

## An optimized animal model for partial and total skin thickness burns studies<sup>1</sup>

Um modelo animal aperfeiçoado para estudo de queimaduras superficiais e profundas da pele

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### ABSTRACT

**PURPOSE:** Development of an improved animal model for studying skin burns in rats.

**METHODS:** Twenty-four male Wistar rats were randomly assigned to four groups (n=6): G1-Control, G2- T100°C, G3-T150°C and G4-T200°C. Two 10 x 10 mm squares were outlined with a sterile surgical marker on each side and along the vertebral column using a prepared template positioned between the anterior and posterior limbs. G2-G4 rats were subjected to 100°C, 150°C and 200°C thermal burns, respectively. G1 rats served as controls. Burns were inflicted by applying a copper plate connected to an electronic temperature controlling device to the dorsal skin of anesthetized rats. Four burns were produced on each animal (total area: 4 cm<sup>2</sup>/ animal) leaving about 1 cm of undamaged skin between burn areas. Analgesia was administered during 24 h after burn injury by adding 30 mg codeine phosphate hemihydrate to 500 ml tap water.

**RESULTS:** The application of 100°C and 150°C resulted in partial thickness skin burns with central reepithelialization of the burned area only at 100°C. In G4 group the whole thickness of the skin was injured without central reepithelialization. However, there was marginal reepithelialization in all groups.

**CONCLUSION:** The model studied is inexpensive and easily reproducible, enabling the achievement of controlled burns with partial or total impairment of the skin in experimental animals.

**Keywords:** Models, Animal. Hot Temperature. Burns. Rats.

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### RESUMO

**OBJETIVO:** Desenvolvimento de um modelo animal aperfeiçoado para estudo de queimaduras cutâneas em ratos.

**MÉTODOS:** Vinte e quatro ratos Wistar, machos, foram distribuídos aleatoriamente em quatro grupos (n=6): G1-Controle, G2-T100°C, G3-T150°C e G4-T200°C. Dois quadrados medindo 10x10 mm foram delineados com um marcador cirúrgico estéril em cada lado e ao longo da coluna vertebral e posicionados entre os membros anteriores e posteriores, utilizando um molde previamente preparado. Os ratos dos grupos G2-G4 foram submetidos a queimaduras térmicas de 100°C, 150°C e 200°C, respectivamente. O grupo G1 foi utilizado como controle. As queimaduras foram infligidas pela aplicação de uma placa de cobre, ligada a um dispositivo de controle eletrônico de temperatura, na pele dorsal de ratos anestesiados. Quatro queimaduras foram produzidas em cada animal (área total: 4 cm<sup>2</sup>/animal), deixando cerca de 1 cm de pele intacta entre as áreas queimadas. Analgesia foi obtida durante 24 horas após a queimadura por adição de 30mg de fosfato hemi-hidratado de codeína a 500 ml de água potável.

**RESULTADOS:** A aplicação 100°C e 150°C resultou na produção de queimaduras profundas comprometendo parte da espessura da pele, com reepitelização central da área queimada, somente a 100°C. No grupo G4 houve lesão de toda a espessura da pele sem reepitelização central. Entretanto, observou-se reepitelização marginal em todos os grupos estudados.

**CONCLUSÃO:** O modelo estudado é de baixo custo e facilmente reproduzível, propiciando a obtenção controlada de queimaduras com comprometimento parcial ou total da pele, em animais experimentais.

**Descritores:** Modelos Animais. Temperatura Alta. Queimaduras. Ratos.

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## Introduction

Induction of experimental burns in animal models is an essential tool to understand the pathogenesis of skin damage due to burns in humans and for designing new methods of treatment. Animal models have been used to investigate burn wound pathology, local therapy<sup>1</sup>, the influence of systemic drug application on the burn wound and the effect of burn trauma on the entire organism<sup>2</sup>. Inconsistency and irreproducibility are commonly found in experimental studies. Two factors may be related to the controversial and different findings presented in the available literature: burn models are not identical or different modalities of treatment are used by researchers.

The present study was therefore undertaken to determine the optimal experimental model of burns heat-induced by direct conduction in rats, correlating standardized temperatures and lesion depths (partial or total skin thickness burn).

