keratinocyte sheets have to be enzymatically released from the dish, mounted to the vaseline gauze and transferred to the wound with the basal layer facing the wound. The enzymatic release can be harmful to the cells (McKay et al., 1994), but the greatest disadvantage of keratinocyte sheets is their fragility, hypersensitivity and difficult handling. Several types of membrane delivery systems were, therefore, developed to solve the problems of graft instability. It has been presumed that only pre-confluent keratinocytes on a membrane can be grafted, because confluent keratinocytes are stratified and differentiated, and therefore cannot change orientation and attach to the wound (Barlow et al., 1992, Harris et al., 1998).

We showed that our RHPS made it possible to successfully treat donor sites and deep dermal burns. Not only pre-confluent but also confluent and post-confluent keratinocytes could be used (Matoušková et al., 1997). The advantage of RHPS in comparison with cultured epidermal sheets (Rheinwald and Green, 1975; Green et al., 1979) and keratinocytes grown on a synthetic membrane (Barlow et al., 1992) is the skin-like consistency, optimum adhesiveness and haemostatic effect (Matoušková et al., 1997). The recombined skin is applied to the wound with inverted orientation: keratinocytes down and dermis up. However, the 'upside-down' application often provoked the question whether keratinocytes can change orientation and temporarily 'take' or whether such a graft stimulates wound healing only by local production of growth factors (Harris et al., 1998).

The ability of keratinocytes to migrate from the inverted confluent keratinocytes-RHPS onto the culture dish was reported already in our previous study (Matoušková et al., 1997) and here it is demonstrated in Fig. 1. A proliferative potential of the confluent keratinocytes on RHPS was proved in the organotypic RHPS model (Matoušková et al., 1998). The temporal 'take' of inverted cells to the wound bed can be further explained by the fact that keratinocytes on the pig dermis, in contrast to multilayered keratinocyte sheets, form usually only 1–2 layers. This may be caused by a poorer medium (H-MEM instead of D-MEM/Ham's F12, high bovine and low foetal bovine serum, absence of adenine, transferrin and triiodothyronine) than is usually applied in Green's 3T3 technique (Matoušková et al., 1989).

Few data are available about early regeneration of human epidermis *in vivo* after cultured keratinocyte grafting. Usually, the earliest biopsies are taken about one week after transplantation at the point when the epidermis is already fully stratified. Therefore, morphological analysis of the early phase of wound healing has been missing. Thanks to the RHPS skin-like consistency, we could afford to take biopsies as early as on day 4 after grafting. Similar early samples were only obtained from the human skin organ culture model *in vitro* (Moll et al., 1998). There appears to be a good correlation between our and these results.

For identification of allogeneic donor keratinocytes, two markers were used: the Y chromosome of male keratinocytes grafted onto a female patient, and the expression of vimentin shown to be characteristic for cultured keratinocytes (Franke et al., 1982; Auböck et al., 1989; Moll et al., 1998). The Y-chromosome signals were detected in nuclei of single cells or islands of cells on days 4 and 6. On day 9 the Y-chromosome signals were no more detected, which indicates disappearance of donor cells. Comparison of morphological and immunohistochemical results with localization of Y-chromosome signals led to the conclusion that we did not succeed in irregular neoepidermis morphology. Otto et al. (1995) reported a similar observation by finding that in more differentiated cells the Y chromosome could not be labelled.

The expression of vimentin is considered to indicate the culture origin of donor keratinocytes (Franke et al., 1982; Auböck et al., 1989; Moll et al., 1998). Vimentin positivity found on day 4 (Fig. 1/B3) in areas without adnexa remnants in cells with cuboidal morphology shows that donor keratinocytes were present on interfaces with human as well as pig dermis. The absence of involucrin and K10 in the same layers further indicates their basal cell character. Persistent expression of vimentin in the 6-day-old epidermis (Fig. 1/C3) indicated that donor keratinocytes were still present while on day 9 they were replaced by host cells (Fig. 1/D3). The absence of vimentin expression on day 4 in areas with adnexa remnants on the other hand confirms the early regeneration of the host epidermis.

Both the Y chromosome and the course of vimentin expression in combination with the overall evaluation of the process of healing (see Table 1) confirmed the early colonization of the wound by allo-keratinocytes, demonstrating their direct involvement in the wound closure. Similarly as in the case of cultured allogeneic keratinocyte sheets, the allo-RHPS did not elicit any visible signs of rejection. This is understandable because the donor cells disappear soon after the wound is healed, between days 6 and 9.

We are well aware of the small number of histological examinations. However, our intention to use the same batch of freshly prepared keratinocytes for treatment of similar affection has limited the number of cases suitable for investigation. Moreover, the required patient's consent has constrained our endeavour to perform one biopsy per case. In spite of these problems the results are in accord with our long-term histopathological evidence about wound healing stimulated by RHPS (Matoušková et al., 1997). The results of FISH and immunohistology for vimentin expression, together with localization of K10 and involucrin, fitted well into the picture of facilitated wound healing. It was clearly shown that confluent allogeneic human keratinocytes delivered to the wound via the RHPS technique have temporarily 'taken' and stimulated regeneration of the epidermis.

Our results demonstrated that allogeneic keratinocytes cultured to confluency on xenodermis and

grafted 'upside-down' can 'take' temporarily, close the wound and, being after about one week replaced by the patient's own cells, positively influence wound healing.

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