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Controlled Partial Skin Thickness Burns: Rabbit Ear as a 2nd Degree Burn Wound Model for Studies of Topical Therapy

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ABSTRACT – This study was designed to prepare an animal model for partial thickness burn wound which can be employed for testing topical therapy. We first evaluated whether rabbit ear and mouse back skin wound model could differentiate the wound healing process in terms of degree of re-epithelialization, required days for complete wound closure, presence of scarring. 2nd degree wet burn were prepared on mouse back skin and rabbit ear by applying 5 mL hot water (85±0.1°C) for 7 sec followed by 5 mL ice-cold 0.5% acrynol solution for cooling and disinfecting the inflicted area. After removing the dead epidermis layer at 24 hr, tested dressings were applied for specified time and wound progression was investigated. In mouse model, wound contraction was the primary wound closing mechanism, which is quite different from human wound healing process. In rabbit ear model, epidermal regeneration was the major wound healing process rather than wound contraction and the difference in wound healing property among tested dressings could be clearly demonstrated. A rabbit ear model could differentiate the wound progression among open, occluded and epidermal growth factor (EGF) treated wound. Four sites of circular wound (diameter: 1 cm) on the anterior part of rabbit ear could be employed for the comparative wound healing study. For obtaining reproducible burn wound, degree of burn depth and burn sites should be carefully controlled in addition, employing rabbits of same strain and weight. The result suggests that rabbit ear could be employed as a reliable and human-resembled wound model.

Key words – Animal model, Burn wound, Wound progression, Rabbit Ear, Mouse

Wound healing in adult mammal proceeds by a series of overlapping highly coordinated events. Dermal wound repair commences with the arrest of hemorrhage followed by an inflammatory response, re-epithelialization of the wound, and formation of granulation tissue within the wound space, remodeling of the wound.[1)]

In order to study the effect of the topical therapy, reliable and reproducible dermal wound model is required. Animal models such as guinea pigs, rats and pigs have been studied to investigate burn wound pathology and local therapy.[2-4)] Lower vertebrates, such as rat and mouse, wound contraction is the primary wound healing process than wound regeneration. Mouse contracts the wound site very rapidly with almost no scarring and closes the wound sites much faster than human. Unlike the lower vertebrates, in human skin, epidermal regeneration is the major wound healing process and contraction proceeds very slowly. Wound heal progression in a deep second degree burn is very slow in human and open wound can not be healed in time without a proper treatment. The experimental result of wound healing process with mouse shows a quite different wound heal characteristics. Thus it can not be directly applied to human. In humans and other mammals, there are regional differences in the thickness of skin layers as well as in the distribution of hair follicles and sweat and sebaceous glands. The hairless porcine skin is very similar to human skin.[5)] However, pigs need more housing space and requires higher maintenance fee than smaller animals. Thus we first studied mouse as an animal model for partial thickness burn to access the preferred local therapy. But we observed the progression of wound healing between mouse and human was quite different. As the mouse species has to survive in very harsh and contaminating conditions, wound contraction which close the wound very quickly might be more efficient way than regeneration of the wound tissue. Considering this, mouse might not serve as a good animal model for comparative wound healing study of local therapy. Thus we tested New Zealand white rabbit as an animal model for studies of burn wound progression. Although the skin of rabbits is more elastic than human skin, the structural configuration of the skin layers in rabbits is similar to human skin[6)] and provides scar in the preliminary study.[7-9)]

In this study, we prepared deep 2nd degree burn animal model and applied the tested topical therapy such as collagen sponge wound dressing containing epidermal growth factor and silver sulfadiazine and evaluate whether rabbit ear and

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mouse back skin wound model could differentiate the wound healing process in terms of degree of re-epithelialization and wound closure among tested topical therapy.

