

Autologous non-cultured epidermal cell suspension combined with platelet rich fibrin for the treatment of stable vitiligo: A case series

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Abstract

Non-cultured epidermal cell suspension (NCECS) is a relatively new cellular grafting technique for vitiligo. Platelet rich fibrin (PRF) is a platelet and immune concentrate gather on a single fibrin membrane which can be used in conjunction with grafts and has several advantages, such as promoting wound healing, haemostasis, and give better handling properties to graft materials. This study was conducted to determine the efficacy of NCECS combined with PRF in patients with stable vitiligo. Seven patients with stable vitiligo which not responding to topical and phototherapy for more than 12 months were included in the study. The melanocytes were harvested as an autologous melanocyte rich suspension from a donor skin. The non cultured melanocyte transplanted to recipient area that had been superficially dermabraded and smeared with PRF gel. Of all 7 patients, 1 patients showed excellent pigmentation (90-100%), 2 had good repigmentation (60-89%), 1 had fair repigmentation (25-59%) and 3 patients had a poor response (0-24%). The procedure is safe and promising surgical modality for stable vitiligo.

Introduction

Vitiligo is an acquired pigmentary disorder of the skin which is characterized by well-defined depigmented macules or patches thought to occur secondary to melanocyte dysfunction and loss. It is the most common depigmentation disorder, affecting approximately 0.5 to 2.0 percent of the population and has no predilection for gender or race.¹ There are several hypotheses in the etiopathogenesis of this disease. Autoimmune, neurohumoral, cytotoxic, oxidative stress and genetic hypotheses are the most popular theory. It seems that the etiology of vitiligo is multifactorial and each of the factors plays an important

role in the pathogenesis. Depigmentation may be the source of severe psychological distress, diminished quality of life, and increased risk of psychiatric morbidity.²

In vitiligo which does not respond to medical treatment, several surgical procedures includes tissue grafts and cellular graft have been reported to be effective.³ Tissue grafts such as full-thickness punch grafts, split-thickness grafts and suction blister grafts are limited to small areas while the cellular grafts included cultured autologous epithelial grafts, cultured melanocytes and non-cultured melanocytes-keratinocytes can cover larger area of vitiligo. The cellular non-cultured graft technique has the additional advantage that a laboratory equipped for cell cultures is not necessary.⁴

Platelet rich fibrin (PRF) is a platelet and immune concentrate gather on a single fibrin membrane, containing all the components of a blood sample which are favorable for healing and immunity.⁵ In this study, we used Gauthier and Benzekri⁶ method with a modifying technique by adding PRF to the autologous non-cultured epidermal cell suspension (NCECS) which never done in other study before to achieve better manipulation of transplanted cells.

Materials and Methods

Study Design and Patients

This interventional controlled study was conducted from December 2017 to June 2018, in dr. Moewardi General Hospital Surakarta. All the patients were screened at the preliminary visit. Seven cases of stable vitiligo were treated by autologous non-cultured epidermal cell suspension (NCECS) combined with PRF transplantation. Inclusion criteria were the stability of vitiligo lesions for more than 1 year, aged older than 18 y.o and never received any treatment in the past one month. Exclusion criteria were the activity of vitiligo, including koebnerization or keloidal tendency, history of bleeding disorders, anticoagulant medications, pregnancy and positive for HIV and HbsAg. Clinical photographs were taken before the procedure and then at week 1, 4 and 16 to compare the pigmentation change and color match. The endpoint selected was 24 weeks after the transplantation procedure.

Donor site

A punch biopsy specimen was taken from the patient's normal pigmented femoral area locally anaesthetized using intralesional lidocaine. An area of epidermis containing minimal underlying dermis was

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punched. It was approximately one tenth of the recipient area. The donor site was covered by topical antibiotic and secured by gauze and adhesive tape.

Non-cultured autologous melanocyte-keratinocyte suspension

Sample preparation

The skin tissue was immediately transferred to a transport medium to the laboratory and washed three times using phosphate buffered saline (PBS) which containing antibiotics and antimikotics (penicillin, streptomycin and amphotericin B). The tissue was cut into tiny pieces and cleansed slowly from the remnants so the epidermis was separated from the dermis and keratin tissues. The epidermis then transferred to the sterile Falcon and incubated with 2.5 ml of 0.25% trypsin - 0.05% ethylene diamine tetraacetic acid (EDTA) at 37° C for 15-30 minutes to prepare a single cell suspension. After 15 minutes, 2.5 ml of trypsin inhibitor was added and left it for 10 minutes. Then filtration was done with a 70 um cell strainer. Next the cell suspension was centrifuged for 5 minutes at 2000 rpm. The supernatant

was discarded to obtain the cell pellet. It was then resuspended in 2 ml of Dulbecco's modified eagle medium (DMEM) as nutrient medium before planted in recipient area. The number of cells in the suspension were counted manually by the haemocytometer. Survival of cell suspension was examined with trypan blue dye exclusion method (Figure 1).

