IMPROVED EXPERIMENTAL BURN MODEL ON RABBITS

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IMPROVED EXPERIMENTAL BURN MODEL ON RABBITS (Abstract): Objective: The goal of our study was to find an easy and suitable skin burn model for rabbits, to identify the temperature and contact time to reach a deep 2nd degree burn, and to find the best wound dressing fixation method. Material and Methods: We used New Zealand male rabbits as experimental models. On a pilot study, with a copper device heated to temperatures between 43-48 Celsius degrees, the burn was inflicted using contact times of 3, 4, 5, and 7 seconds. The burn depth was evaluated histologically. Twenty-four rabbits were used to evaluate four dressing fixation methods: regular dressing with retention bandages; skin stapler; collars and body resin cast. Results: A 2nd degree deep burn was obtained at 43 degrees for 4 seconds. The retention elastic bandages were removed within hours and the skin staplers were removed with teeth leaving wounds on the skin. The collars were more successful but produced superficial neck lesions. The body resin casts were the most successful. Conclusions: The best contention method was the body resin cast. Keywords: SKIN BURN MODEL, RABBIT, DRESSING CONTENTION METHOD, BODY CAST

We aimed at finding the ideal experimental burn model to identify an animal with skin properties similar to humans and possibility of good wound dressing contention. On Pubmed, most experimental models are rats (1-5), Guinea pigs (6), rabbits (7-12) and pigs (13). In the last 15 years of searching on Pubmed, we have found few articles dealing with rabbit skin burn models. Finding a suitable burn model in animals is not an easy task. A review of the literature revealed several studies that attempted to describe a model of skin burns in rodents, most commonly using Wistar rats. Most did not explain how the dressings were kept in place without hurting the animal. Our goal was to find the proper temperature to produce a deep 2nd degree burn and to identify the best dressing contention method for rabbits.

MATERIAL AND METHODS
The study was performed on New Zealand male rabbits. The experiment was approved by the Ethics Committee of our institution and by the University of Agri-
cultural Sciences and Veterinary Medicine Ethics Committee and is part of a research national grant project.

Study design

A) We investigated the ideal temperature to reach a 2nd degree deep skin burn

Five male rabbits with an average weight of 2.5 kg were used to identify the ideal temperature to reach a deep 2nd degree skin burn. For the entire period, the animals were fed with rabbit food and water ad libitum. All procedures were performed under general anesthesia with intramuscular Ketamine 50 mg/kg and Xylasine 5 mg/kg.

Each rabbit had hair removed on each flank. A very fine and short hair of 0.20mm remained on the skin. For removing this hair, we used a commercial depilatory cream. The contact time with the cream was 25 minutes, according to the manufacturer’s instructions. The cream was then removed and the skin was washed with water and then dried (fig. 1). For the next 24 hours no procedures were performed, allowing time for the skin irritation to disappear.

![Fig. 1. Preparing the skin for the burn to be inflicted](image)

A. The hair is removed with an electric shaver; B. The depilatory cream is in place; C. The skin appearance immediately after washing. Note some skin irritation.

The area to be burned was marked with a permanent skin-marker. Each animal had four burns, two on each flank (fig. 2). A 350 g copper device was used to inflict the burns (fig. 3). The device was introduced in boiling water and kept for 30 seconds. The temperature was measured using an infrared thermometer (Fluke 62 mini infrared

1048
thermometer, accuracy: ±1°C). When the device reached the desired temperature, it was applied. Because the copper has a better thermal conductivity compared with other materials, the temperature decreased slowly with an average of 1°C per three seconds at room temperature.

![Fig. 2. The skin area to be burned is marked](image1)

Fig. 2. The skin area to be burned is marked

![Fig. 3. The device used for the burn. The temperature was infrared measured.](image2)

All the procedures were performed under aseptic conditions, after cleaning the skin with diluted Povidone Iodine solution. No additional pressure was applied manually, except for the device’s own weight. The device’s copper volume was 19.28 cm³ and the contact surface was 14.4 cm². Using contact times of 3, 4, 5 and 7 seconds and the device’s temperatures of 43°C, 44°C, 45°C, 47°C, and 48°C (for each timing), burns were inflicted on the marked areas (two on each flank). The time between measuring the device’s temperature to skin application was less than one second on a digital stopwatch.