Experimental

Collagen sponge preparation

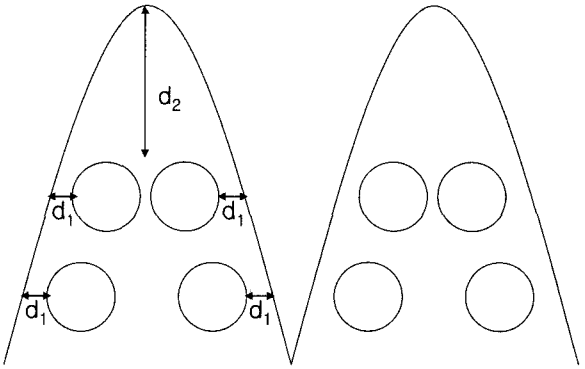
Collagen used in the present study was acid-soluble fraction of pig skin, which was extracted by hydrochloride and acetic acid solution, precipitated by saturated sodium chloride solution and moderately desalted. The concentration of collagen stock solution was 13 g/L and collagen sponge was prepared by freeze drying 3.8 g-stock solution in 35-mm petridish. The resulting sponge was crosslinked by dipping in 5-mL hexamethylene diisocyanate (1% in methanol) for 10 min. Subsequently, the sponge was washed twice with MeOH and water, and lyophilized sponge was stored at −20°C until used. Epidermal Growth Factor (EGF) was incorporated into collagen sponge by suspending the sponge in the solution containing EGF at specified concentration (0, 2, 4 and 8 ug/1.7 cm^2 circle).

Preparation of 2nd Degree Burn Wound Model

Mouse model– Deep second-degree burn circular wounds (1 cm diameter) were prepared on the back skin of ICR mouse of 5 weeks old. On the dosal side, two sites of burn wound were induced to each mouse by applying 85±0.1°C water for 7 sec. After 24 hr, the dead epidermis layer was scrapped off with curette. Specified dressing formulations were applied for 96 hr. For each tested dressing, at least 6 wound sites were employed.

Rabbit ear model– New Zealand white rabbit (average body weight, 2.0±0.2 kg) were housed in individual cages and acclimated for 1 week prior to the study. The rabbits were fed and watered *ad libitum*. Rabbits were anesthesized by ethyl ether inhalation (4-8 mL) for 30 sec and rabbits became sedated.

Four sites of circular wound (1 cm diameter) were induced on the anterior surface of both ears of rabbit by applying 5 ml hot water (85±0.1°C) for 7 second as illustrated in Scheme I. Total 24 wound sites were prepared for comparative wound healing study among various dressings. Thermostat System (Polyscience Co., Illinois, USA) was used to control the temperature of the applied hot water. And open 1 cm diameter cylinder was placed on the treated area and 5 mL hot water was placed for 7 seconds. And the burn area was cooled and disinfected with 5 mL 0.5% acrinol aqueous solution which was chilled in ice-bucket, for 30 seconds. Burned animals were placed in fixant for a day.



Scheme I–Preparation of deep 2nd degree burn wound model. Four circular wounds (diameter: 1 cm) were inflicted on the anterior part of rabbit ear. For a reliable comparative wound healing study, the burn wound position should be well controlled to have same distance from the top and the side.

Location of burn lesions– The burn was inflicted on the anterior part of the rabbit ear with a diameter of 1 cm; two horizontal burn sites were prepared as shown in Scheme I.

Harvesting of skin and assessment of the depth of burn

At least three histological sections were cut out of each tested region and stained with hematoxylin-eosin to differentiate vital and damaged skin cells. The healthy dermis has a characteristic collagen structure. As partial-thickness burns cause a characteristic change in the collagen structure of the skin, the presence of weave like collagen fibers and irregular collagen bundles were investigated.