## Methods

Approval for experimental use of laboratory animals was obtained on September, 2010 (Protocol #37/10) from the Ethics Committee on Animal Research (CEPA) of the Federal University of Ceara, now Ethics Committee on the Use of Animals (CEUA), in view of the Federal Law No. 11794 of October 8, 2008, [http://www.planalto.gov.br/ccivil\\_03/\\_Ato2007-2010/2008/Lei/L11794.htm](http://www.planalto.gov.br/ccivil_03/_Ato2007-2010/2008/Lei/L11794.htm) and Decree No. 6689 of July 15, 2009 that regulated the Law 11794, available at: [http://www.planalto.gov.br/ccivil\\_03/\\_Ato2007-2010/2009/Decreto/D6899.htm](http://www.planalto.gov.br/ccivil_03/_Ato2007-2010/2009/Decreto/D6899.htm).

The study was designed to minimize the number of animals required for the experiments. The animals were housed in polypropylene cages at ambient temperature of 24°C on a 12 h light-dark cycle.

### Study design

Twenty-four male Wistar rats were randomly assigned to four groups (n=6): G1-Control, G2-T100°C, G3-T150°C and G4-T200°C. Two 10 x 10 mm squares were outlined with a sterile surgical marker on each side and along the vertebral column using a prepared template (an X-ray film with a 10 x 10 mm grid) positioned between the anterior and posterior limbs. G2-G4 rats were subjected to 100°C, 150°C and 200°C thermal burns, respectively. G1 rats served as controls. Immediately before inflicting the burn, the area was shaved with a standard electric shaving machine to obtain a smooth surface and hairless skin. The dorsal skin was surgically prepared with successive applications of 2% chlorhexidine scrub. A previously described model<sup>3,4</sup> was used with some modifications. The original tip of an ordinary 40W soldering iron was replaced with tip and square 10 x 10mm copper plate. An electronic temperature controller with a thermocouple-type feedback sensor was connected 2 mm above the plate tip in order to allow precise optimal temperature monitoring at the tip of the instrument. Additionally, a digital multimeter with a K type thermocouple was fixed to the copper plate to assure real time optimal control of the temperature applied to the skin (surface counter). The desired stamp temperature (100°C, 150°C and 200°C)

was reached 5 min after switching on the electric current. The device was positioned vertically under its own weight (85g) and applied to each skin burn site during 9 seconds to inflict the burns as outlined. Immediately after each burn injury, the respective wound was cooled off during 1 min with gauze embedded in isotonic saline at 22°C, as described elsewhere<sup>5</sup>. The plate produced a burn area of approximately 1 cm<sup>2</sup>/ wound. Four burns were produced on each animal (4 cm<sup>2</sup>/animal) leaving approximately 1 cm intact skin between burn areas. Following conclusion of the procedure, the animals were returned to their individual cages for recovery with free access to rat chow and tap water. Analgesia was administered during 24h after burn injury by adding 30 mg codeine phosphate hemihydrate to 500 ml tap water.

### Anesthesia

Anesthesia was induced with an intramuscular injection of ketamine hydrochloride (90 mg/kg) and xylazine (10 mg/kg) to all rats.

### Macroscopic analysis

Anesthetized rats were photographed in the seventh day post-burn under anesthesia and standard lighting conditions using a tripod-mounted digital camera (Sony DSC-W35, 7.2 mega pixels) with a constant 1.0 x zoom at 35 cm distance from the dorsum of the animal. The Images (format TIFF, 512 x 384 pixels) were calibrated and the wound surface area (mm<sup>2</sup>) and perimeter (mm) were calculated by computerized planimetry using the software Image Tool 3.0 (University of Texas Health Center at San Antonio).

### Histopathology

At the end of the experiments all animals were killed by an overdose of anesthetics (Ketamine+xylazine). Tissue samples were collected from the dorsum with a surgical blade, removing skin fragments (20 mm x 5 mm), including the central scar, adjacent healthy tissue and underlying panniculus carnosus muscle. Tissue samples were fixed in formalin for 24 hours before being transferred to 70% ethanol solution. Further processing included paraffin embedding and sectioning, to generate 5-µm-thick tissue coronal sections to be mounted on glass slides. The slides were stained using hematoxylin and eosin. The extent of skin damage was assessed by a blind pathologist using a light microscope (Olympus, X100).

### Statistical analysis

Graphpad Prism 5.0 (GraphPad Software, [www.graphpad.com](http://www.graphpad.com)) was used for statistical analysis and graphics design. Data from images (wound surface area and perimeter) were tested for distribution (Kolmogorov-Smirnov test). Results were expressed as mean+SD. Groups were compared at different temperature (100 °C, 150 °C and 200 °C) using Student's *t* test for unpaired variables. The level of statistical significance was set at 5%. Histopathology findings were tabulated for descriptive analysis.

