



## A study on new experimental induction of wound using metal surface contact in a rat model

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### ABSTRACT

Burns injury is a global issue; when burns left unmonitored, it may lead to sepsis & hypovolemia. It majorly contributing to the country's morbidity and mortality. Accidental burns are quite common throughout the world. The degree of burns differs variably, and to identify a possible therapeutic option, it is of great importance for the researchers and scientists. Achieving a uniform burn wound model is thus required to study the burns injury at different levels involving epidermis, dermis, and hypodermis. The goal of this study is to create a simple third-degree contact burn in Wistar albino rats. Third-degree contact burn wound was monitored on 7th, 14th, and 21st day for natural wound healing under gross and histopathological observation. Gross observation reported damaged skin to appear dry, leathery, and charred. Wound closure was observed to be delayed and incomplete on day 21. Histopathology revealed a wound healing process by hematoxylin and eosin method. This metal surface contact method revealed a simplified and cost-effective method for inducing a third degree of a wound in Wistar rats. The study is thus a milestone that could enable research progress in the area of wound healing.



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### INTRODUCTION

Proteins form the basic building block of the body. The group of cells forms tissue, and a group of tissues forms organs; the largest body organ is skin. Skin consists of upper - epidermis, middle - dermis, and lower- hypodermis. Its primary function is to protect the body and keeps it hydrated with a desmin tight junction. Burns are acquired tissue injuries when the skin is subjected to chemicals, radiation, hot liquids, hot solids, flames, etc. (Rowan *et al.*, 2015). Heat injury on the skin causes complete damage to the skin resulting in loss of internal milieu.

According to the depth of injury caused in differ-

ent layers of skin, burns are categorized into 1st, 2nd, and 3rd-degree burns. First degree burns involve epidermis. The wound appears to be red, painful, warm, soft and blanched when in contact; generally, there are no blisters (Reich *et al.*, 2017). Second-degree burns comprise epidermis and dermis. These burns are characteristically blistered, very painful, red, soft, moist, and blanch when touched (Allahverdi *et al.*, 2018). The third degree involves epidermis, dermis, and hypodermis. Burns have slight pain or no pain, and can be brown, white, or burned and feel firm and rubbery to palpation devoid of blanching (Stone *et al.*, 2018). During the burn, injury tissue organization is lost with cell injury, inflammation, necrosis, and loss of collagen fibers. Wound healing is an ordinary phenomenon that comprises inflammation, proliferation, hemostasis, and tissue remodeling. The study aims to observe the wound healing pattern of metal plate contact burn injury in the skin of Wistar rats.

## MATERIALS AND METHODS

### Animals

The test facility has been registered with the committee for the controlling and supervision of tests on an animal, ministry of environment, forest and climate change, animal welfare division, Govt. of India (No. 1182 /PO/Rc/S/08/CPCSEA). The IAEC approval number for this study is MTR/IAEC/PRU/013-15. Animals were accustomed in room one weeks prior to the procedure. Room temperature and relative humidity were maintained from 19.9°C to 24.5°C and from 51 to 67%, respectively. The test room facility was provided with a photoperiod of 12 hrs light and 12 hrs dark conditions throughout the experiment. Sterilized standard polypropylene cages having a non-corroding steel grill on top was used for housing the animals. Sterilized, clean paddy husk was taken as a material for bedding. The grills, water bottles, and cages were kept on changing in alternative days. Each animal was kept separately throughout the experimental time. Animals were given standard rodent pellets, food, and regular UV purified RO clean water ad libitum.

### Burn injury induction

A total of 6 male Wistar rats being used to induce a simple third-degree contact burn model. Two days prior to the start of the procedure, hairs from the dorsum area of all rats were trimmed, using an electric hair clipper. Approximately 15 minutes before creating the wound, rats were anesthetized using a combination of xylazine at the dose of 10 mg/kg and ketamine at the dose of 80 mg/kg intraperitoneally

with a total volume of 1.5 ml/animal and the volume were injected as per the bodyweight of each animal. Burn wound was created on the dorsum region of each rat. In this simple burnt model, a metal plate of size in dimensions of 2.5 cm (length) x 2.5 cm (breadth) x 6 mm (depth) equipped with a wooden handle (Figure 1) was heated to 300°C on the burner and was placed on the shaved area for 10sec with a little pressure on animal and the same method was adopted for all rats resulting in simple third-degree contact burn. No pain management medication is given to animals because of third-degree burns.

