

Grafting of *In vitro* Cultured Melanocytes onto Laser-ablated Lesions in Vitiligo

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A variety of grafting procedures using autologous melanocytes have achieved promising results in the treatment of vitiligo. We here report on the preparation of an adequate graft recipient bed by pulsed Erbium-YAG laser skin ablation. In particular, for irregular lesions on delicate sites, which cannot be approached by utilization of suction blisters or dermabrasion, this technique may offer a distinct advantage. Key words: melanocytes grafting; laser skin ablation.

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Transplantation of autologous cultured melanocytes has been reported to successfully repigment vitiliginous skin lesions in a variety of settings (1–10). Common to all grafting methods is that the epidermis has to be lifted or otherwise separated from the dermis in order to insert new melanocytes at the dermo-epidermal junction. To achieve this purpose several modes of de-epithelialization have been described, including the induction of suction blisters (1), blister formation by liquid nitrogen application (2) or dermabrasion of the vitiligo areas (3–6). The application of these techniques is, however, limited to those areas which are easily accessible. Critical sites like the periorbital region or within the nasal fold present a problem for mechanical abrasion, as do spotted and irregular lesions for creating corresponding suction or freezing blisters. For these more delicate zones or unsuited lesion types, we

questioned whether controlled ablation of the skin surface by the use of pulsed laser technology could provide an adequate recipient site for melanocyte grafting.

PATIENTS AND METHODS

Patients

Three patients, 38 to 62 years of age, with generalized vitiligo since 1–35 years involving 12–25% of the body surface, were studied. In all cases the disease had stabilized, with some follicular repigmentation after previous photochemotherapy, which had been discontinued 1 month prior to study entry. After written consent punch biopsies ($\varnothing$ 6 mm) were taken from the pigmented skin in the inguinal, scrotal or axillary region for cell culture.

Cell culture

The tissue samples were placed in HBSS-buffered 2.5% trypsin solution and incubated for 1 h at 37°C. The epidermal sheet was then separated from the dermal face and the cells were released by vigorous pipetting. Soluble cells were harvested by centrifugation (200 g, 5 min, RT), seeded in two 1.9 cm²-wells and cultured with a melanocyte growth-promoting medium according to Lerner et al. (1). After 10 days a homogeneous confluent melanocyte culture was achieved. Cell count was approximately 10⁵ cells/well. Melanocytes were trypsinized and seeded onto a perforated absorbable hyaluronic acid matrix (Laserskin®, Marka, Italy). The matrix was cut into circles of 1.5 cm diameter, which fitted exactly into 24-well microdishes. Melanocytes were allowed to further propagate for 7 days, producing a density of approximately 5×10^4 cells/cm². Pure melanocyte cultures were achieved without visible contamination by any other cell species (Fig. 1a,b). Prior to transplantation the melanocyte-containing matrix was washed three times in sterile PBS.

