Deep partial skin thickness burns: a reproducible animal model to study burn wound healing

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A reproducible deep partial skin thickness burn model using guinea-pigs to study the healing process of this injury is described. Round aluminium templates heated to 75°C and applied for 5 s to the moistened, clipped and depilated dorsal skin produced the desired depth of injury. This model is applicable for the study of the three main components of the burn wound healing process: epithelialization, contraction and scar formation. It is recommended that the India ink injection technique be used to confirm the depth of the burn wound.

Introduction

Not infrequently, inconsistency and irreproducibility of experimental data are shown by different groups of investigators using the same topical agents or dressings in the field of research into burn wound healing. The different and controversial findings may be attributed to two main reasons: First, the burn wound model employed is not identical and, secondly, the modality of treatment differs among the researchers.

A deep partial skin thickness burn is an excellent wound model to study the three main components of the burn wound healing process, i.e. epithelialization, contraction and scar formation. Moreover, prevention of ischaemia and promotion of the microcirculation in the stasis zone as mediated by various agents can be studied in great and careful detail using this model.

This report describes a reproducible deep partial skin thickness burn in guinea-pigs which has been used in our laboratories for over 1200 wounds during the past 8 years.

Methods

Animals

Female albino Hartley-derived guinea-pigs, weighing at least 500 g, were used. They were housed in individual cages for 2 weeks prior to commencing the study and were weighed three times a week. The animals were fed regular guinea-pig chow and water *ad libitum* enriched with vitamin C (1 g/week). Sodium bicarbonate was added to the water (in a similar amount) to ensure that the vitamin was dissolved. At the end of the adaptation period, those animals which failed to thrive or did not meet the narrow range of the required weight were excluded from the study. Generally, about 30 per cent of the animals were excluded.

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Preparation of the skin

Twenty-four hours prior to the injury, the animals were anaesthetized (ketamine HCl, 150 mg/kg, i.m.) and the back and abdomen were thoroughly clipped using an electrical clipper (Oster-Golden A-S, model 5-55E, 35 Watts, Head No. 80, Blade size 40). Then, the back and both flanks were depilated using a regular depilatory cream. This approach ensured a thorough and uniform removal of the animal's fur. Since clipping and the depilation procedures produce marked oedema of the skin, it is recommended that 24 h should elapse before the burn is inflicted.

The burn injury

The thermal source

Cylindrical aluminium templates (diameter, 3.76 cm; height, 3.78 cm; length of the handle, 24 cm; total weight, 500 g) were heated in a waterbath for 2 h prior to the injury at a constant temperature of 75°C. By this time, the template surface temperature equalled that of the surrounding heating water. Five or six templates should be heated concurrently and used alternatively one for each injury, and then be returned to the heating water, to ensure maintenance of the desired temperature of the template surface. About 5 min should elapse between reusage of the same template.

Wound location

In order to reduce significantly the biological variables among the various treated groups of burns in a set of experiments, we inflict two mirror-image wounds on the back of each animal so that each treated burn has its own control on the contralateral side of the animal. The anaesthetized guinea-pig (using identical dosages) is restrained and stretched on a metal meshed board, and the midline corresponding to the spine is marked on the animal's back as well as the horizontal upper limits of both sacroiliac joints (*Figure 1*). At the level of the mid distance between the twelfth rib and the horizontal drawn line, 1.5 cm are measured and marked mirror-image on both sides of the midline. These dots correspond to the medial borders of the burn wounds, providing a distance of 3 cm between them.

Inflicting the burn wound

The heated and moistened template was applied at right angles to the skin of the animal's back according to the

Figure 1. Marking the anatomical guidelines on the dorsal skin. The two burns are mirror images, while their outer boundaries are accentuated by a dye.

