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Treatment of vitiligo lesions by ReCell® vs. conventional melanocyte–keratinocyte transplantation: a pilot study

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Conflicts of interest
S.V.M. was reimbursed travel and accommodation expenses to conduct a workshop in Singapore in December 2007 and a seminar in London in March 2007 by Clinical Cell Culture (U.K.).

Summary

Background Surgical procedures are indicated to treat stable vitiligo, refractory to medical treatment. In addition to conventional surgical techniques, noncultured cellular grafting is gaining wider acceptance among dermatologists.

Objectives To assess the efficacy of the ReCell® kit (Clinical Cell Culture, Cambridge, U.K.) and to compare it with conventional melanocyte–keratinocyte transplantation (MKT) for the treatment of vitiligo.

Methods Ten lesions in five patients at the same anatomical localization (left vs. right, or two separate lesions at the same anatomical location) were treated with ReCell® and conventional MKT and repigmentation compared at 4 months post-transplantation.

Results Of the five lesions treated with ReCell® two lesions showed 100%, one 65% and one 40% repigmentation, and one lesion failed to repigment. Of the five lesions treated by conventional MKT three showed 100% and one 30% repigmentation and one failed to repigment.

Conclusions ReCell® may be an effective method to treat vitiligo. Studies on larger series of patients are required to confirm its efficacy. Further research is required to establish the effective dilution of the cell suspension.

Several surgical procedures are reported for treating vitiligo which is refractory to medical treatment. Transplantation techniques which use noncultured autologous melanocyte or epidermal cell suspension offer an advantage of treating a larger recipient area with a smaller donor site. These procedures have two steps: (i) preparation of cell suspension and (ii) transplantation of the same on to the recipient area. The technique used by Olsson and Juhlin[1] and van Geel *et al.*[2] uses a thin shave biopsy specimen, transported to a laboratory in Spinner minimum essential medium or Dulbecco's modified Eagle's medium (DMEM) supplemented with penicillin and streptomycin. The sample is then trypsinized and incubated for about 45 min to separate the skin layers. The cells obtained are delivered in melanocyte medium or DMEM. Thus the first step of cell separation requires transportation of skin samples to a separate laboratory set-up. Mulekar[3,4] reported a modified procedure, in that preparation of the cell suspension and the transplantation procedure were performed in the operative room without the need for a separate laboratory. Cell delivery was made in DMEM without any additives. However, all authors used melanocyte medium or DMEM as a transportation and cell delivery system, which is believed to help survival and multiplication of the cells obtained. The concentration of resultant cell suspension is arbitrary, which gives a donor/recipient ratio of 1 : 10. The new ReCell® kit (Clinical Cell Culture, Cambridge, U.K.), a portable battery-operated cell-harvesting device, has eliminated the need for either a separate laboratory set-up or equipment such as a centrifuge or incubator to process the cell suspension preparation. The major difference between the ReCell® technique and conventional cellular transplantation techniques is the use of sodium lactate as cell delivery system and for dilution of the cell suspension, which gives a donor/recipient ratio of about 1 : 80.

We report a comparative study in five patients treated by ReCell® and by conventional melanocyte–keratinocyte transplantation (MKT).

Materials and methods

Five otherwise healthy patients (three men and two women) with stable vitiligo were included in the study, which was approved by the ethics committee of the National Center for Vitiligo and Psoriasis. The clinical profile of the patients and a description of the lesions treated are shown in Table 1. All the patients gave written informed consent. Patients were treated between 30 March and 24 July 2006. Patients were assessed at 4 months post-transplantation. Four patients had generalized and one had segmental vitiligo. All the patients had a

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Table 1 Clinical profile of patients treated by ReCell® and melanocyte–keratinocyte transplantation (MKT) with analysis of results

				Transplanted area (cm^2)		Percentage repigmentation at 4 months		
Patient	Age (years)/sex	Type of vitiligo	Anatomical site	ReCell®	MKT	ReCell®	MKT	Stability (years)
1	23/M	Segmental	Shoulder	8·0	13·0	100	100	3
2	40/M	Generalized	Foot	9·0	9·0	40	30	Doubtful
3	40/F	Generalized	Shoulder	4·0	8·0	0	0	Doubtful
4	32/M	Generalized	Maleolus	12·0	16·0	65	100	5
5	18/F	Generalized	Foot	6·0	8·0	100	100	1

minimum of two lesions at the same anatomical localization (left vs. right, or two separate lesions at the same anatomical location). One lesion was treated by the conventional method using DMEM and the second lesion was treated by ReCell® using sodium lactate as a cell delivery system. Patients were assessed by two physicians using photographs taken before and at 4 months after transplantation. In addition, the lesions were measured directly to assess the approximate area before transplantation. The nonpigmented area was measured at 4 months after transplantation to arrive at the percentage of repigmentation.

