-cultured autologous melanocytes. The previous surgical and cultured techniques can be time-consuming and, in some cases, aesthetically unsatisfying or painful for the patients [3].

Melanocytes play a crucial role in determining skin pigmentation by producing and distributing melanin to neighboring keratinocytes. Melanocytes are specialized cells responsible for producing and distributing the pigment melanin, which is crucial in determining skin color, hair, and eyes. In the skin, melanocytes are primarily located in the epidermis, the outermost layer of the skin. Their location in the basal layer of the epidermis and their interaction with other skin cells are essential for maintaining the color and tone of the skin [4]. It is important to note that melanocytes are not limited to the basal

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layer of the epidermis. Melanocytes are also present in some regions of the body, such as the hair follicles. These specialized melanocytes contribute to hair pigmentation by transferring melanin to the growing hair shaft [5].

In recent years, new non-cultured autologous epidermal techniques have been developed with similar results to the cultured melanocyte techniques, but they are simpler, less expensive, and less time-consuming. These techniques do not require a laboratory but only a split-thickness thin biopsy that is pretreated with trypsin solution [6].

Indications for cellular grafts are similar to those used for tissue methods. The commonest indication is stable vitiligo, refractory to standard medical and physical treatment modalities. It is particularly useful as a primary treatment for segmental and focal vitiligo [7,8]. Therefore, in this study, we aimed to evaluate the applicability of cell therapy using non-cultured epidermal transplantation regarding the aesthetic outcome and possible complications in the treatment of postburn hypopigmentation.

2. Methods

This prospective study included 35 cases of postburn hypopigmentation. It was conducted at the Plastic and Reconstructive Surgery Department in Menoufia University Hospitals from February 2020 to April 2023. Informed consent was taken from all patients regarding the information on the procedures and the possible complications. Approval was obtained from the ethical committee of the scientific research of the faculty of medicine, Menoufia University, under code no. (2/2020surg21).

All the patients underwent full history taking, including (personal, present, past medical and surgical history, and family history). Complete clinical examination: general and local examination (determine site, size, and shape of the lesion). Investigations: (complete blood count, SGOT, SGPT, urea, creatinine, PT) for routine preoperative evaluation.

The inclusion criteria for the present study were patients with postburn hypopigmentation in any area of the body. Exclusion Criteria were patients who underwent previous grafting over the hypopigmented area, associated skin lesions, and immunocompromized patients (diabetes mellitus, hypertension, cancer patients, previous strokes).

2.1. Methods of harvesting

The entire procedure consisted of harvesting the donor skin sample, preparation of cell suspension and recipient area, and transplantation were performed in a clean procedure room no special laboratory setup was used for the cell separation procedure. The procedure was done under adequate sterilization.

2.2. Harvesting donor skin sample

The donor area of one-tenth of the recipient area was marked on the lateral aspect of the gluteal region of the thigh and was anesthetized with a 1 % lidocaine injection. The skin was stretched, and a very superficial skin sample was taken with a skin graft knife superficial wound with a thickness of about 0.1–0.8 mm, then covered with the appropriate coverage.

2.3. Preparation of cell suspension

The thin skin sample was transferred to a Petri dish containing ~5 ml of 0.25 % (w/v) trypsin solution. The sample was incubated for 25 min at 37 °C in an incubator. After incubation, the Petri dish was taken out, and the contents were removed with a Pasteur pipette. The skin sample was washed with a Dulbecco Modified Eagle medium F12 (Sigma, Japan) to remove traces of trypsin. The dermis was separated from the epidermis. The dermis was then discarded, and then the epidermis was broken into multiple pieces with tweezers the epidermal pieces were then transferred to a 15 ml centrifuge tube and centrifuged at 2000 rpm for 5 minutes. The epidermal cells settle at the bottom of the centrifuge tube in the form of a pellet. The size of the recipient area determined the quantity of the suspension that was then prepared.

2.4. Preparation of the recipient area

The recipient site was cleaned with povidine iodine and 70 % ethanol and anesthetized with lidocaine 1 %. Then dermabrasion was done using a motor dermabrader to a level indicated by pinpoint bleeding spots. The speed of the motor dermabrader varied from 15 000 rpm (for sites such as the dorsum of feet) to 5000 rpm (for sites such as lips). The denuded area was then covered with a sterile gauze piece moistened with isotonic sodium chloride solution until the transplantation of the cell suspension.

