

Fig. 1. Migration of keratinocytes from the inverted RHPS after 2-week storage at room temperature Keratinocytes on the acellular porcine dermis were cultured to confluency at  $37^{\circ}$ C in a  $CO_2$  incubator. Then some dishes were kept for 2 weeks at room temperature ( $22^{\circ}$ C approx., the dishes closed with parafilm, medium changed twice a week). After this 'cold' storage three RHPS samples ( $2 \text{ cm}^2$ ) were placed 'upside-down' onto new culture dishes containing a 373 feeder layer, weighed down with a piece of cover glass and put at  $37^{\circ}$ C in the  $CO_2$  incubator. The keratinocytes started to migrate from the RHPS after 3 days. They continued to migrate and proliferate on the culture dish for at least another 3 weeks. The picture shows migrating and proliferating keratinocytes after two weeks of 'cold' storage at room temperature followed by 3 weeks at  $37^{\circ}$ C in a  $CO_2$  incubator. The same result was achieved with the control RHPS kept for the whole period of time (2 weeks normal orientation and 3 weeks 'upside-down') at  $37^{\circ}$ C in a  $CO_2$  incubator.

treated with silver sulfadiazine cream. The areas grafted with RHPS ranged from 12 to 250 cm<sup>2</sup> (1–6% total body surface area (TBSA)).

## Grafting with RHPS

The recombined skin was placed 'upside-down' (the keratinocyte layer facing the wound) on the wound bed and covered with tulle gras and gauze wetted with cholera toxin-free medium. The grafts showed a strong haemostatic effect.

## Wound preparation

- a) <u>Tangential excision</u>: laminar excision with a Watson knife, gentle enough to avoid losing deep epidermal cells. Seventeen wounds were covered with RHPS and, for control, 5 wounds with xenografts and 4 with Aquagel. Early excision (up to day 5) was performed in 12 out of 17 wounds.
- b) <u>Surface dermabrasion:</u> blunt abrasion with the longitudinal edge of forceps. The wounds hardly bleeded. Eight wounds were covered with RHPS, 4 with xenografts and 3 with Aquagel. One wound was treated with silver sulphadiazine cream.

c) <u>Deep dermabrasion</u>: in the early phase the wounds were treated with antibacterial creams (silver sulphadiazine), which prevent infection but also encourage necrolysis; blunt dermabrasion to the level of capillary bleeding was performed 4–8 days later, in the phase in which the thin necrotic layer had started to dissolve. Six wounds were covered with RHPS.

## Results

## Case reports

<u>Case 1</u> (Fig. 2, patient No. 1) demonstrates the healing effect of RHPS compared to xenografts. A 20-year-old male suffered flame burns over 11% of TBSA. On day 5 the wound on the left calf was tangentially excised and covered with xenografts. On day 7 a part of the wound was treated with one 30-cm<sup>2</sup> RHPS graft (Fig. 2a); the rest of the wound was covered with xenografts. The area treated with RHPS healed within five days (Fig. 2b) while the area covered with xenografts had to be autografted on day 14. Histology of the area healed under RHPS is shown in Fig. 2c.