ReCell® kit

ReCell® is a single-use battery-operated autologous cell-harvesting device. It consists of a processing unit with built-in heating mechanism for warming the enzyme solution to optimum working temperature (37 °C) and a removable insert to act as a sterile Petri dish for use when separating and scraping the skin biopsy. In addition it contains a sealed glass vial of enzyme, lyophilized trypsin 0·75% (minimum activity 3000 Tu mL^{-1}, equivalent to 50 μkat mL^{-1}), 1 × 10 mL ampoule of sterile water, 1 × 10 mL vial of compound sodium lactate, needles, syringes, cell strainer, and size 23 disposable surgical scalpel. The kit does not contain sterile surgical instruments such as forceps.

Biopsy and cell separation using ReCell® kit

The ReCell® kit is shown in Figure 1. With routine aseptic precautions a thin split-thickness biopsy (1 × 1 cm) was harvested with silver's knife from the lateral upper third of the thigh. The harvested biopsy sample was transferred to a well (labelled A+E) containing trypsin, which was heated to approximately 37 °C by turning the device on. The biopsy sample was thus incubated for about 15–20 min, rinsed in the middle well (labelled B) containing sodium lactate solution to remove the residual trypsin solution, and then returned to the Petri dish. The dermis and epidermis were separated mechanically using both pairs of forceps. Using the forceps to anchor the biopsy layers, the cells were scraped from the junctional surfaces with the sterile scalpel to develop a plume of

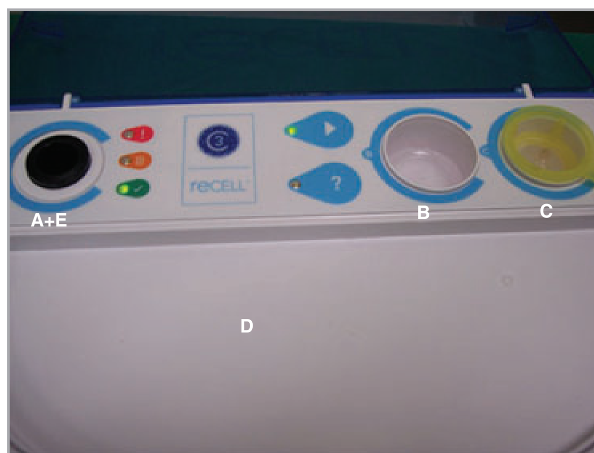

Fig 1. ReCell® kit. A+E, well at extreme left, containing trypsin solution; B, middle well containing compound sodium lactate solution to remove residual trypsin; C (extreme right), conical well with

cell, which was drawn up into the syringe and aspirated several times to a create a cell suspension. The cell suspension was dispensed into the cell strainer in the right conical well (labelled C). The filtered cell suspension was aspirated from the conical well, using an insulin syringe. The quantity of cell suspension prepared was 0·2 mL.

Biopsy and cell separation by conventional melanocyte–keratinocyte transplantation

With routine aseptic precautions a thin split-thickness biopsy (1 × 1 cm) was harvested with silver's knife from the lateral upper third of the thigh. A separate site close to the previous one was selected to obtain the biopsy. The sample obtained was transferred to a Petri dish containing 4 mL trypsin–ethylenediaminetetraacetic acid and incubated at 37 °C for 15–20 min in an ordinary incubator. At the end of incubation, the Petri dish was removed from the incubator, and trypsin solution was aspirated using a Pasteur pipette. The sample was then washed by DMEM to remove the traces of trypsin. The dermis and epidermis were separated mechanically using both pairs of forceps. The dermis was discarded; the epidermis was broken into small pieces, transferred to a

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centrifuge tube containing DMEM–Ham's F12 and then centrifuged to prepare a suspension. The cell suspension (0·2 mL) was drawn up in a 1-mL insulin syringe with detachable needle.

Preparation of recipient area

With routine aseptic precautions, the vitiligo patches under study were dermabraded under local anaesthesia (1% lidocaine without adrenaline) using a diamond fraise wheel to the dermoepidermal junction, which was identified by the presence of pinpoint bleeding. The same method was used to prepare the recipient site treated by ReCell® and by conventional MKT.

Application of cells

The cell suspension prepared by both methods was evenly spread on to the respective recipient dermabraded area, and covered with dry collagen (Collomedica Laboratories, Bangalore, India). This was then covered with sterile gauze pieces and held in place with micropore tape.

