Necrobiotic Process Causing Burn Wound Conversion May Be Prevented by Allogeneic Keratinocytes Delivered by the Recombined Human/Pig Skin

(necrobiosis / cultured keratinocytes / delivery system for keratinocytes / recombined human/pig skin / grafting upside-down / wound healing)

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Abstract. The spontaneous necrobiotic process frequently causes conversion of DDB (deep 2nd degree wounds) into full-thickness skin loss (3rd degree wounds). We found that this process may be positively influenced by the activity of living human allogeneic keratinocytes cultured on acellular pig dermis. This RHPS, if applied 'upside-down' with the epidermal layer facing the wound, provides an opportunity for keratinocytes to influence the healing. The aim of the present study was to find conditions, in terms of timing and wound-bed preparation, for optimum healing activity of RHPS. The wound beds were prepared either with tangential excision, surface dermabrasion or deep dermabrasion. Out of 17 wounds grafted with RHPS after tangential excision, 15 (88%) healed in 4–10 days; early excised wounds (up to day 5) healed within less than 10 days after the injury. Out of 8 wounds grafted after surface dermabrasion, only 2 (25%) healed. Out of 6 wounds grafted with RHPS after deep dermabrasion, 4 (67%) healed. The optimum healing effect of RHPS and prevention of conversion was achieved in early tangentially excised wounds.

Deep dermal burns (DDB, deep 2nd degree wounds in which residual adnexa are present) frequently tend to convert into full-thickness skin loss (3rd degree wounds, no adnexa present) by the necrobiotic process (Deitch et al., 1983; Muller et al., 1996). Although early excision and grafting dramatically changed local care, this procedure is still restricted by the difficulty in diagnosing the burn depth, by limited donor sites and by technical skills to excise certain areas. The depth of the burn wound is a determinant of the late prognosis, causing fatal outcome in very extensive burns and influencing the quality of life due to scar formation in survivors. The depth is a variable factor, being altered by many additional insults. A wound that appears superficial on day one may appear deep on day three or four. Conversion of superficial dermal into deep dermal or even into full-thickness skin loss is encountered during the whole course of burn treatment (Deitch et al., 1983; Muller et al., 1996). Prevention of this conversion has so far been rather difficult.

Treatment of DDB consists either in the application of antibacterial creams or in gentle laminary excision and immediate coverage of the excised area with biological or synthetic covers, or with thin split-thickness autografts (in these cases 'overgrafting' may occur) (Janzekovic, 1970). Treatment with antibacterial creams prevents development of early infection, but actual healing is prolonged (Muller et al., 1996), because the necrolysis of the thin necrotic layer has to take place and thus the spontaneous epithelization from residual adnexas is delayed. If the healing takes too long, granulation tissue develops, resulting in hypertrophic scars and following contractures. On the other hand, early excision removes the thin necrotic layer and an appropriate immediate coverage of the wound bed prevents dehydration, which would otherwise result in microthrombosis followed by conversion into full-thickness skin loss. The quality of the cover applied on excised areas influences healing decisively (Janzekovic, 1970) - the majority of temporary covers protect the wound but do not sufficiently stimulate epithelization; meshed autografts have unwanted cosmetic consequences.

In the last two decades it was shown that cultured human keratinocytes (Green et al., 1979) can serve as an effective biological cover of burns and other types of wounds (O'Connor et al., 1981). Autologous as well as allogeneic keratinocytes strongly stimulate healing (O'Connor et al., 1981; Madden et al., 1986; Bolivar-Flores et al., 1990; Nuñez-Gutiérrez et al., 1996),

Received February 19, 2001. Accepted April 3, 2001.

This work was financially supported by grant No. 4368-3 from the Grant Agency of the Ministry of Health of the Czech Republic.

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Abbreviations: DDB – deep dermal burns, FISH – fluorescence *in situ* hybridization, RHPS – recombined human/pig skin, TBSA – total body surface area.

although they do not solve (without allograft skin pregrafting) the problem of full-thickness skin loss (Navsaria et al., 1995). However, they may decrease its size by preventing the conversion of a deep dermal into a full-thickness wound. An integral therapy using allogeneic cultured keratinocytes for treatment of deep dermal burns and donor sites resulted in decreasing time of hospital stay (Nuñez-Gutiérrez et al., 1996). However, wide application of cultured epidermal sheets is hampered because the graft preparation prior to operation is complicated (sheets must be enzymatically released from the culture substratum and mounted on the backing material), grafts are very thin, difficult to handle and hypersensitive to all kinds of stress and, particularly, to infection (Navsaria et al., 1995).