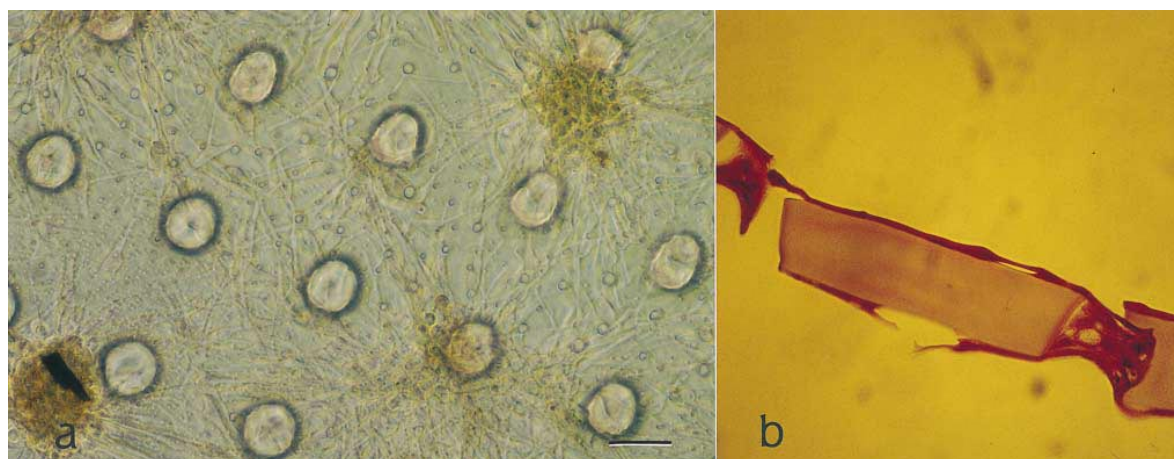


Fig. 1. Melanocyte culture on perforated hyaluronic acid matrix (laserskin®). (a) Cells partly accumulate to form clusters. The matrix leads to a reduction in image resolution and features orderly arrayed laser-perforated microholes. Bar is 50 μ m. (b) Paraffin-embedded section stained with hematoxylin and eosin, showing melanocytes colonizing both sides of the matrix as well as the microholes.

Skin ablation

Test lesions were ablated without anaesthesia. The entire epidermis of a circular area ($\varnothing$ 1.5 cm) of vitiliginous skin was removed using 3–5 passes of a pulsed Er:YAG laser (Dermablate®, Aesculap-Meditec or Superb®, Baasel Laser Technologies). Pulse energies varied between 300–450 mJ, repetition rate was 5 Hz and spot size 1.6–3 mm. The procedure was stopped when small capillary bleedings indicated that the papillary dermis had been reached (Fig. 2a,b). A total of nine circular areas, situated on different involved sites of the trunk and the extremities, were ablated.

Grafting procedure

After preparation of the wound bed the melanocyte-containing matrix sheet was lifted with forceps from the microdish and turned upside down onto the freshly ablated wound bed. Then the sheet was fixed to the wound bed using non-adhesive gauze and a sterile tape, which was left for at least 5 days. Three weeks after transplantation, phototherapy (UVA-light) was initiated with doses up to 12 J/cm² and a frequency of 3 times weekly. Post-grafting evaluations were performed after 1, 2 and 3 months.

RESULTS

In 2 patients a good growth of melanocytes was achieved in culture (Fig. 1). In these patients the skin biopsies had been taken from the inguinal or scrotal area, respectively. Two of the nine areas treated showed complete repigmentation, three areas were partly pigmented, and four areas exhibited no pigmentation at all (patients #2 and #3). Fig. 2 demonstrates an original test lesion before grafting (2a), after removal of the entire epidermis (2b) and the outcome 3 months after the intervention (2c). The grafted circular skin area was completely repigmented, whereas the adjacent vitiliginous skin remained unchanged despite of the exposure to photochemotherapy.

DISCUSSION

Among the pulsed infrared lasers used for superficial tissue removal and in skin resurfacing procedures, the Er:YAG laser provides an extremely efficacious and controlled ablation avoiding unwanted thermal injury due to its high absorbance in tissue water (11, 12). Hence, in contrast to CO₂-laser vaporization, an accurately defined ablative lesion with excellent depth control producing wounds comparable to those obtained by dermabrasion can be created (13, 14). The precision of this technique in generating superficial lesions with maximum sparing of adjacent tissue structures led us to test Er:YAG laser ablation for preparation of a wound bed for melanocyte grafts in the treatment of vitiligo.

Melanocyte grafts were first introduced by Lerner et al. in 1987 (1), who injected cultured cells into a suction blister of a patient with piebaldism. Thereafter, different approaches for melanocyte grafts were published (2–10). Also different culture conditions and grafting materials (e.g. collagen film, epidermal sheet) were employed. Olsson & Juhlin were the first in 1992 to introduce a medium free of phorbol esters, pituitary extract and serum (3). The various transplantation techniques mainly differed in the way in which the donor tissue was harvested (shave biopsy, punch biopsy, suction- or cryo-induced blister, split skin) or the recipient site was created (dermabrasion, suction- or cryo-induced blister).