2.5. Transplantation of the cell suspension

The cell suspension was applied to the dermabraded recipient area and covered with a non-adherent surgical dressing. A secondary dressing

with sterile gauze pieces secured with micropore adhesive tape was applied, and the dressing was removed after one week. Absolute immobilization was not necessary.

2.6. Outcome measurement

Preoperative and postoperative size evaluation Image processing software (imagine-Pro Plus) was applied to measure preoperative and postoperative depigmentation at three months. The Kahn grading system was also used to evaluate the postoperative results [9].

2.7. Statistical analysis

Data collected were reviewed, and the collected data was coded using IBM SPSS statistics software version 28.0 (IM Corp., Armonk, NY, USA). Quantitative data were tested for normality using the Shapiro–Wilk test, then described as mean ± SD (standard deviation) and minimum and maximum of the range, and compared using the ANOVA test. Qualitative data were described as numbers and percentages and compared using the χ^2 and Fisher's exact tests for variables with small expected numbers. The Bonferroni test was used for post hoc comparisons. The significance level was taken at a *P*-value of less than 0.050, which was significant. Otherwise, nonsignificant.

3. Results

The current study included 35 burn patients aged between 19 and 49 years with a mean value of 32.40 ± 7.46. 40 % were females, and 60 % were males. Among our studied 35 patients, the preoperative dyspigmentation ranged between 6.2 and 24.5 cm^2 with a mean value of 14.2 ± 4.65 cm^2, and the postoperative dyspigmentation we were ranged between 2.36 and 16.17 cm^2. The repigmentation% ranged between 20 and 95 %, and the color matching% ranged between 30 and 80 % (Fig. 1, Table 1). Most cases had good repigmentation, 60 %, while 22.9 % had fair repigmentation (Fig. 2, Table 1). Excellent repigmentation was detected in 14.3 %, while 2.9 % had poor repigmentation. There is a significant association between the development of complications and the burn site (more in the face and neck), the presence of complications, and the severity of the burn (more in full thickness than partial thickness burn). There is a statistically significant association between the development of complications and poor outcomes in the studied cases. No statistically significant association exists

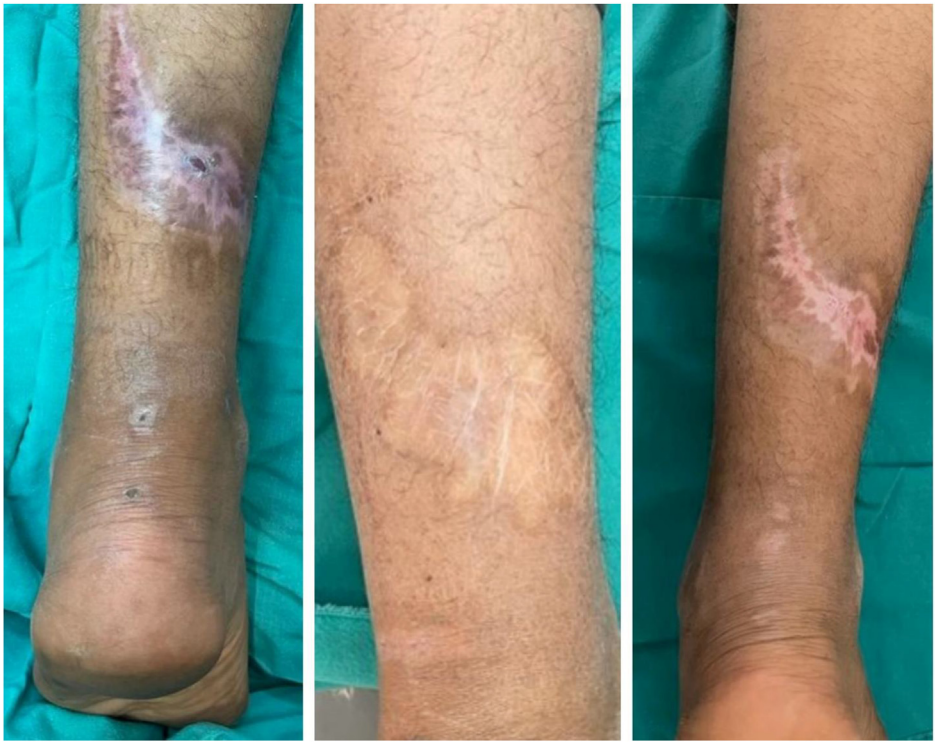

Fig. 1. A Preoperative post burn hypopigmentation on left lower limb, B First dressing on the Seventh Postoperative day, C, A 3-month follow-up indicating Excellent repigmentation.

