Table 1. Pigmentation of vitiligo lesions grafted with RHPS

Case No.	Sex	Size of the grafted area (cm^2)	UVA irradiation	Pigmentation (% of grafted area)
1	M	10	–	65
2	M	12	–	60
3	F	25	–	80
4	F	30	+	100
5	F	20	+	100

fixed 15 min with 4% paraformaldehyde, washed 3 times in PBS, and incubated for approximately 5 h in fresh 0.1% DOPA solution in PBS at 37°C, changing the DOPA solution after the first hour. Keratinocytes were counterstained with May-Grünwald (5 min) and Giemsa-Romanovski (10 min).

Five patients (2 male, 3 female, age of 25–40 years) with stable vitiligo (new symptom-free period of at least 2 years) were treated. The first symptoms of vitiligo occured in the patient group between the last 3 to 8 years (the clinical activity of the disease was 1 to 6 years). The mean age of the patient group was 36.7 years. The medical history, as well as clinical and laboratory examination, showed no concomitant diseases, including other autoimmune disorders. The area of skin intended to be treated was localized in lower leg (2 patients), in fingers (2 patients) and in the abdominal area (1 patient). Depigmented skin (size of 10–30 cm^2) was dermabraded using the Aesculap device until papillary bleeding occurred, which was subsequently reduced by an application of wet gauze. RHPS was applied "upside-down", i.e. with epidermal cells facing the wound, and covered with one layer of a vaseline gauze and several layers of gauze wetted with cholera toxin-free culture medium. The external bandage was changed every second day. The vaseline gauze was removed after one week.

Confluent primary or secondary autologous epidermal cells on porcine dermis were used for vitiligo treatment. Primary cultures reached confluence in about 10 days, secondary cultures during the following 8–9 days. The cell suspension was not cryopreserved prior to subculture because, according to the literature, melanocytes do not survive freezing in liquid nitrogen (DeLuca et al., 1994). In fact, in our recent experiments, we have observed secondary melanocytes growing from a cryopreserved suspension of epidermal cells. In all types of culture (primary, secondary fresh or cryopreserved), melanocytes were regularly dispersed between keratinocytes grown on the tissue culture dish (Fig. 1a) or on porcine dermis (Fig. 1b). One melanocyte was surrounded by approximately 30–40 keratinocytes (Fig. 1a). After reaching confluence, auto-RHPS (porcine dermis covered with 1–2 layers of autologous epidermal cells) was applied "upside-down" on the vitiligo lesion (Fig. 2a), which was dermabraded to the level of capillary bleeding. Within 24 h after grafting, the RHPS adhered firmly by active attachment of epidermal cells to the wound bed. The dermal layer dried and peeled off spontaneously within 8–10 days, leaving new epithelium underneath. The wound healed usually with slight reactive erythema, which disappeared after several weeks. Five patients were treated with auto-RHPS. Pigmentation had been checked weekly and final results after 23 weeks were summarized in Table 1. The pigmentation started to be visible 4–6 weeks after grafting. In three cases 60–80% of the grafted area was homogenously pigmented. In two cases islands of diffuse pigmentation were observed six weeks after grafting (Fig. 2b). Ninety days post RHPS application, UVA irradiation followed the surgical procedure (because of the seasonal lack of natural sunshine). The total dosage of four sessions per each patient was 20 J/cm^2 in the course of two weeks. The pigmentation increased during irradiation sessions and the region was sufficiently pigmented one week after the UVA treatment (Fig. 2c). During the follow-up period of two years no pigmentation reduction in the treated areas was observed. As a control, 1–2 cm^2 of depigmented area of all subjects was dermabraded and covered with acellular porcine dermis. From the statistical point of view, the dummy-treated area size was sufficient for evaluation because of the easier repigmentation tendency in smaler vitiligo lesions compared with the larger ones. No pigmentation was observed in lesions where only surgical application of the cell-free dermis was performed.

The treatment of vitiligo is not easy and in most cases not successful. A recently developed possibility is to transplant autologous melanocytes to the depigmented skin areas. The advantage of the newly described methodology is the possibility to recolonize the melanocyte-free skin areas with autologous melanocytes, which are able to start pigmentation in the diseased areas again. In comparison with standard methods, this is definitely the most successful therapeutic procedure in the cases of stable vitiligo, where additionally the repigmentation tendency after conventional treatment is very low. Presumably, in the period of a decreased disease activity the pigmentation efficacy is quite high. Melanocytes can either be transplanted as a suspension kept in place by a net or a gel (Olsson and Juhlin, 1993; Zachariae et al., 1993), as epidermal cells on a collagen-coated membrane (Plott et al., 1989), or as simple cultured epidermal sheets (Fallabela et al., 1992; Zachariae et al., 1993; Kumagai

and Uchikoshi, 1997). Our modification of such a procedure permits us to transplant cells grown on a very natural substrate – acellular porcine dermis. The porcine dermis protects the wound from the top and simultaneously provides the graft with mechanical properties of normal skin. High adhesivity, no secretion under the graft and quick cell attachment to the dermabraded wound enhances the probability of take and pigmentation, even in mechanically stressed areas. The grafts are ready to be used for at least two weeks, without complicated enzymatic preparation, just by pealing off the dish with two forceps. Melanocytes are not present in the epidermis in a density comparable to the application of enriched melanocyte suspension, but UVA irradiation satisfactorily improves the pigmentation results. The pigmentation of wounds grafted with auto-RHPS is a visible proof that the epidermal cells applied in the "upside-down" orientation can take. Even four weeks after reaching confluence the epidermal cells were able to migrate from the porcine dermis into the freshly prepared dermo-epidermal wound. As the dry dermis is an ideal culture substrate for all adhesive cell types, it could probably serve as a delivery system for melanocytes cultured by any tissue culture method. The simplification of the application methods for epidermal grafts containing melanocytes brings new hope to patients psychologically suffering from vitiligo.

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