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Autotransplantation in Vitiligo: Treatment with Epidermal Grafts and Cultured Melanocytes

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Previous studies have shown the usefulness of autologous grafts carrying melanocytes for treatment of vitiligo. In our study repigmentation was obtained in three patients using epidermal sheets from suction blisters. The repigmentation was found stable in follow-ups from one to three years. The most homogenic repigmentation, however, was found in a patient treated by grafting autologous melanocytes applied to the dermabraded areas of vitiligo by collagen film.

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Vitiligo, which is an acquired pigmentary abnormality of the skin manifested by depigmented white patches, is characterized histologically by the absence of epidermal melanocytes in affected areas. The etiology is unknown, although autoimmunity is presumed to be involved. Current treatment with PUVA or topical steroids in general has been disappointing. Within recent years there have been reported several attempts to use autologous grafts containing melanocytes from normally appearing skin (1–5), as treatment of vitiligo, and recently autologous cultured epithelial grafts have also been tried (6, 7).

The present paper contains a report on four grafting procedures with epidermal tissue obtained by the suction blister technique in three patients and one grafting procedure using autologous cultured melanocytes applied by a collagen film.

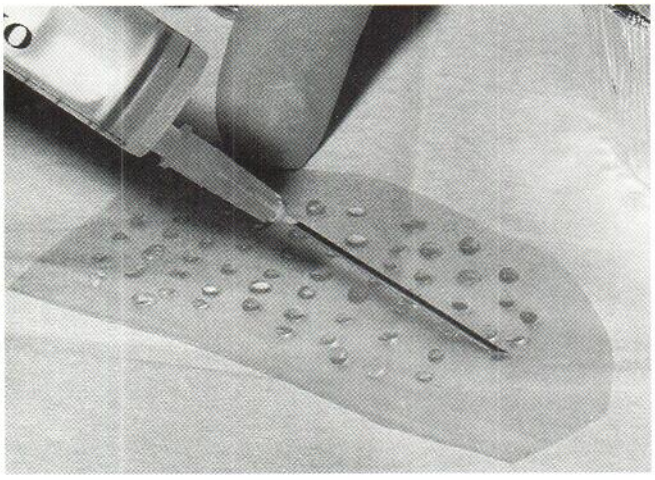

Fig. 1. Collagen film carrying a suspension of cultured melanocytes. Approximate density 50 000 cells/cm^2.

PATIENTS AND METHODS

The patients were two adult Caucasian females aged 46 and 60 years and a young girl born by Tamil parents in Sri Lanka. The latter was 12 years old when treated for the first time with epidermal grafts and 15 years old when she received autologous cultured melanocytes. All patients were otherwise healthy and their vitiligo had been stable for several years.

Epidermal tissue for grafting was obtained from normal pigmented abdominal skin. The total area of the grafts varied from 3.6 to 7.2 cm^2. Epidermis was separated from dermis by suction blister technique as described by Kiistala (8). The recipient sites, which were on the face, the neck, the chest, and arms were prepared for grafting by superficial dermabrasio. The roof of the blisters were placed on the dermabraded skin and covered by a collagen film supplied by TRIPASIN AB, Malmö, Sweden (9). The film was left in place for approximately two weeks and then removed. Our first patient was treated on a 3 × 3 cm area on the left wrist. A symmetrical area on the right wrist was dermabraded as a control but not grafted. All patients returned at various intervals for control and clinical photographs.

For cell culture the epidermal tissue from suction blisters was immediately placed in MCDB-153 medium (Biochrom, Berlin) and stored at 37°C for no longer than 2 h. The material was then placed in a trypsin/EDTA solution (0.25% w/v trypsin, 0.02% w/v EDTA in PBS pH 7.0) and incubated for 40 min at 37°C. The trypsination was stopped by adding MCDB-153 medium supplemented with 0.6 ng/ml basic fibroblast growth factor, 8 nM 12-0-tetrade-canoylphorbol-13-acetate, 5 μg/ml insulin, 5 μg/ml transferrin, 1.0 μg/ml α-tocopherol all from Sigma, St. Louis, USA and 30 μg/ml Crude bovine pituitary extract, 0.5 μg/ml hydrocortisone from Clonetics Laboratories, San Diego, CA and 5% heat inactivated fetal calf serum (Biochrom, Berlin). In primary cultures 20 μg/ml Catalase from bovine liver (Sigma, St. Louis, USA) was also added for the first 6 days in culture. The cultures were fed twice weekly. When they reached 80% confluency they were passaged, by first washing them twise in PBS followed by trypsination with a 0.1% w/v trypsin$^+$, 0,02% w/v EDTA