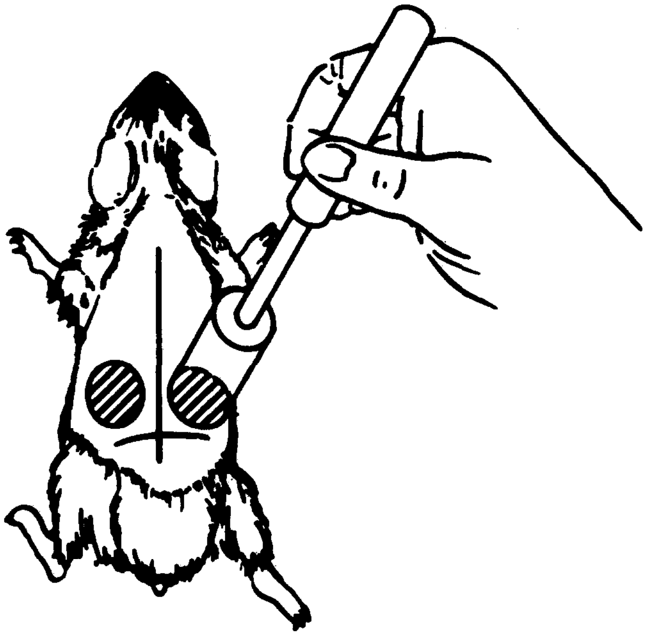


Figure 2. Deep partial skin thickness burns produced by applying cylindrical aluminium templates heated to 75°C for 5 s to the clipped and depilated moistened skin.

pre-marked locations, while an assistant is monitoring, aloud, 5 s (*Figure 2*) using an analogue stopwatch (Fisher Heuer, Switzerland). Only minimal pressure is required to ensure a perfect contact between the template surface and the underlying dorsal skin. In addition, it is recommended that the investigator should inflict both burns without crossing the animal's midline, i.e. it is necessary to turn by 180° the board with the animal, in order to produce the wound on the opposite side of the back.

Results

Assessing the burn wound depth

To establish the burn model, the depth of the burn wound is confirmed using the India ink injection technique (Kaufman et al., 1984). Briefly, at intervals of 0, 8, 16, 24, 36 and 72 h postburn the ink, in a volume of 2.5 times the total blood volume of the animal is injected into the aorta at a constant pressure of 400 mmHg. Tissue biopsies are harvested from the burn and the surrounding unburned tissue, and the extent of the unfilled dermal arterial plexus is compared with that of the healthy tissue.

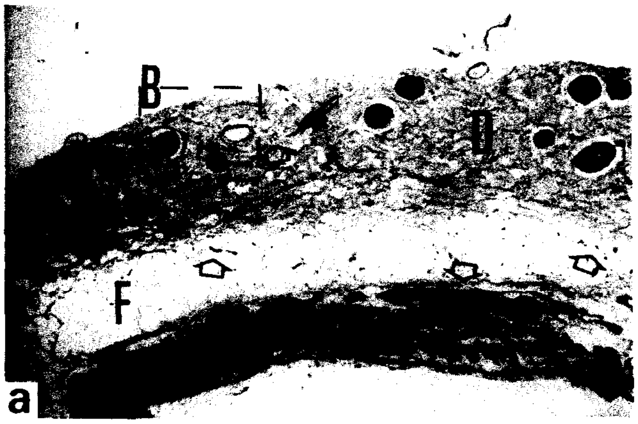
By applying the heated aluminium templates for 5 s to the dorsal skin, the India ink fills the subdermal plexus and only the deeper half thickness of the dermis 24 h postburn (*Figure 3*), while the injury deepens at 36 h postburn (*Figure 4*), indicating that a deep partial skin thickness burn has been produced. In contradistinction, when local humidity is applied to the wound surface, dermal ischaemia is prevented, resulting in survival of the hair follicles and, hence, the microcirculation (*Figure 5*).

Discussion

With the advent of new generations of drugs, topical agents and synthetic dressings, it is essential to use a proper experimental model to evaluate their potential beneficial effects on the healing process of burn wounds. It is obvious that if the agent is capable of reversing the microcirculatory stasis in the stasis zone via pharmacological, biochemical or

Figure 3. Histological section of an experimental deep partial skin thickness burn, harvested 24 h postburn. Note the pseudo-blister (B), the India ink filled subdermal plexus (SDP) and only the lower half thickness of the dermis (D). PCM, panniculus carnosum muscle; HP, hair follicles; F, fat tissue. (H & E, × 25.)

Figure 4. Histological section of a deep partial skin thickness burn, harvested 36 h following the injury. Note the progressive deepening of the burn and its conversion into a full skin thickness burn. Hair follicles are absent. E, eschar; D, dermis; F, fat; PCM, panniculus carnosum muscle. (Mason Trichrome, × 25.)