All rats were observed for third-degree contact burnt healing. Healing measurement of an individual rat was calculated for every seven days throughout the experiment period and was recorded on day 1, followed by day 7, 14, and 21. Wound healing was detected by histopathology and gross observation.

### Wound contraction

Erythema, edema, and clinical signs were grossly observed under a simple contact burn model. The wound surface area was measured by radial planimetry. By locating the wound margins on acetate film, wound contraction was observed all the days.

Percentage of Wound contraction is calculated using formula;  $\frac{\text{Initial wound area} - \text{specific day wound area}}{\text{initial wound area}} \times 100$  (Chen *et al.*, 2015).

### Histology study

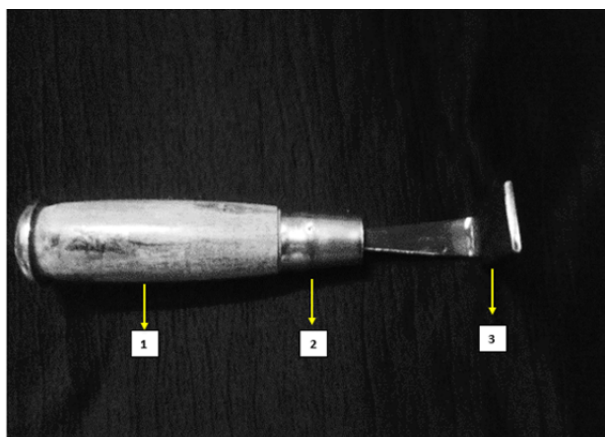
At the end of experimentation, the third degree burn treated model rats were sacrificed by an overdose of CO<sub>2</sub> anesthesia. The treated area of wounded skin samples was taken from each group on day 7, 14, and 21. The Skin tissue measuring 5-6 μm thickness was collected and stored formalin neutral buffer (10%) for the histopathological process. Tissue, once embedded with wax, were subjected to microtome and were stained using hematoxylin and eosin (H&E) dye for histological examination.

## RESULTS AND DISCUSSION

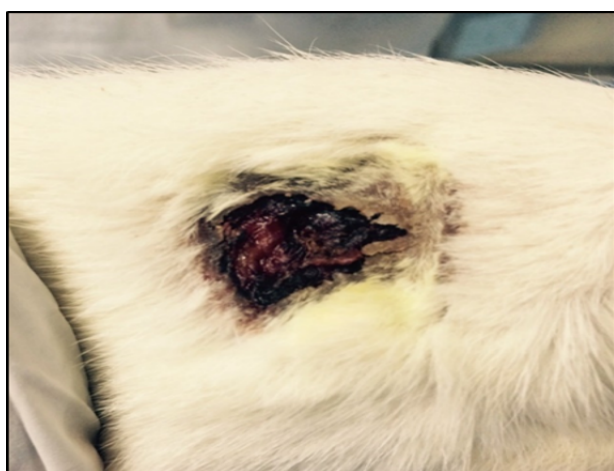
### Simple metal plate contact burn device

Burn wound was created on the dorsum of the shaved Wistar rat using a simple metal plate contact burn device (Figure 1). The simple metal plate contact burn device consists of three parts, namely A= Wooden handle, B=Neck, C=Metal plate. Wooden handle in the device serves as an insulator to protect the hand from the hot metal plate during metal plate contact burn injury. The neck is the constricted part between the wooden handle and metal plate. Metal plate of size 2.5cm (length) x 2.5cm (Breadth)

x 6mm (depth) was used for creating third-degree contact burn injury (Figure 2).