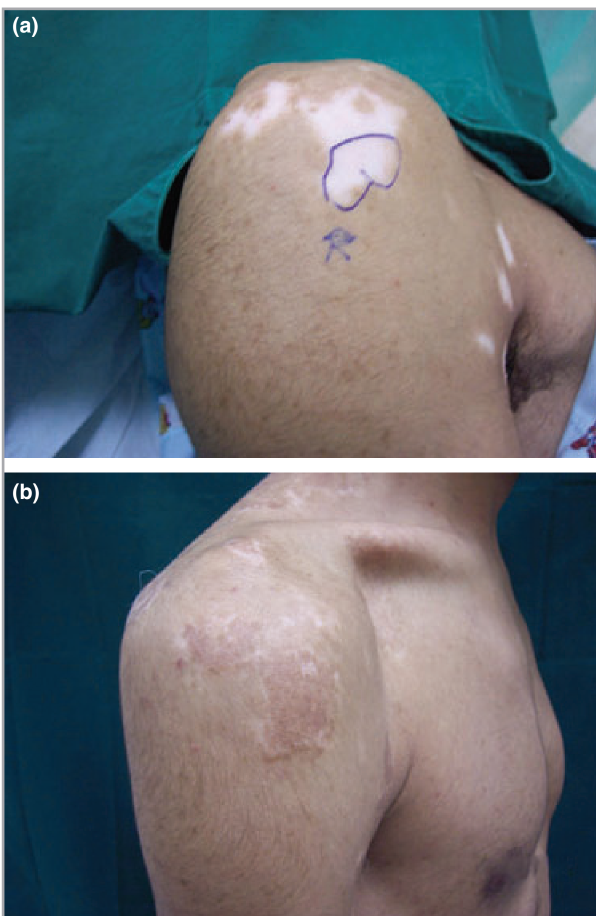

Fig 2. Patient 1. (a) Before transplantation. Marked area was transplanted by ReCell®. (b) At 4 months post-transplantation.

Dressing removal and follow-up of the patients

The dressing was removed after 7 days. Collagen dissolves within this period and was not removed. All the patients were treated with the prophylactic antibiotic cefazolin 500 mg twice daily for 1 week starting 1 day prior to the cell transplantation. Recipient sites healed within 7 days and did not require another dressing thereafter. There were no special precautions postoperatively in any of the patients. Postoperative exposure to ultraviolet or solar radiation was not advised.

Results

The main parameter to measure the efficacy of treatment was the percentage area of repigmentation in the test lesions.

Patients 1, 4 and 5 (Table 1) had clinical stability of 3, 5 and 1 year. Patients 1 (Fig. 2a, b) and 5 (Fig. 3a, b) repigmented completely at both the sites treated by ReCell® and conventional MKT. Patient 4 showed complete and 65% repigmentation at the site treated by conventional MKT and ReCell®, respectively.

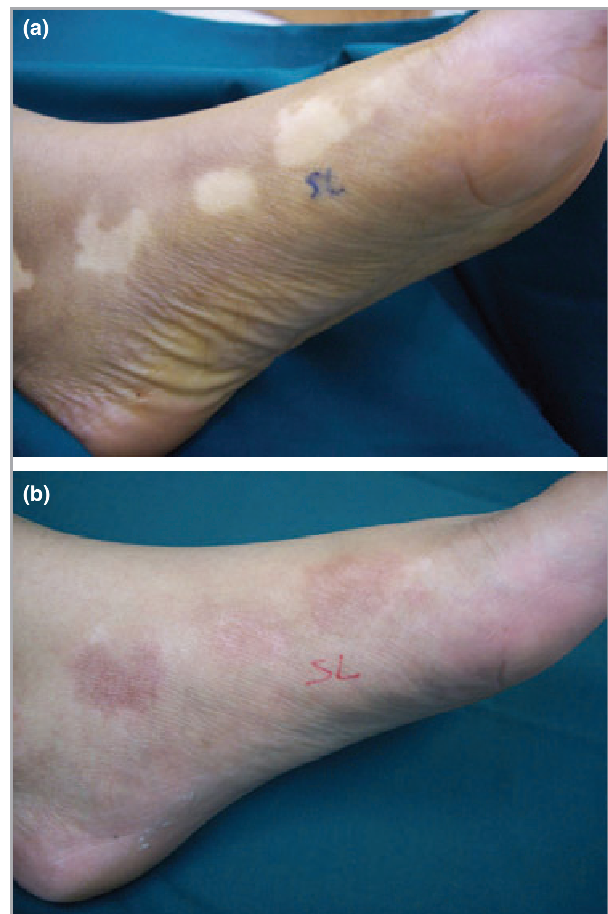

Fig 3. Patient 5. (a) Before transplantation. The macule marked by 'SL' was transplanted by ReCell®. Other lesions were treated by conventional melanocyte–keratinocyte transplantation. (b) At 4 months after transplantation.