Application of tested dressing

After 24 hr of post burn time, the dead epidermis layers were scrapped off with curette followed by an application of 5 mL of iced cold 0.5% acrinol solution for 30 seconds to prevent the bacterial contamination. Each freeze-dried collagen sponge (1 cm diameter) was wetted with 200-600 μL sterile water and applied on the wound area. An occlusive dressing Duoderm® (Convetec Co., NJ, USA) was finally placed. Since wound healing process was affected by the occlusion condition and the exudate absorption capacity of the dressing, all the treated area were covered with an occlusive dressing except open, untreated wound as a comparison. As an occlusive dressing, Duoderm® (Convetec, NJ, USA) was employed. The tested dressings were applied for 96 hr and wound area remained open thereafter. After 120 hr post burn time, tested dressings were removed and the wound sites were investigated in terms of degree of re-epithelialization and wound sizes were measured by tracing on the cellophane film: the tracing area was

put on the graph paper and counted. The required days for complete wound closure and the state of healing in terms of wound contraction and scar formation have been continuously measured until complete wound closure.

Histological evaluation

The skin specimens were excised and processed for histological evaluation either immediately after the removal of the dressing (i.e., 96 hr after the induction of burn wound) or after the complete wound closure.

At the time of the removal of the dressing, the burn wound area remained moist and the skin appeared viable as evidenced by the fact that healing process was apparently ongoing in the specimens. Presence of inflammatory cells and degree of epidermal regeneration were investigated in the specimens. Olympus microscope (model BX41, Japan) and image recording equipment (models DP11 and PM10SP, Japan) were employed.

Results and Discussion

Figure 1 shows the progression of wound healing between collagen sponge impregnated with EGF with Duoderm® (head) and Duoderm® only (caudal). With EGF treated, more epidermal regeneration with less contraction is observed as compared with the one without EGF. Although EGF treated wound demonstrated a little favorable effect on wound healing, still wound contraction is predominant in both wounds.

Figure 2 shows the wounds either treated with collagen with silver sulfadiazine with Duoderm® (head) or Duoderm® only (caudal) after 12 days post burn. As the degree of wound contraction overwhelms the wound regeneration, no epidermal regeneration was progressed.

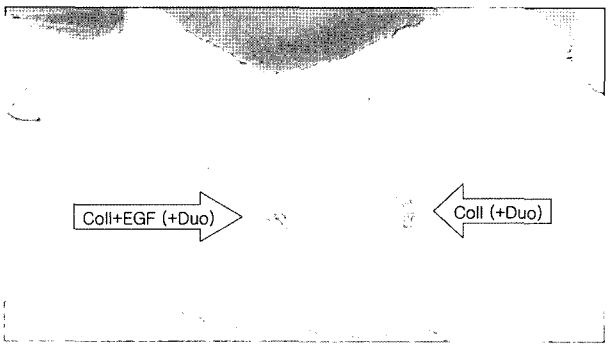


Figure 1–Comparison of wound healing after 12 days post burn between EGF treated and non-treated in mouse. In mouse, wound contraction is the primary wound closing process. In EGF treated wound, epidermal regeneration with less contraction is observed as compared with occlusive dressing only. However, wound contraction overwhelms epidermal regeneration; enhancement in wound healing is not clearly demonstrated.

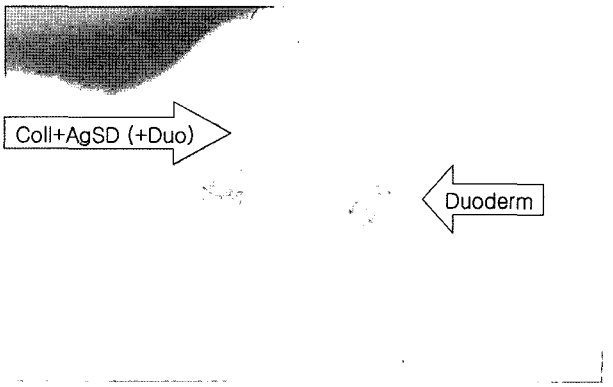


Figure 2–Wound contraction in open wound and in silver sulfadiazine (Ag-SD) treated wound. In mouse, wound contraction is the primary wound healing process; no significant difference could be observed between open wound and Ag-SD treated wound.

As shown in this result wound contraction is the primary wound closing process. In mouse, considering this, mouse might not be a desirable animal model for human-resembled skin.