Table 1. The repigmentation of the studied population.

	Minimum	Maximum	Mean	SD
Preoperative dyspigmentation area size (cm^2)	6.2	24.5	14.20	4.65
Postoperative dyspigmentation area size (cm^2)	2.36	16.17	8.87	3.85
Re-pigmentation%	20	95	61.49	16.25
Color matching%	30	80	56.71	12.00

between complications and poor outcomes in the studied patients. There is no significant correlation between the repigmentation% and the clinical data of the studied population (Fig. 3).

4. Discussion

The current study included 35 postburn patients; their ages ranged between 19 and 49 years, with a mean value of 32.40 ± 7.46. About 40 % of the patients were females, and 60 % were males.

While in the study of Busch et al. [10], 20 patients are included. Fifteen subjects were women, and five were men. The average age was 33 years, ranging from 6 to 60 years. Whereas, in the study of Shraddhamayananda [11], most of the patients aged between 11 and 30 years (64.0 %), with slightly higher (56.0 %) partaking of female patients.

The present study showed that among our studied 35 patients, the most frequent site of burn was the trunk and distal extremities at 31.4 %, proximal extremities in 28.6 %, face at 5.7 %, and neck at 2.9 %. 51 % had full thickness, and 48.6 % had partial thickness burn. 40 % had obstacles in the form of contracture in 11.4 % and keloid scarring in 28.6 % of cases. The mean value of the burn duration was 3.51 ± 1.30 years.

However, in the study of Chen et al. [12], most cases had lesions in the face with a mean duration of 7 years.

Dyspigmentation, in either hyperpigmentation or hypopigmentation, is often a serious psychological problem for patients' self-image. This is especially the case after a burn injury. The most common alteration in skin color occurs due to changes in the epidermal melanin or the underlying vascular bed.

Fig. 2. A. A 12-year-old female presented postburn hypopigmented area on the lateral aspect of left thigh. B. First dressing on the seventh postoperative day indicating good pigmentation.

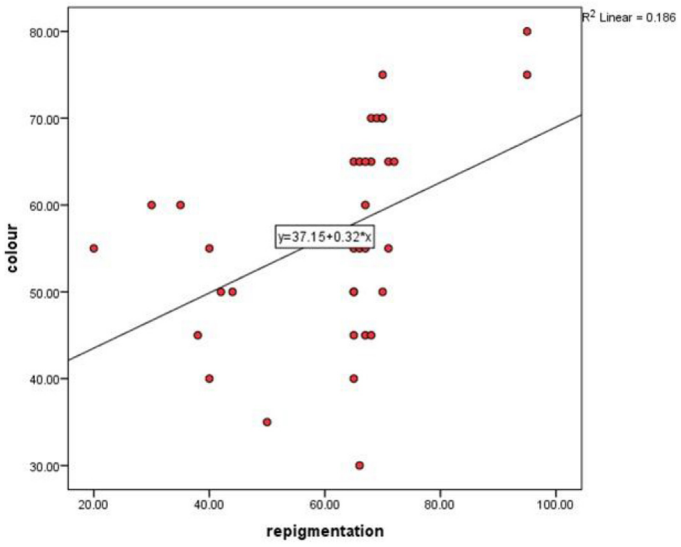

Fig. 3. Correlation between the color matching % and repigmentation.

Deep burns often result in hypopigmentation, referred to as leucoderma, which has a similar psychosocial impact on the patients as that of vitiligo. It is especially distressing for black-skinned individuals. After a burn injury, depigmented skin has been reported to contain little melanin pigment in the basal cells and marked thickening of all skin layers [13].

Several techniques have been described for the treatment of postburn leukoderma with variable outcomes. Small lesions can be peeled off and replaced with an epidermal or conventional sheet skin graft. Such lesions are often extensive donor site is needed. Other techniques have been described for the treatment of postburn leukoderma, such as split-thickness skin grafting, chip skin grafting, sheet grafting, and cultured epithelial autografts in addition to cosmetic camouflage and tattooing [14].