The wounds were covered with dry gauze to keep them clean. On the third day, biopsies were taken from the burned areas to evaluate the depth. From each burned area one biopsy was taken.

B) Evaluation of the best dressing contention method

We compared four dressing contention methods on 24 New Zealand white male rabbits with an average weight of 2.5 kg, separated into 4 groups. Each rabbit’s flank was inflicted with a burn.

Group I: We used the simplest method: dressing and retention bandages circumferentially (Peha Haft®, Hartman) (fig. 4). Each dressing was planned to be changed every 2nd day.

![Fig. 4. Group I: Retention bandages placed circumferentially](image3)

1049
Group II: The dressing was fixed with skin staplers (fig. 5) and retention bandages circumferentially (Peha Haft®, Hartman) applied on top. Each dressing was planned to be changed every 2nd day.

**Fig. 5.** Group II: The dressing fixed with skin staplers

Group III: The same as described in group II plus collars (fig. 6). Each dressing was planned to be changed every 2nd day.

**Fig. 6.** Group III: The collar in place

Group IV: The same as in group II, with fewer skin staplers and a body resin cast on top. The cast was placed circumferentially with narrower openings compared to the middle part. After the resin hardened, the cast was cut longitudinally and the sides connected with Omnifix® (Hartman) (partial elastic adhesive dressing) to allow better thoracic movement during respiration (fig. 7). The dressings were changed every 2nd day under general anesthesia for a period of 2 weeks.

**Fig. 7.** Group IV: Body resin cast placed circumferentially

**RESULTS**

A) The ideal temperature to reach a deep 2nd degree skin burn

The skin biopsies collected were histological evaluated and it was found that a deep 2nd degree burn can be obtained at 43°C for 4 seconds (fig. 8). Since the burn surface is not uniform, we considered a deep 2nd degree burn if more than 75% of the area was involved.

B) Evaluation of the best dressing contention method

Group I: The dressing fell down within hours after the rabbit woke up. The dressings were set again, but under a bit more tension. It was hard to find the proper tension in the retention bandages. This way, the dressings were in place, but four of the rabbits died 24 hours later. The autopsy revealed hemorrhagic ulcers in the stomach and bowel, most probably due to post-traumatic stress and administration of NSAIDs. Pleural effusion and pulmonary edema was associated. The treatment with
ketoprophen was stopped to the other groups and Phytomenadione and Omeprazole was initiated for the next days.

**Group II:** The dressings were in place for a longer time (hours up to one day). In the end all rabbits were able to remove them with the teeth.

**Group III:** The collars were useful and stopped the rabbits from removing the dressing for the next 48 hours. All the rabbits were very agitated and had superficial neck wounds at the site of collar. Their movements were highly restricted.

**Group IV:** This was the best contention method. After the first 48 hours, the cast was shortened in four rabbits, in order to prevent pressure sores at the hip joint.

After the dressings fell down twice in groups I-III, the contention method was changed to body resin casts and all animals (groups I-IV) were involved in a further study on topical creams for burn wounds.

**DISCUSSION**

Several types of burn models are described in the literature, most focusing on thermal injuries. Such injuries could be obtained after direct contact with hot water or with a hot surface. The contact temperature and the time are the main factors influencing the burn depth. There is a wide variety of reported contact temperatures and time to reach a 2nd degree burn.

Knabl et al (7) reported partial skin thickness burn on 9 New Zealand rabbits using an aluminum stamp of 85g heated up to 80°C and 14 seconds contact time. No details about the methods of dressing fixation were described. They recommend using rabbits of the same strain and weight in the anagen hair growth phase for better evaluation. Von Bullow et al (8) described a skin burn model on 10 New Zealand rabbits. Full thickness burns were achieved by the application of heated brass probes at 100°C for more than 30 seconds on the backs of the rabbits. No details about the weight and volume of the probe are described. According to the same author (8), superficial partial thickness burns can be produced with a contact time of 7 seconds. There are no data about the type of dressing. In their study on 15 rabbits, Aksoy et al (9) obtained 3rd degree skin burn injuries using a brass plate weighting 500 g, with a surface diameter of 3.5 cm. Before applying it to the skin, the instrument was left in boiling water at 100°C for 15 minutes. A contact time of 15 seconds was used to obtain a full thickness burn injury. It was mentioned that no topical antimicrobial treatment was applied. In this
study a delayed wound healing model was obtained by removing the panniculus carnosus layer, and three weeks later a 3rd degree burn was inflicted on the skin flaps.

