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 han-
dling. Several types of membrane delivery systems were,
therefore, developed to solve the problems of graft insta-
bility. It has been presumed that only pre-confluent ke-
ratinocytes on a membrane can be grafted, because con-
fluent 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 suc-
cessfully 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
erpidermal sheets (Rheinwald and Green, 1975; Green et
al., 1979) and keratinocytes grown on a synthetic mem-
brane (Barlow et al., 1992) is the skin-like consistency,
opportunity of adhesiveness and haemostatic effect
(Matoušková et al., 1997). The recombined skin is
applied to the wound with inverted orientation: ke-
ratinocytes 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 inveto-
ed 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 prolifer-
ating 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 ke-
ratinocytes 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 triiodothyro-
nine) 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 cul-
ture 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 cul-
tured 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 immuno-
histochemical results with localization of Y-chromosome
signals led to the conclusion that we did not succeed in
reported a similar observation by finding that in more dif-
ferentiated 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 inter-
faces with human as well as pig dermis. The absence of
involutrin 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) indi-
cated 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
ey earlier 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, demonstra-
ning 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 con-
sent has constrained our endeavour to perform one biop-
sy 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 immunohisto-
lumination for vimentin expression, together with localization of
K10 and involucrin, fitted well into the picture of facili-
tated 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 ke-
ratinocytes cultured to confluence 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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References