**Figure 1: Simple metal plate contact burn device. 1- Wooden Handle, 2 - Neck (Constricted Part Between Wooden Handle & Metal Plate), 3-Metal Plate**



**Figure 2: Simple metal plate contact burn model of Wistar rat. Day 1: Wound created from the device appears to be leathery, dry non-elastic and charred**

### Simple metal plate contact burn model of Wistar rat

A simple metal plate contact burn device was used to create third-degree burn on the shaved dorsum of Wistar albino rats. The depth of metal plate contact burns involved epidermis and dermis of the skin. The wound created from the device appears to be leathery, dry, non-elastic, and charred appearance (Figure 2).

Figure 3 A depicts the Day 7 wound - Extensive loss of epidermis with large scab consisting of necrotic cellular debris in fibrin coagulum (arrow mark) bordered with numerous degenerated and intact neutrophils; increased mast cells and histiocytes with moderate fibroblasts in the hypodermis.

Figure 3 B depicts the Day 14 - Extensive necrosis of epidermis with fatty changes disorganization of collagen and loss of adnexa in dermis; large scab with necrotic debris and fibrin coagulum, numerous neutrophils and bacterial clumps distorting the muscle and adipose tissue in the hypodermis (circle); increased fibroblasts, neovessels, mast cells and histiocytes in the hypodermis.

Figure 3 C depicts the Day 21 - Extensive loss of epidermis with the scab, increased fibroblasts, neovessels in the dermis with immature collagenisation and a moderate number of neutrophils, mast cells and histiocytes (arrowhead).

Healing measurement of an individual animal was measured and recorded on day 1 of burn wound followed by day 7, 14, and 21 (Table 1). A delayed wound healing pattern with incomplete closure of the wound was witnessed in rats with 21 days. The wound contraction and reduction in healing were observed on different days. There was a slight reduction in the size of the wound on day 21 in rats when compared to day 1. Wound closure was observed to be delayed and incomplete.

Burns injury is universal; to study simple third-degree burns, it is vital to create a simple third-degree burns model. This was achieved by designing a device with a metal plate of size 2.5cm (length) x 2.5cm (Breadth) x 6mm (depth). The metal plate was equipped with a wooden handle, which served as a source of insulation from heat.

The device was heated to 300°C on the burner. The hot metal plate of the device measuring size 2.5cm (length) x 2.5cm (Breadth) x 6mm (depth) was placed on the shaved dorsum area of Wistar rat for 10sec with a little pressure on an animal to create simple third-degree contact burn model. The adopted method of wound creation, however, has a minor disadvantage with the pressure that is induced for imposing a third-degree wound. It will be impossible for generating consistent pressure for creating the third-degree wound. This can be minimized by involving the same person to perform the procedure and also generating a similar amount of pressure. It was encountered during the procedure that there wasn't much impact when the same person was involved in creating the third-degree wound.

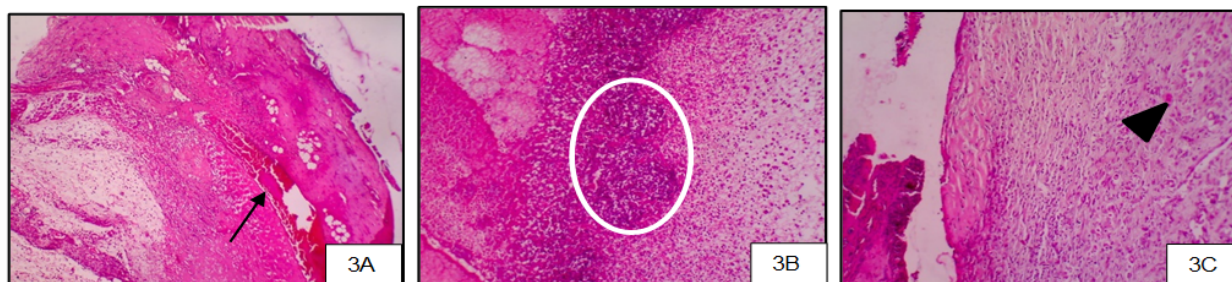
In our study, simple third-degree contact burns' injury was created 6mm deep on the skin using hot metal plate devices involving hypodermis, dermis, and epidermis of shaved skin of Wistar rat. When epidermis of thin skin is exposed to moderate sunburn or ultraviolet light, it causes capillary, venules in the papillary and sub papillary region to dilate