Recipient site

The recipient site was surgically cleansed and was anesthetized using topical EMLA (lidocaine 2.5%, prilocaine 2.5%). The recipient area was abraded manually until tiny pinpoint bleeding spots were seen. Using an 18 g needle attached to a tuberculin syringe or a pipette, few small drops of suspension were placed over the denuded surface, flattened to cover all the dermabraded area, then covered with fibrin matrix and dressed with first sofratulle, then cell suspension again followed by the second dressing of sofratulle, finally covered with topical antioxidant gel and sterile cotton pad. After the procedure, patients were instructed to lie at least 2 hours to allow successful attachments of cells to the transplant area and advised to keep the dressing dry and minimize local manipulation. An oral broad spectrum antibiotic was given for 7 days to avoid infection of the donor and grafted areas. All dressings were removed after 7 days.

Results

Demographic and disease characteristics

Demographic characteristics are shown in Table 1. The patients were 2 males and 5 females with age ranged from 18 to 78 years (mean 33.4). Three patients had segmental vitiligo, two had acrofacial localized vitiligo, one focal vitiligo vulgaris, and one focal vitiligo. The stability duration of the vitiligo

ranged from 13 to 180 months (mean 49.1) (Table 1).

Repigmentation

The patients were planned to be followed up regularly for a period of 6 months. We used Photoshop-based image analysis using the color information contained in each individual pixel and allowed the separation of colours at the pixel resolution level. And the degree of repigmentation was evaluated qualitatively on the basis of visual analogue system score for the extent of pigmentation and color match. Most of our patients started developing repigmentation within 4-24 weeks, it is characterized by the appearance of normal pigmented macules and/or patches (Figure 2).

Laboratory analysis

Average total cell count using Neubauer chamber was $2-30 \times 10^5$ /ml. Small cells were identified as keratinocytes and melanocytes, and large pale cells were identified as fibroblasts. Cell viability was assessed with trypan blue staining, and the percentage of viability was 97%.

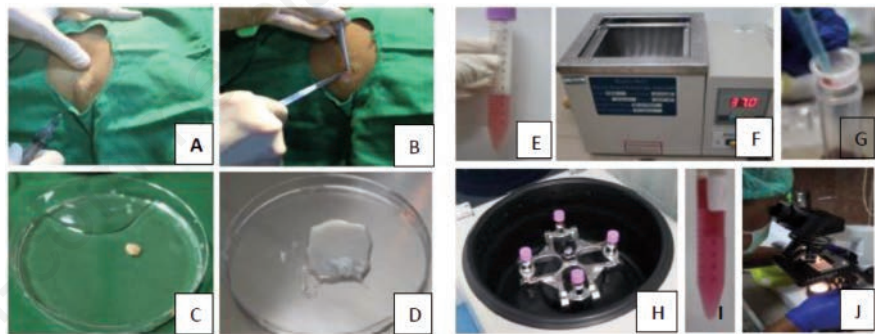


Figure 1. Preparation of autologous NCECS. A-B. Donor area were anaesthetized and punched. C-D. Punched skin was cut into tiny pieces. E-F The epidermis tripsinized then incubated at 37° C. G-H. Filtered and centrifuged for 5 minutes at 2000 rpm. I. Pellet cell resuspended with 2 ml DMEM. J. Manually counted by haemocytometer

Table 1. Demographic distribution of patients and the percentage change of the depigmented areas (from baseline) in the patches treated with melanocytes transplantation.

Patient	Sex	Age (years)	Area treated	Type	Duration of stability (Months)	Repigmentation after 6 months (%)
1	M	42	Left hand	Acrofacial	36	10
2	F	78	Hand	Acrofacial	18	25
3	F	22	Back	Focal	60	5
4	F	25	Right forehead	Segmental	180	94
5	F	31	Right hand	Vulgaris	13	85
6	F	18	Left shoulder	Segmental	24	70
7	M	18	Face	Segmental	13	15

BEFORE



AFTER



Figure 2. Repigmentation in the week 24 of follow up, it is characterized by the appearance of normal pigmented macules and patches. (A) Before; (B) after.

go. In our present study, we observe and continue follow up the patients until at least 24 weeks. In a study by Leelavathy *et al.* among NCECS group, majority of patients showed poor pigmentation (53.33%) followed by 46.67% with good pigmentation at 6 weeks. At 12 weeks, excellent repigmentation was observed in 6.67% of patients and 30 % patients showed very good repigmentation.¹¹ In the study by Van Geel *et al.*⁹ 13.55% patients showed 70% repigmentation at 12 weeks post op. At 24 weeks, excellent repigmentation (>90%) was observed in 66.67%, very good response (with 75%-90% repigmentation) in 16.67% and good response (with 50%-75% repigmentation) in 13.33% of patients.

PRF consists of a fibrin matrix polymerized in a tetra molecular structure, with consolidation of platelets, leucocytes, cytokines, and circulating stem cells.^{12,13} Clinical studies showed that its biomaterial would be a great matrix for the development of a coherent healing, without any inflammatory excess. PRF in the form of a platelet gel can be used in conjunction with grafts, which has several advantages, such

as promoting wound healing, haemostasis, and give better handling properties or scaffolding to graft materials.¹⁴

Conclusions

This NCECS transplantation combined with PRF is a promising technique in vitiligo surgical intervention. The procedure is simple without complicated laboratory facility.

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