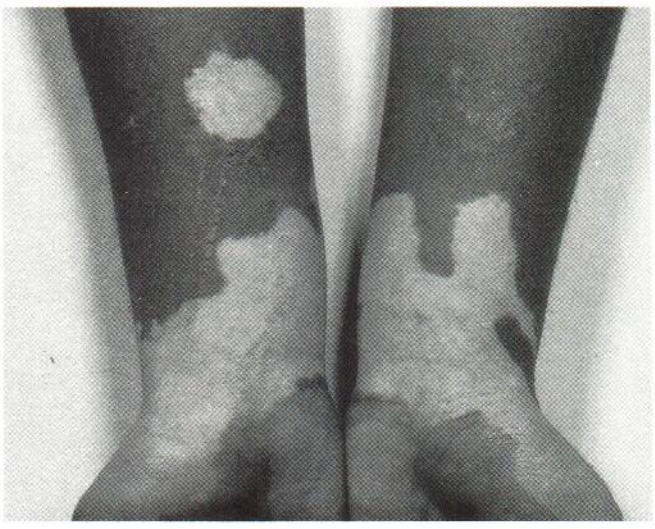

Fig. 2. Symmetrical areas of vitiligo treated with dermabrasion and epidermal grafts and control dermabrasion one year after treatment.

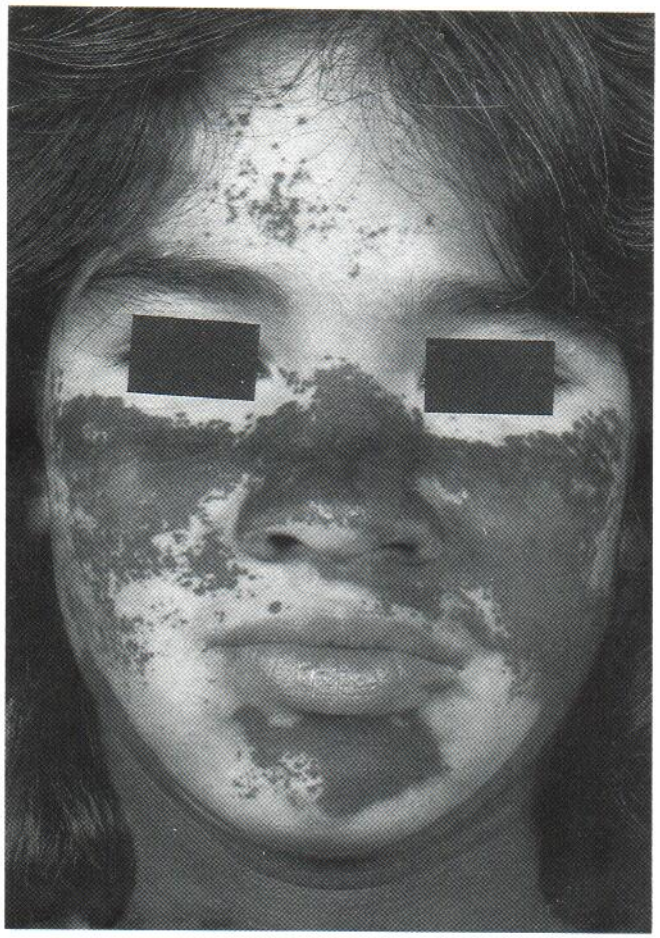

Fig. 3. Face of vitiligo patient before treatment with grafts of cultured melanocytes.