In our study we tried to optimize the technique of recipient site preparation by using the Er:YAG laser, allowing a stepwise and controlled ablation. Good cosmetic results (com-

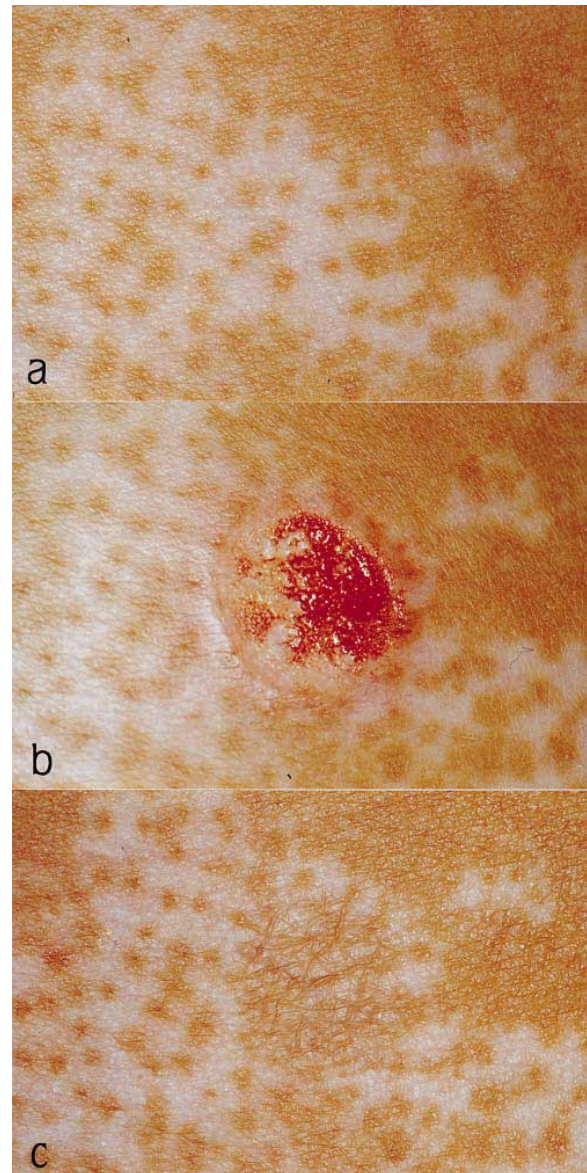


Fig. 2. Grafting of vitiligo following laser ablation. (a) Right lower quadrant of the abdomen of a 38-year-old female patient with vitiligo prior to therapy. (b) Lesion following Er:YAG laser ablation. The de-epithelialized area is 1.5 cm in diameter. (c) Same lesion 4 months after grafting.

plete and partial repigmentation) were achieved in five of nine treated areas. Three of the four test sites which showed no repigmentation belonged to the 63-year-old patient, who had only minor proliferation of melanocytes in culture obtained from the axillary skin. Otherwise, the response was as good as expected from conventional techniques reported thus far by other groups. Apart from the clinical success, this approach offers some distinct advantages. Since there is nearly no pain perception while ablating the epithelium, the entire procedure can be performed without local anaesthesia in most areas or it otherwise requires only minor pain relief using topical

anaesthetic creams. Infiltration anaesthesia should be considered only in sensitive sites, such as the eyelid skin. Epithelial ablation is a fast procedure and the operation can be performed on an outpatient basis. The Er:YAG laser offers an optimal tool for depth control when ablating the skin surface and so helps in providing perfect transplantation conditions. Moreover, the precision of the technique enables us to ablate also delicate areas such as the periorbital region of the face or the back of the hands. In addition, we were able to exactly follow the borders of each vitiligo lesion, which can be irregularly shaped. Only recently, Khan has demonstrated a good take of epithelial split-thickness skin grafts on vitiliginous skin after de-epithelialization has been achieved by pulsed carbon-dioxide laser vaporization (15). However, this technique still produces some degree of underlying tissue necrosis due to the amount of residual thermal damage created because of the approximately 10-fold less absorption of the 10640 nm wavelength in tissue water as compared to the 2094 nm Er:YAG laser radiation. Though most of the necrotic tissue debris can be mechanically wiped away, resulting in a clean surface, CO₂-laser removal of the skin surface still results in additional collagen shrinkage, which might be beneficial in some cosmetic skin resurfacing procedures but which could on the other hand interfere with proper depth control or wound healing when treating larger areas in critical sites (e.g. neck, periorbital skin, back of the hands).

In conclusion, the application of the Er:YAG laser presented here provides an additional surgical tool in the generation of erosive skin defects necessary for cell graftings in vitiligo patients. We believe this method to be of particular value for critical anatomical sites, where the use of dermabrasion and other related techniques is impractical.

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