The current study showed that regarding the outcome, among our studied 35 patients, the preoperative dyspigmentation ranged between 6.2 and 24.5 cm^2 with a mean value of 14.2 ± 4.65, and the postoperative dyspigmentation ranged between 2.36 and 16.17 cm^2. The repigmentation% ranged between 20 and 95 %, and the color matching% ranged between 30 and 80 %. Most cases had good repigmentation, 60 %, while 22.9 % had fair repigmentation. Excellent repigmentation was detected in 14.3 %, while 2.9 % had poor repigmentation. According to Kahn's grading system, 40 % were poor, 28.6 % were fine, 25.7 % were good, and 5.7 % were excellent. Complications occurred in 28.6 % of cases in the form of atrophy in 14.3 %, scare in 11.4 %, and infection in 2.9 %.

Busch et al. [10] revealed that the patients evaluated the color of the scars treated with needling and non-cultured autologous skin cell suspension preoperatively with a median of 8.0 ± 2.1 SD (standard deviation) points. Postoperatively they rated 4.0 ± 2.5 SD points which is an improvement of 50 % and statistically significant with P less than 0.05. Additionally, the patients rated the overall opinion of their scars preoperatively with a median of 7.0 ± 2.6 SD points. Postoperatively they evaluated 3.0 ± 2.3 points which is an improvement of 57.1 %. With P less than 0.05, the improvement is statistically significant.

Also, our results were supported by a study by Chen et al. [12]. They conducted their study to design to generalize the experience of tiny epidermal particles graft (TEPG) for treating postburn depigmentation. From 2012 to 2013, 30 consecutive patients with depigmentation caused by burn injuries were divided into two groups. Group (I): 15 cases (11 males and 4 females) were treated by the TEPG. Group (II): 15 patients (10 males and five females) were treated by suction blister epidermal skin graft (SBEG). The size of preoperative dyspigmentation was 14·62 ± 3·28 (cm^2) in group I and 14·29 ± 3·13 (cm^2) in group II ($P > 0·05$). The size of repigmented sites three months postsurgery was 13·36 ± 3·02 (cm^2) in group I and 9·64 ± 2·96 (cm^2) in group II ($P = 0·002 < 0·05$). No significant difference was found in the gender ($P = 0·690$), location ($P = 0·549$), duration of depigmentation (0·700), and old proportion ($P = 0·388$). According to the Kahn grading system, 86 % of Group I and 73 % of Group II scored excellently, while 7 % of Group I and 7 % of Group II obtained good scores. With 7 %

of Group I and 20 % of Group II in the fine score, there were no poor scores in the two groups. Optimum repigmentation (RP > 75 %) was seen in 12 patients in group (I) and nine patients in group (II). Excellent repigmentation was achieved in 14 cases in group (I) and 10 patients in group (II). The biopsies of the preoperative depigmented area and postoperative depigmented area at 12 months were performed. Pathological results showed that melanocytes existed at the basal layer of resurfacing skin. In their study, the technique of TEPG is found to produce a larger size of excellent repigmentation than SBEG. The following hypotheses may explain the obtained results: (i) the communication between keratinocytes and melanocytes is essential for the melanin depositing and transferring process. However, the epidermal graft would be off 1-week postsurgery, with no keratinocytes remaining virtually, while in TEPG, the two cells coexist and help each other to generate excellent repigmentation; (ii) it is harmful to the keratinocytes and melanocytes in SBEG under the condition of continuous suction for a long time (about 1·5–2 h). We designate the time to reduce the cell loss rate as 40 minutes. However, the rate of repigmented area in SBEG is still lower than in TEPG; (iii) the contact epidermal graft in SBEG cannot diffuse freely like a tiny broken graft. Because of the even transfer to the recipient area in TEPG, there may be no obstacle to cell migration. In the study of Mulekar et al. [13], patients were treated with 18 sessions of excimer laser starting one month postoperatively to hasten the repigmentation. Three of the ten patients treated with melanocyte-keratinocyte transplantation (MKTP) were lost to follow-up. The remaining seven patients showed repigmentation ranging from 90 % to 100 % with good color matching. MKTP is an effective method to treat post-burn leucoderma. No special precautions are required to treat any anatomical site or uneven scarred surface.

