

# Tissue-Engineered Skin in the Treatment of Vitiligo Lesions

( recombined human/porcine skin / upside-down grafting / melanocyte / keratinocyte / tissue culture )

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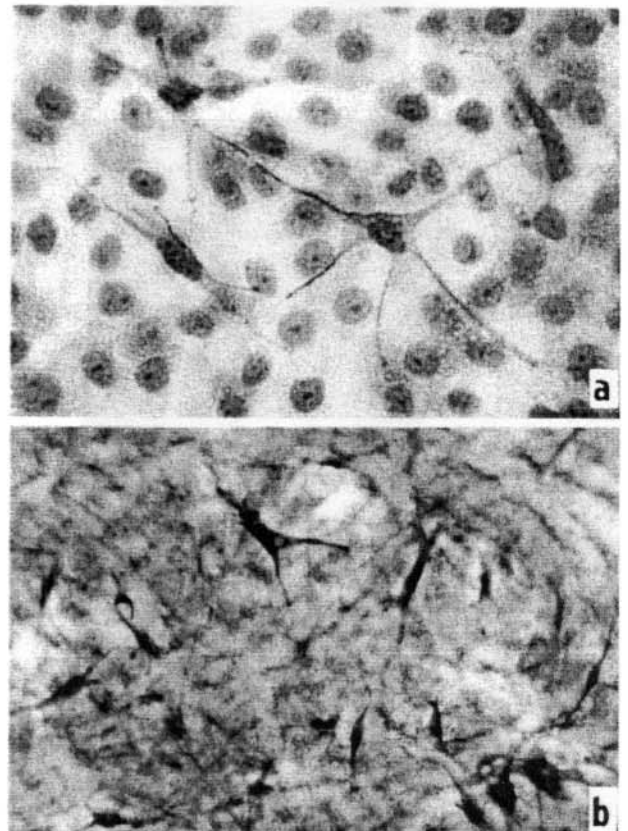
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**Abstract.** Vitiligo is characterized by the loss of skin pigmentation due to the destruction of melanocytes. Its treatment is usually difficult. For stable cases, melanocyte transplantation is the method of choice. A newly developed treatment with recombined human/porcine skin methodology, permitting easy handling of the graft, is described in the present work. In five vitiligo patients, autologous epidermal cells were obtained from pigmented thin skin biopsies. The cells were cultured on a dried cell-free porcine dermis by the 3T3 feeder layer technique. After 10 days melanocytes were regularly dispersed in confluent keratinocyte cultures. Upside-down delivery of epidermal cells was used. The epidermal layer was directly applied onto a dermabraded vitiligo lesion, with porcine dermis covering the lesion. Pigmentation started to be visible 4–6 weeks after grafting. After using the above described methodology, the pigmentation appeared in the range of 65–80% of the grafted area. Additional UVA irradiation enhanced the treatment success up to 100%. The surgical vitiligo treatment appears to be a reasonable method of choice in stable vitiligo cases of a disease lasting for at least two years, which means for approximately 5% of all vitiligo patients.

Vitiligo is a skin disease characterized by the loss of skin pigmentation due to the destruction of melanocytes. Standard vitiligo treatment is seldom successful and pigmentation is difficult to achieve (Kenney, 1971; Pathak et al., 1984). None of the current therapeutic methods makes it possible to solve the main problem, which is the absence of melanocytes in vitiligo spots. Therefore, attempts were performed recently to implant autologous melanocytes into the skin as enriched melanocyte cultures

(Olsson and Juhlin, 1993; Zachariae et al., 1993; Löntz et al., 1994), as cultured epidermal cells on a collagen-coated membrane (Plott et al., 1989), or as standard cultured epidermal sheets grown by the feeder-layer technique (Kumagai and Uchikoshi, 1997; Pellegrini et al., 1998). We have developed recombined human/porcine skin (RHPS), consisting of human epidermal cells cultured on acellular porcine dermis by the 3T3 feeder-layer technique (Matoušková et al., 1993). RHPS has been serving as a delivery system for allogeneic (allo-RHPS)



*Fig. 1.* Melanocyte growth in the keratinocyte culture. (a) Growth on the tissue culture dish; melanocytes stained with DOPA, keratinocytes counterstained with May-Grünwald and Giemsa-Romanowski, (b) growth on acellular porcine dermis; melanocytes stained with DOPA, keratinocytes are not stained and therefore not visible.

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Abbreviations: DOPA – L-3,4-dihydroxyphenyl-alanine, PBS – phosphate-buffered saline, RHPS – recombined human/porcine skin.