**Table 1: Simple metal plate contact burn wound measurement**

Days	Day 1	Day 7	Day 14	Day 21
Degree of Burn	6.25 ± 0.15	5.45 ± 0.14	6.06 ± 0.21	6.12 ± 0.16

Results were expressed as Mean ± SEM

**Figure 3: Photos depicts the histology study of simple metal plate contact burn**

by making it turn red – erythema. With severe burns, the capillaries and venules allow plasma to leak through them, resulting in accumulation of fluid between the epidermis and dermis, resulting in blisters (Widgerow *et al.*, 2015). In our study, the skin after third-degree burn appeared to be dry, leathery, and charred. Skin grafts are a common option for third-degree burns (Venter *et al.*, 2016). Under natural conditions, the epidermal cells surviving in the lower part of hair follicles regenerate over the blisters surface resulting in healing. Acute wound fluids have a positive effect of the wound; this could be due to increased stem cells. The rate of wound closure in simple third-degree contact burns was delayed in day 21 than the original at day 1. This could be due to the lack or non-availability of stem cells from the basal layer (Li *et al.*, 2017).

If the burn injury involves the superficial part of the dermis, epithelial cells from a deep part of the hair follicle proliferate to grow along with the interface between living and the dead dermis. However, if the burn damage extends beyond the base of the hair follicle, regrowth of epidermis can occur only at the perimeter of the burns. Delayed healing in chronic wounds is due to stem cell deficiency.

A delayed wound healing pattern of simple third-degree contact burns with incomplete wound closure was observed in rats with 21 days. This could be due to extensive loss of epidermis with large scab necrotic cellular debris with fibrin coagulum bordered in the epidermis.

Numerous degenerated and intact neutrophils were observed on day 7 of simple third-degree contact burns. Dermis showed numerous neutrophils in day 14. There is a moderate amount of increased in neutrophils was detected on the 21st day in the burn site. This could be due to a response of neutrophils

to the acute phase of inflammation (Rosales, 2018).

Mast cells secrete histamine and other substances during an inflammatory and allergic reaction (Benly, 2015). Mast cells slowly increase in hypodermis from day 7 of burns and seen moderate in day 21. This could be due to inflammatory mediators released from mast cells, causing inflammation and healing of burns. Inflammatory mediators (TNF- $\alpha$ , IL-1), platelet-derived growth factor (PDGF) growth factors (TGF- $\beta$ 1), and proteases are released during tissue injury (Nagar *et al.*, 2016).

A number of mast cells directly related to the degree of angiogenesis. In the present study, neovessels are formed in hypodermis on day 14. Vascular endothelial growth factor excites angiogenesis during hypoxia and shear stress (Hein *et al.*, 2015). Neovessels were addressed to have formed in the dermis on day 21.

Fibroblasts are an important cellular component in a connective tissue. In our study, the moderate fibroblast is appreciated in hypodermis on day 7. The fibroblast tends to increase in number on day 14 in the hypodermis of the skin. Fibroblast perpetuate to the burn site of the skin to produce ground substance and fibers. The dense collagen fiber produced by the fibroblast arranges irregularly to maintain the integrity of the skin. In the present study, day 21 shows increased fibroblast with immature collagenisation in the dermis. The closure of the wound was detected to be delayed and incomplete on day 21.

## CONCLUSIONS

The device is cheap, affordable, and can be used by researchers and scientists to study third-degree burn by a simple method of contact metal plate burn

injury. The device can be used for assessing the efficiency of topical medication and its healing pattern in third-degree contact metal plate burns.

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