To solve these problems, we have developed recombined human/pig skin (RHPS) composed of human allogeneic keratinocytes cultured *in vitro* on sterile acellular porcine dermis (Matoušková et al., 1993). If the RHPS is applied in an 'upside-down' manner, an effective keratinocyte delivery system is achieved (Matoušková et al., 1997). Keratinocytes temporarily 'take' to the wound bed and stimulate epithelization from the patient's own cells, while pig dermis protects the wound from mechanical damage, desiccation and infection (Matoušková et al., 1997). Temporary incorporation of allogeneic keratinocytes into regenerating epidermis (temporary 'take') was proved by positive fluorescence *in situ* hybridization (FISH) for the Y chromosome in female donor sites grafted with male keratinocytes delivered by RHPS (see Pokorná et al., in this issue).

However, to achieve an optimum effect, the wound bed has to be prepared in a suitable manner. In our previous study we used RHPS for treatment of DDB and donor sites (Matoušková et al., 1997). Healing of DDB after dermabrasion of the wound surface varied and was sometimes unsuccessful, whereas freshly harvested bleeding donor sites always healed quickly, even in patients with prolonged wound healing (Matoušková et al., 1997). This experience indicated that RHPS has to be applied differently from usual biological covers (e.g. xenografts) and that the wound has to be prepared in a way similar to donor sites. Gentle excision of the wound surface, saving the deep epidermal appendages, seemed to be crucial for the keratinocyte 'take'.

The purpose of the present study was to define the proper type of wound-bed preparation and its timing in order to secure an optimal healing effect of RHPS grafts. The main aim was to initiate the healing process in DDB before the necrobiotic process becomes irreversible and thus to prevent conversion into full-thickness wounds.

Material and Methods

Preparation of RHPS

Keratinocytes were obtained from the redundant skin of healthy donors after plastic operations screened for HIV and hepatitis B (between 1989–1994). More recently (from 1995), the donors were tested for hepatitis A, B and C, BWR, and twice for HIV with a 6-month interval, while keratinocytes were kept frozen in liquid nitrogen. RHPS was prepared in a manner described previously (Matoušková et al., 1993; Matoušková et al., 1997). Briefly, thin aseptically harvested strips of pig skin were trypsinized to remove epidermis and fibroblasts (Matoušková et al., 1993). The dermis was washed in sterile water and by drying attached to a tissue culture dish. Before starting the cultivation the dermis was disinfected for 10 min with 70% ethanol for antiviral safety. Human keratinocytes were cultured on the dermis using the modified Green's 3T3 feeder layer technique (Matoušková et al., 1989; Matoušková et al., 1993). Confluent RHPS grafts of 40–50 cm^2 can be stored alive for about 2 weeks at room temperature or at 37°C if the medium is changed 2–3 times weekly. The viability of stored cells was proved by their high metabolic activity (the pH of the medium was dropping regularly) and by the ability of cells to proliferate and migrate from the inverted RHPS onto the culture substratum (Fig. 1).

Cover types

RHPS: recombined human/pig skin composed of human allogeneic keratinocytes cultured on accellular porcine dermis to subconfluence or confluence (described above).

Xenografts: 0.2–0.3 mm thick sterile strips of pig skin prepared in the Skin Bank of the Prague Burn Centre, used routinely as a temporary cover of burns. Xenografts were used as control covers because they have similar composition and consistency as RHPS, but do not contain cultured keratinocytes.

Aquagel: hydrogel cover composed of 6% polyvinylpyrrolidone, 1.5% polyethylene glycol, 1% agar, and water (Rybus and Rybus, Lodz, Poland). Aquagel was chosen for control as one of the most effective synthetic covers used in the Prague Burn Centre especially for the slowly healing residual granulation areas.

Patients

The RHPS treatment has been approved by the Ethical Committee of the 3rd Medical Faculty, Charles University, Prague. Forty seven wounds in 22 patients (15 males, 7 females; age range 1–20 years) with deep dermal burns (19 scalds, 3 burns) were grafted, 31 of which with RHPS. Xenografts (9 wounds) or Aquagel (7 wounds) were used as controls. One wound was