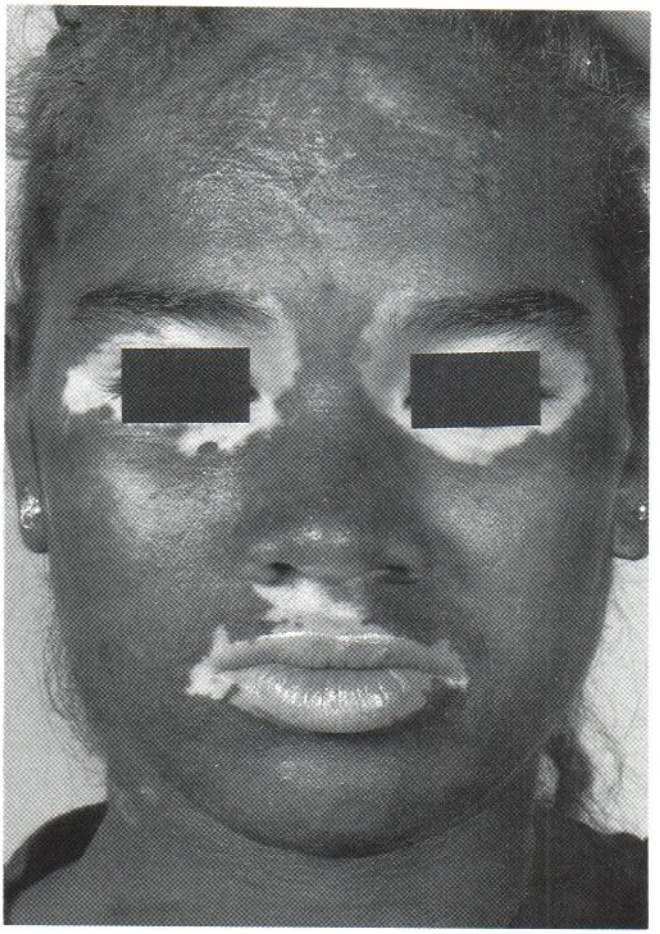

Fig. 4. Face of same patient as in Fig. 3 two months after grafting.

solution in PBS. The trypsination was stopped by adding complete growth medium (as above). The total time in culture was 8 weeks.

Before transplantation the procedure was the same as for trypsination except that the trypsination was stopped with MCDB-153 medium containing only 5% FCS, and the cells were washed twice in PBS before resuspending them at density of 2 mill/ml. The cell suspensions were seeded onto collagen films (Fig. 1) at a density of 50000 cells/cm^2, and the collagen films were applied to the patient in the same manner as mentioned above, and left in place for 2 weeks. The areas treated were the forehead, the chin and partly vitiligo around the lips.

RESULTS

In our first patient, the young girl, a clinical repigmentation took place at both the transplanted and non-transplanted control area within four weeks. The control area, however, lost its repigmentation after 10–12 months, while the transplanted area retained its pigment at even the latest follow-up three years after transplantation (Fig. 2). Also a later transplantation covering areas of the face was successful, but due to the relatively large areas involved, it was difficult to obtain complete repigmentation. Therefore the patient was chosen for treatment with cultured melanocytes. Fig. 3 shows her face before treatment, and Fig. 4 two months after transplantation. Areas around the eyes and minor perioral vitiligo remain to be treated.

Pigmentation was also obtained in the two patients treated with epidermal grafts alone, but the repigmentation was not so complete and not so even as by treatment with cultured melanocytes. The pigmentation remained evident at one-year follow-ups. All donor sites healed without scarring showing only a slight hyperpigmentation.

DISCUSSION

Successful grafting of in vitro-cultured epidermis has been reported in three papers (6, 7, 10). The repigmentation, however, was seen to be somewhat uneven in several cases. The use of cultured melanocytes applied to the dermabraded tissue on a collagenfilm in our hands gave a quick and even repigmentation of vitiligo. Cultured melanocytes may allow treatment of larger areas than other grafting techniques.

In the majority of reported cases (1–7, 10) repigmentation following the different types of autologous grafting has been found stable for several years. The practical use of our technique is only limited to the expense of the culture procedure and by obtaining the skill required to culture and graft melanocytes.

At present we will conclude that the technique of transplanting autologous cultured melanocytes seems promising, and our observations together with those by other workers

indicate, that repigmentation obtained by grafting with epidermal sheets or cultured melanocytes is long-lasting.

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