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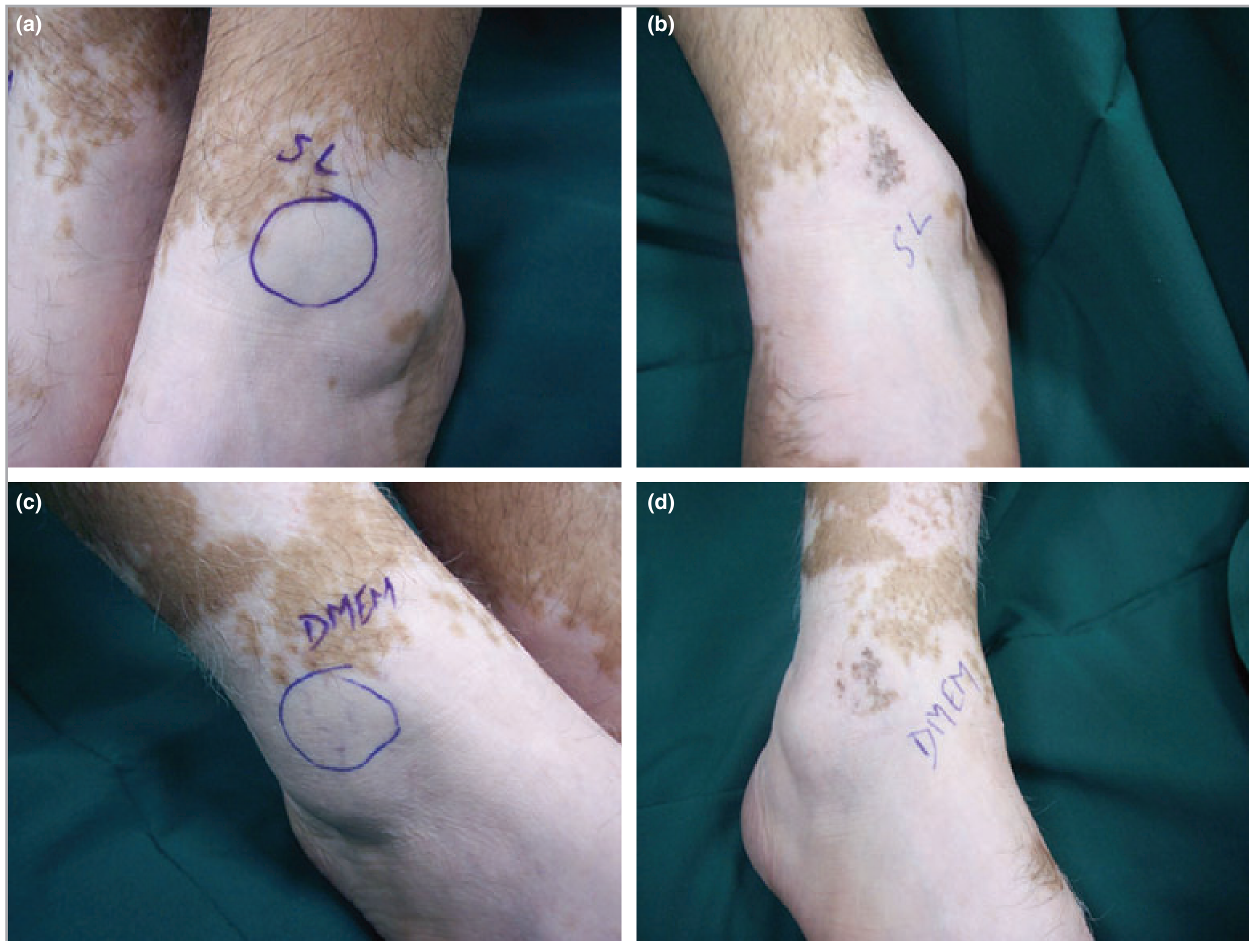

Fig 4. Patient 2. (a) Marked area (left ankle) before ReCell® transplantation. (b) At 4 months following ReCell® transplantation. (c) Marked area (right ankle) before conventional melanocyte–keratinocyte transplantation. (d) At 4 months post-transplantation.