Wang et al (10) obtained a full thickness burn area after 10 seconds of contact with 90-93°C water. The dressings on the burned areas were fixed with the tie-over method. No details about follow-up of wound dressing were reported. In another study on 16 New Zealand male rabbits where no burns were inflicted but full thickness wounds were performed to check different topical agents, the wounds were covered with a self-adhering wrap-around bandages. The rabbits were checked twice a day for the bandages to be in place and the dressings were changed daily without anesthesia.(11). The same type of dressing fixation was used by Jurjus et al in their study on deep 2nd degree skin burns obtained using an aluminum stamp heated to 80° and a contact time with the skin of 25 seconds (12). Unlike the study mentioned before, these dressings were changed after sedating the animals.

A possible explanation for this great variety of temperature and time can be explained by using different instruments for obtaining the burn, each with a different capacity for releasing the heat. Heated brass (1, 8, 9, 13), heated copper instruments (3), aluminum instruments (6, 7, 12), or even contact with hot water (10) were all tested to obtain an experimental burn model. It is important to know the time that elapsed from measuring the device’s temperature until the inflicted burn. We chose to use a copper device because it has better thermal conductivity compared to other nonferrous metals or iron.

We decided to use rabbits for this experimental research, because they are larger than other rodents, easy to handle, and allow good follow-up. When working on small animals for burn models, some results can be negatively affected by the burn size. The contraction can be significant enough to alter the wound evaluation. In small animals the burn size can be a limiting factor for the experiment. Researchers dealing with different models of burns know that maintaining the dressings in place for a longer period of time (days) is a difficult task.

CONCLUSIONS

According to our results, this burn model seems to be better than those existing on rabbits and offer details about obtaining a deep 2nd degree burn and describes a good contention method. The best contention method was the body resin cast. We hope this information will be useful for those researchers working on rabbits.

DECLARATION OF INTEREST

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**CYTOLOGICAL ANALYSIS OF THE PERIODONTAL POCKET IN PATIENTS WITH AGGRESSIVE PERIODONTITIS AND CHRONIC PERIODONTITIS**

Oral exfoliative cytology includes the study and interpretation of the features cells exfoliated from the oral mucosa. The aim of a study realized by a group of argentinian researchers was to analyze cytological changes in the periodontal pocket of patients with different clinical stages of aggressive periodontitis and chronic periodontitis. Patients, aged between 24-54 years, of whom 41 were diagnosed with aggressive periodontitis, 40 with chronic periodontitis, sub-classified as mild, moderate and severe periodontitis, and 40 healthy individuals who were the control group. For the cytological study were taken samples of the epithelium of the periodontal pocket. Superficial and intermediate cell values were significantly greater in patients with aggressive periodontitis than in patients with chronic periodontitis or the control group. Histiocyte number was higher in patients with chronic periodontitis than in those with aggressive periodontitis, and differed significantly in both types of periodontitis compared to the control group. There were significant differences in polymorphonuclear neutrophil leukocytes when both types of periodontitis were compared to the control group. Microbial flora was statistically higher in patients with chronic periodontitis than in those with aggressive periodontitis, and there were differences between patients with periodontitis and the control group. The cytological study demonstrated that patients with aggressive periodontitis had greater tissue damage, shown by the increase in intermediate and superficial cells of the epithelium of the periodontal pocket compared to the group of healthy subjects and to a lesser extent, to patients with chronic periodontitis. Only superficial cells made it possible to differentiate the sub-stages of the disease (Cecilia EC, Myriam AK, María EL. Cytological analysis of the periodontal pocket in patients with aggressive periodontitis and chronic periodontitis. *Contemp Clin Dent*, 2014; 5(4): 495-500).