Pigmentation change is an undesirable consequence of cutaneous wound healing after a deep burn injury. Although there are several reports on hypopigmentation, repigmentation of the hypopigmented lesion is still a big challenge. Several clinical trials in this aspect were undertaken, but most of them either led to disappointing results or ended up with other complications [15].

The present study had some limitations. The relatively small sample size is the main limitation. Also, the lack of a control group is another limitation. It is a single-center study. In addition, lack of multiple previous similar studies to be compared with.

4.1. Conclusion

From the findings of this study, we can conclude that cell therapy using non-cultured epidermal transplantation is effective in the treatment of postburn hypopigmentation. There is a significant association between the development of complications and the site of the burn (more in the face and neck), the presence of obstacles, and the severity of burn (more in full thickness than partial thickness burn) Tables 2 and 3.

Table 2. Complications of the clinical data between complicated and noncomplicated cases.

	Complications ($n = 10$)		No complications ($n = 20$)		Independent student t/chi square test	
	Mean	SD	Mean	SD	t	*P*-value
Age	30.80	7.73	33.04	7.41	−0.784	0.445
BMI	28.90	4.20	27.24	4.61	1.026	0.318
Duration of burn	3.50	1.43	3.52	1.29	−0.038	0.970
	***N* (%)**		***N* (%)**		**X2**	***P*-value**
Sex						
Male	7 (70 %)		14 (56 %)		0.583	0.445
Female	3 (30 %)		11 (44 %)			
Site						
Face	2 (20 %)		0			
Trunk	3 (30 %)		8 (32 %)			
Distal extremities	1 (10 %)		10 (40 %)		9.565	0.048*
Proximal extremities	3 (30 %)		7 (28 %)			
Neck	1 (10 %)		0			
Complications						
No	8 (80 %)		6 (24 %)		9.333	0.002*
Yes	2 (20 %)		19 (76 %)			
Burn						
Partial thickness	1 (10 %)		16 (64 %)		8.338	0.004*
Full thickness	9 (90 %)		9 (36 %)			

Table 3. Complications of the outcome data between complicated and noncomplicated cases.

	Complications ($n = 10$)		No complications ($n = 20$)		Independent student t/chi square test	
	Mean	SD	Mean	SD	t	*P*-value
Re-pigmentation%	42.70	14.23	69.00	9.60	−5.376	<0.0001
Color matching %	49.50	8.32	59.60	12.16	−2.820	0.009
Preoperative dyspigmentation	11.30	3.53	15.36	4.59	−2.811	0.010
Postoperative dyspigmentation	4.87	2.54	10.47	3.03	−5.575	<0.0001
	N (%)		*N* (%)		X2	*P*-value
Re-pigmentation						
Excellent	0 (0 %)		5 (20 %)			
Good	2 (20 %)		19 (76 %)			
Fair	7 (70 %)		1 (4 %)		21.846	<0.0001
Poor	1 (10 %)		0			
Kahn grading system						
Poor	10 (100 %)		4 (16 %)			
Fine	0		10 (40 %)		21.000	<0.0001
Good	0		9 (36 %)			
Excellent	0 (0 %)		2 (8 %)			
	Obstacles ($n = 14$)		**No obstacles ($n = 21$)**		**Independent student t/chi square test**	
	Mean	**SD**	**Mean**	**SD**	**t**	***P*-value**
Re-pigmentation%	52.29	16.84	67.62	12.87	−2.891	0.008
Color matching %	54.29	9.58	58.33	13.35	−1.043	0.304
Preoperative dyspigmentation	12.96	3.87	15.03	5.02	−1.369	0.18
Postoperative dyspigmentation	7.01	3.64	10.11	3.54	−2.495	0.019
	N (%)		*N* (%)		X2	*P*-value
Re-pigmentation						
Excellent	1 (7 %)		4 (19 %)			
Good	6 (43 %)		15 (71 %)		7.560	0.056
Fair	6 (43 %)		2 (10 %)			
Poor	1 (7 %)		0			
Kahn grading system						
Poor	9 (64 %)		5 (23.8 %)			
Fine	2 (14 %)		8 (38.1 %)		6.607	0.086
Good	3 (21 %)		6 (28.6 %)			
Excellent	0		2 (9.5 %)			

Conflicts of interest

There are no conflicts of interest.

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