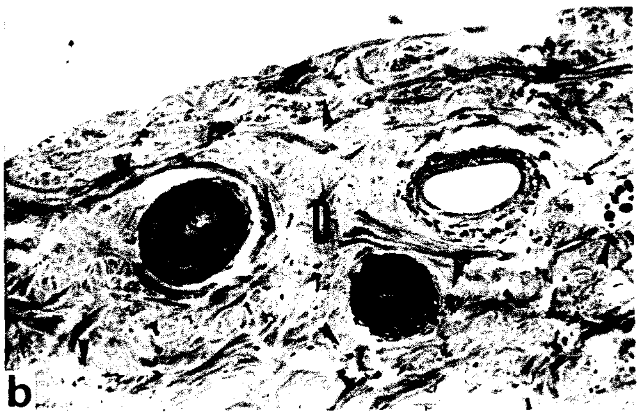


Figure 5. Section of a high humidity-treated deep partial skin thickness burn harvested 36 h postburn. Note the maintenance of the hair follicles and the prevention of deepening of the injury as compared to *Figure 4*. India ink fills the entire wound thickness suggesting prevention of ischaemia in the stasis zone. F, fat tissue; PCM, panniculus carnosum muscle; D, dermis; arrows indicate India ink. (H & E: Fig. 5 *a*, × 25; Fig. *b*, × 25.)

physical mechanisms, prevention of deepening of the burn wound is accomplished and spontaneous healing would be expected. Practically, it implies that a deep partial skin thickness burn should not be converted into a full skin thickness injury which requires excision and skin grafting. More superficial burns generally heal spontaneously unless local infection is superimposed. Hence, using a deep partial skin thickness experimental burn is mandatory for the evaluation of the effects of any agent which might directly or indirectly affect the healing of this injury.

The guinea-pig is an excellent model to study the healing of burn wounds because it maintains an almost constant skin thickness when its weight exceeds 450 g (Kaufman, 1982, unpublished data). In addition, its size permits the production of at least two relatively large wounds on its back, being 3 cm or more apart from each other, precluding any potential reciprocal effects. One might refrain from using this model in view of its dissimilarity to human skin. However, since we are investigating the basic physiological processes of healing, the accumulated data are pertinent concerning the physiology of human skin.

Supplemental vitamin C is recommended in view of the high demands by the animal and in order to preclude impairment of healing due to its deficiency.

Thermal energy transfer by conduction from a solid to the skin surface does not depend on the pressure employed, but mainly (Davies, 1982) on the temperature gradient between the skin and the solid and the path length through which the heat flows as shown by the following equation:

$$q = \frac{K}{L}(T_1 - T_2)$$

where:
K = thermal conductivity
L = path length through which the heat flows.
T_1 = temperature of the solid.
T_2 = tissue surface temperature.

Henriques and Moritz (1947) showed that the fast responses in the skin develop rapidly following application of a heated block. For example, a heated block with a temperature of 80°C induces a temperature of about 67°C in the skin within 0.5 s, whereas an additional 9.5 s exposure only raises the temperature to about 73°C, a very moderate rise.

Any variations of the light pressure of the heating template employed to produce the present animal model burn can be neglected. Moreover, since the burn wounds are selected at random by picking a card, the various injuries are equally distributed between the treated and control agents, as well as between the right and left sides of the animal's back.

According to previous findings (Henriques and Moritz, 1947), it would appear that the depth of the wound mainly depends on the thermal energy transferred as a function of time. In our experience, India ink injection showed that the application of aluminium templates heated to 60°C for 5 s produced a partial skin thickness burn (Kaufman et al., 1983; Kaufman et al. 1988a). On the other hand, application of 75°C for 5 s inflicted a deep partial skin thickness burn in over 1200 wounds (Kaufman et al., 1985a,b; 1988a,b; unpublished results).

For the study of burn wound healing, it is recommended that any interference with the natural and anatomical blood supply to the wound areas before inflicting the injury should be avoided. Some animal models have been described in which pneumoderma was induced under the dorsal skin by injecting 25–40 ml of air or nitrogen (Selye, 1959; Smahel, 1976), or undermining of the skin was carried out to enable the introduction of a Pyrex glass spatula (Stadtler et al., 1972; Kistler et al., 1988). Any compromise of the *in situ* anatomical blood supply could significantly impair or affect the healing process of this wound and hence, in our opinion, should be avoided.

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