Scheme I shows the preparation of deep 2nd degree burn wound model on rabbit ear. Four circular wounds (diameter : 1 cm) were inflicted on the anterior part of rabbit ear. For a reliable comparative wound healing study, the burn wound position should be well controlled to have same distance from the top (d_2) and the side (d_1).

Figure 3 shows that rabbit ear can serve as a reliable wound model to assess the effect of topical epidermal growth factor. Wound healing proceeds more favorably in 4 μg/1.7 cm^2 EGF treated wound as compared with the one of occlusive dressing only. While partial regeneration from the edge of the thermal wound (85°C, 7 sec) starts to appear in occlusive dressing only (A), almost complete epidermal regeneration appears in EGF+ occlusive dressing treated wounds (B) after 16 days

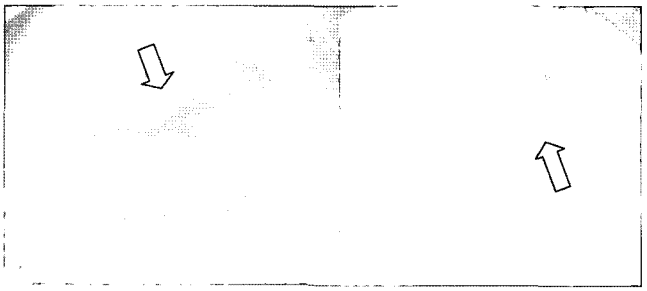


Figure 3–Rabbit ear for an evaluation of the effect of topical epidermal growth factor as compared with occlusion only.

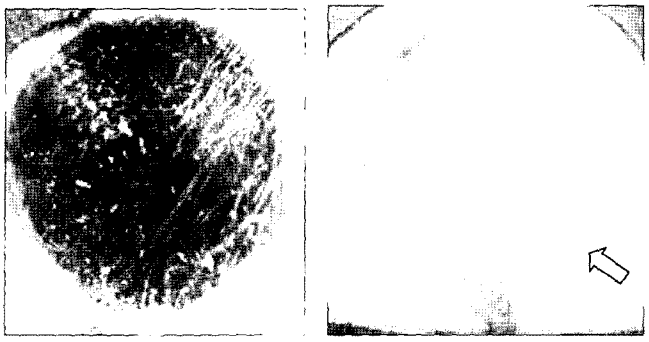


Figure 4–Effect of occlusion on the wound healing: comparison of epitherialization between open wound and silver sulfadiazine (Ag-SD) 2% cream+vaseline dressing treated wound at 16 day post-thermal injury (85±0.1°C for 7 sec).

post burn.

And in EGF treated wound, the re-epithelialization proceeds more rapidly and no sign of wound contraction alongside of the wounds were observed while a significant wound contraction was observed in without EGF treated wound. Based on these observations, EGF seems to have an inhibitory effect on wound contraction.

Figure 4 shows how rabbit ear wound model could differentiate the wound progression between open wound and tested dressing which is silver sulfadiazine impregnated collagen sponge with occlusion at 16 day post burn injury (85± 0.1°C, 7 sec). On occluded wound which provides moist environment suitable for epidermal regeneration, partial regeneration of the epidermis layer is observed, while in open wound, no epithelial regeneration is proceed. Hardy[1] reported that epithelialization can be facilitated by maintaining moist dressings, protecting the wound from minor repetitive trauma, avoidance of infection.

Unlike the significant wound contraction observed in mouse back skin model (Figure 1 and 2), wound contraction did not proceed in rabbit ear model. If we consider that wound contraction is the primary wound closing mechanism in mouse, no significant wound contraction observed in open wound in rabbit ear model suggests that the anterior part of rabbit ear could be employed as a human-resembled wound skin model.

Conclusion

In mouse, contraction plays a major role in wound healing process. A rabbit ear model could differentiate the wound progression among open, occluded and EGF treated wound. This result suggests that rabbit ear model could be employed as a reliable and human-resembled wound model.

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