## Results

No infection or mortality was observed during the experiments and no systemic complications were clinically detectable.

### Macroscopic analysis

Wound surface area and perimeter (Figures 1 and 2) shown by digital planimetry did not differ significantly when comparing G2 (T100°C) and G3 (T150°C) groups ( $p > 0.05$ ). However, wound surface area in G4 (T200°C) rats was larger than in G2 group by Student's paired t test ( $p < 0.03$ ). The same was true for wound perimeter ( $p < 0.04$ ) (Figure 3).

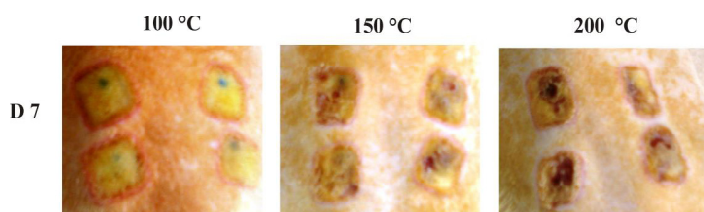


FIGURE 1 - Macroscopic appearance of burns on post-burn day 7.

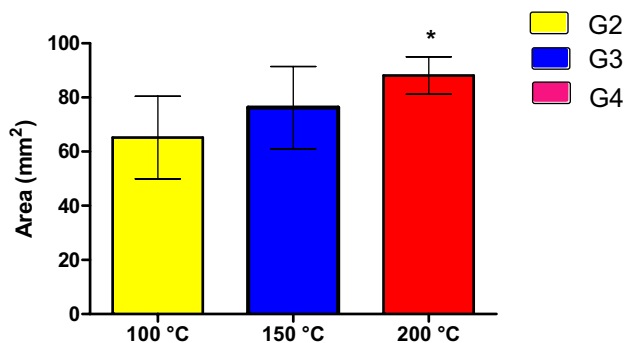


FIGURE 2 - Mean wound surface area injury in rats burned. Bars represent mean  $\pm$  SD. \* $p < 0.03$ .

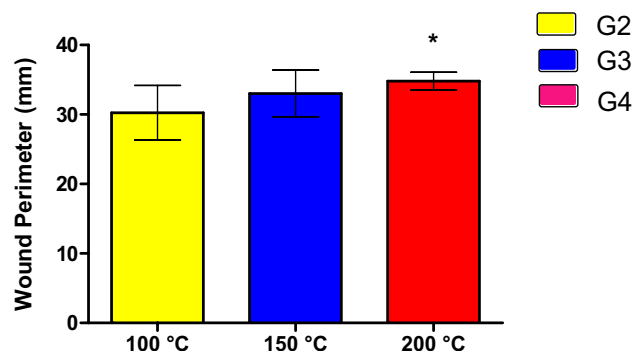


FIGURE 3 - Mean wound perimeter injury in rats burned. Bars represent mean  $\pm$  SD. \* $p < 0.04$ .

### Microscopic analysis

Scars were present in all wounds along incipient marginal reepithelialization (Figure 4, images E, G and J). Incipient central reepithelialization was present in G2 only (Figure 4, image F). The dermis presented obliterated vessels and partially damaged pilosebaceous units in groups G2 and G3 (Figure 4, images C, E and H). Total dermis and muscle damage were found in G4 rats (Figure 4, images J and L). Musculature layers were preserved in G2 and G3 rats (Figure 4, images D and I, respectively). Table 1 depicts the Presence/absence of skin structures and reepithelialization in G2-G4 rats.