Patients 2 and 3 (Table 1) had doubtful clinical stability at the time of cell transplantation. Patient 3 did not repigment at all and patient 2 showed $<50\%$ repigmentation at sites treated by ReCell® (Fig. 4a, b) and conventional MKT (Fig. 4c, d).

Discussion

Surgical methods, which include minigrafting, split-thickness grafting and blister roof grafting, are practised at many dermatology centres. Cellular grafting is gaining a wider acceptance among dermatologists, with an increasing number of publications.

Since 1987 there have been many reports of transplantation of cultured autologous melanocytes.[5–8] As it involves *in vitro* multiplication of cells, the use of various culture media was obligatory. Culture techniques require a state-of-art laboratory, highly trained staff and two visits by the patient, and are expensive. As a result they are restricted to very few centres and are mainly useful for research purposes. Subsequently, the culture technique was modified by eliminating the step of *in vitro* culture of melanocytes, and cells were transplanted directly on to the affected depigmented area. Other steps of cell separation and preparation of the recipient area remained the same with minor modifications. There was no attempt to interfere with the cell delivery system or the resultant dilution of cell suspension. This is logical as research and development is a step-by-step process. All the authors retained the use of either DMEM or melanocyte medium with or without additives to transport the skin biopsy sample and to prepare the cell suspension. It was presumed that the medium plays a crucial role for the survival and multiplication of transplanted melanocytes and keratinocytes even though the cells remain in the media for a very few minutes. The use of sodium lactate as a cell suspension medium was first initiated by Clinical Cell Culture for ReCell®. This device was developed to treat superficial burn injuries with the help of epidermal cell transplantation. During the management of superficial burn by ReCell® it was observed that the resultant healing was accompanied by good repigmentation. This led to use of this device for the treatment of stable vitiligo macules.

Ten lesions located at the same anatomical localization (left vs. right, or two separate lesions at the same anatomical location) were treated by ReCell® and conventional MKT and the repigmentation was compared at 4 months post-transplant-

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ation in a pilot study. We found that the repigmentation was comparable for both the techniques used. Patient 3 failed to produce any repigmentation when treated by both methods, and the probable reason was the active disease. The initial results suggest that the properties of DMEM or melanocyte medium have minimal or no role in the survival or multiplication of transplanted epidermal cells. An isotonic solution such as sodium lactate may be as effective as DMEM or melanocyte medium in noncultured cell transplantation methods. The various growth factors released as a result of injury and the physiological conditions at the transplanted site are the main factors responsible for survival and multiplication of the transplanted epidermal cells.

In conclusion, ReCell® may be an effective method to treat vitiligo. Studies on larger series of patients are required to confirm its efficacy. Further research is required to establish the effective dilution of the cell suspension.

Acknowledgments

Clinical Cell Culture Europe Ltd (Cambridge, U.K.) donated five ReCell® kits to the National Center for Vitiligo and Psoriasis to treat the five patients in this study.

References

1 Olsson MJ, Juhlin L. Leucoderma treated by transplantation of a basal cell layer enriched suspension. *Br J Dermatol* 1998; **138**:644–8.

2 van Geel N, Ongenae K, De Mil M, Naeyaert JM. Modified technique of autologous noncultured epidermal cell transplantation for repigmenting vitiligo: a pilot study. *Dermatol Surg* 2001; **27**: 873–6.

3 Mulekar SV. Long-term follow-up study of segmental and focal vitiligo treated by autologous, noncultured melanocyte–keratinocyte cell transplantation. *Arch Dermatol* 2004; **140**:1211–15.

4 Mulekar SV. Long-term follow-up study of 142 patients with vitiligo vulgaris treated by autologous, non-cultured melanocyte–keratinocyte cell transplantation. *Int J Dermatol* 2005; **44**:841–5.

5 Lerner AB, Halaban R, Klaus SN, Moellmann G. Transplantation of human melanocytes. *J Invest Dermatol* 1987; **89**:219–24.

6 Olsson MJ, Juhlin L. Repigmentation of vitiligo by transplantation of cultured autuologous melanocytes. *Acta Derm Venereol (Stockh)* 1993; **73**:49–51.

7 Lontz W, Olsson MJ, Moellmann G, Lerner AB. Pigment cell transplantation for treatment of vitiligo: a progress report. *J Am Acad Dermatol* 1994; **30**:591–7.

8 Guerra L, Primavera G, Raskovic D *et al.* Erbium:YAG laser and cultured epidermis in the surgical therapy of stable vitiligo. *Arch Dermatol* 2003; **139**:1303–10.

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