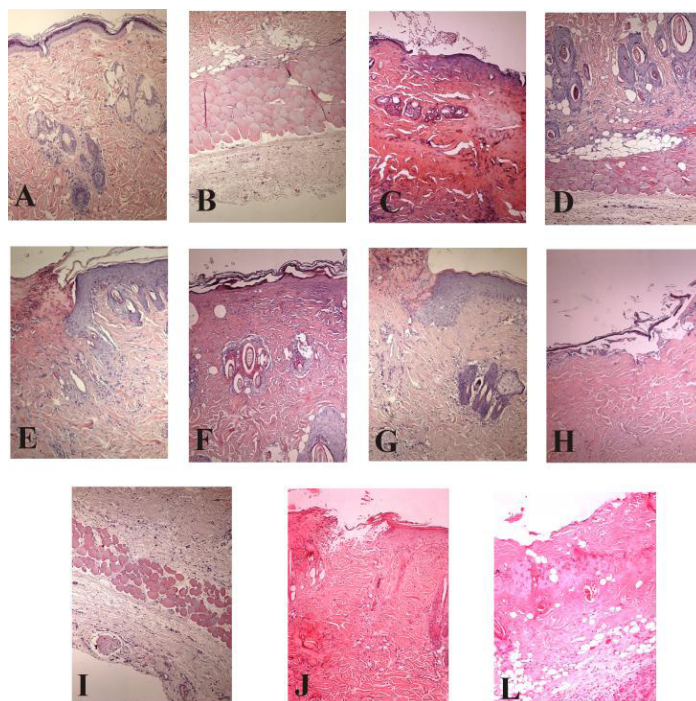


FIGURE 4 - Photomicrography of burns on post-burn day 7 (x100, magnification). **A:** Epidermis, dermis and pilosebaceous units from animal control (normal); **B:** Skeletal muscle and connective tissue from control animal; **C:** Partially damaged epidermis, dermis and pilosebaceous units (animal burned at 100°C). **D:** Skeletal muscle without injury (animal burned at 100°C). **E:** Incipient marginal reepithelialization (animal burned at 100°C). **F:** Central reepithelialization (animal burned at 100°C). **G:** Marginal reepithelialization with epidermis and dermis injury, see pilosebaceous units (burned at 150 °C). **H:** Central ulcer and epidermis/dermis injury (burned at 150°C). **I:** Skeletal muscle and hypodermis injury (burned at 150°C). **J:** Marginal reepithelialization (burned at 200°C). **L:** Injury in all layers with central ulcer and damaged muscle layer (burned at 200°C).

**TABLE 1** - Presence/absence of skin structures and recovery (reepithelialization) in burned rats

	G2 (100 °C)	G3 (150 °C)	G4 (200 °C)
Epidermis	yes	yes	yes
Dermis	yes	yes	yes
Hypodermis	no	yes	yes
Musculature	no	no	yes
Marginal reepithelialization	yes	yes	yes
Central reepithelialization	yes	no	no

Groups: n=6

## Discussion

Over the past decade much has been discovered concerning the pathophysiology and forms of treatment of burn lesions. Different burn models have been presented. Methods for producing experimental burns included boiling water<sup>6</sup>, heated brass bar<sup>7</sup>, heated (130°C) aluminum plate<sup>8</sup>, a constant-temperature hot plate (170°C) placed over stainless steel rods<sup>9</sup> and scalding over 45% of the body surface<sup>10</sup>.

In the present study, skin burns were inflicted by transference of energy (heat) using direct conduction (Copper/skin) utilizing different increasing temperatures (100°C, 150°C and 200°C). Thermal energy transfer by conduction from a solid structure to the skin surface does not depend on the pressure employed, but mainly on the temperature gradient between the skin, the solid structure and the distance between those elements<sup>11</sup>. To the best of our knowledge, this is the first work where a copper template is used to induce thermal burns. Copper thermal conductivity is more efficient than other metals used by researchers<sup>1,7,12</sup>.

The results clearly demonstrated that this experimental model provided excellent contrast between normal and burned tissue at different temperatures. Thus, the highest temperature used (200°C) producing lesions in all skin layers with damaged pilosebaceous units resulting in poor central reepithelialization, making being this method a good choice to evaluate drug effects in severe burnings.

Moreover, it was possible to obtain partial skin thickness burn with lesser temperatures (100°C and 150°C). However, burning at 150°C was more deeper than 100°C and the central reepithelialization occurred only when the plate was heated to 100°C. The partial-thickness burn model (using 100°C and 150°C) in this study spares some epithelial elements within the dermis, allowing for spontaneous regeneration (reepithelialization) via proliferation and migration of epithelial cells from dermal appendages remaining at the wound site and from the wound margins<sup>13-14</sup>. Practically, it implies that a deep partial skin thickness burn should not be converted into a full skin thickness injury.

In order to achieve consistent reproducibility, the stamp pressure should be identical in all lesions and positions to ensure maximal skin contact. A major advantage of the hand-held stamp using the device weight to induce the burn lesion is that it remains constant throughout the procedure and is not related to the pressure exerted by the researcher that can present some changes due to the difficulty of figuring out the intensity of pressure that should be applied to the skin of the animal.

## Conclusion

The model studied is inexpensive and easily reproducible, enabling the achievement of controlled burns with partial or total impairment of the skin in experimental animals.

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