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Original Article

Creating a Thermal Burn Wound Model in Rabbits for Application in Wound Treatment

Ho My Dung, Vu Thi Thom, Pham Thi Thuy An, Phan Hong Minh, Ta Thi Phuong, Vu Hong Duc, Dang Quynh Anh, Do Nhat Long, Do Thi Quynh*

VNU University of Medicine and Pharmacy, 144 Xuan Thuy, Cau Giay, Hanoi, Vietnam

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Abstract: Currently, burn injuries have become a concerning issue in many countries, affecting everyone at different ages and occupations. This study aimed to create a heat burn model in rabbits to optimize burn procedures, which is a premise for developing new treatment methods for burn wounds in preclinical and clinical practices. Healthy white New Zealand male rabbits were maintained in the appropriate living conditions and diet for 7 days before the experiment. The rabbits were anesthetized with chloral hydrate at 150 mg/kg via the ear vein. A metal block (200g in weight, 3cm in diameter) heated by boiling water to 100 °C was used to cause 4 burn wounds on the shaved skin of the rabbit's back, with exposure times of 10 seconds, 20 seconds, and 30 seconds. The wound healing process was monitored, and histopathological analysis at the burn site was evaluated on the 7th and 14th days. The results indicated that the exposure times of 10 and 20 seconds caused second-degree burns (damage to the dermis layer), while that of 30 seconds caused third-degree burns (damage to the hypodermis layer). Thus, the research successfully created a burn model on rabbit skin, laying the groundwork for future testing phases of new burn treatment methods.

Keywords: Burn wounds, thermal injury, rabbit model.

1. Introduction

According to the World Health Organization (WHO), burns are a global public health problem, with an estimated 11 million burn injuries occurring each year worldwide, including 180.000 deaths. The majority of burn cases occur in countries with low and middle per capita income, and Africa and Southeast Asia account for nearly two-thirds of these incidents [1, 2]. According to the definition of the

* Corresponding author.
E-mail address: dohungquynh@gmail.com

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International Society for Burn Injuries, burn is an injury damaging to the skin or other organic tissues, generally caused by heat or other acute trauma [3]. Burns can be caused by various agents, both physical and chemical agents. There are four different degrees to assess burn injuries. First-degree burns affect only the epidermis layer; second-degree burns extend into the dermis; third-degree burns destroy all skin layers; fourth-degree burns involve the subcutaneous layer, including the subcutaneous fat, muscle, and bone [4]. The different burn degrees determine the treatment regimen and post-burn care. Therefore, many experimental models have been established to find the responses of the body, cells, or molecules to treatment methods. The use of animal models is a crucial prerequisite in burn research before conducting clinical trials. Globally, although there have been many *in vivo* models with diverse burn induction methods such as direct flame burns, scald burns, and chemical burns, there is still a lack of comprehensiveness, specificity, and consistency in technical procedures as well as in evaluating and monitoring methods. Currently, in Vietnam, animal burn models still have many limitations, such as insufficient assessment of burn degrees and a lack of diversity in burn induction methods.

Therefore, to supply more information about the burn induction process as well as to create an *in vivo* model for testing new burn treatment therapies, we conducted this study to create a burn wound model on the skin of healthy rabbits and evaluate the results of the thermal injury model.

2. Subjects and Methods

2.1. Experimental Animals

Three-month-old male New Zealand white rabbits, weighing 2-3 kg in healthy condition, were used in this research. The study was conducted in the animal laboratory of National Institute for Food Control, Vietnam. Rabbits were kept at 26 ± 2 °C with a 12-hour light-dark cycle and given a standard diet for 7 days to acclimate to the laboratory conditions before the experiment.

2.2. Machines and Chemicals

Machines: Burn induction tool (A metal block with 200 grams in weight and 3 cm in diameter), Water bath, Ohaus PA213 analytical balance (Ohaus - USA), HistoStar embedding machine (Thermo Scientific, USA), HM325 rotary microtome (Thermo Scientific, USA), Slide staining kit (Bio Optica, Italy), Olympus IX73 microscope (Olympus - Japan)

Chemicals: Chloral hydrate (China), Formaldehyde solution (China), Ethanol (Vietnam), HE staining kit (Germany), Xylene (China), Bouin (Vietnam).

2.3. Research Methods

Twelve healthy rabbits were randomly divided into 3 groups, with each group consisting of 4 rabbits. Each rabbit was induced with 4 burn wounds with burn timings of 10 seconds, 20 seconds, and 30 seconds, respectively. One day before the experiment, rabbits were shaved and cleaned fur on their backs by hair removal cream, with the shaving area measuring about 12 cm x 12 cm. On the day of the experiment, rabbits were anesthetized with chloral hydrate at a dose of 150 mg/kg (1 mL/kg) intravenously through the ear vein. After the rabbits were unconscious, the burn induction was performed on the hair-removed skin by using a metal block (200 grams in weight and 3 cm in diameter) dipped in boiling water at 100 °C until reaching a constant temperature (about 10 minutes) and placed perpendicularly on the rabbits' back without applying additional force. Four burns were induced on both sides of the back of each rabbit.

To evaluate the percentage of burn area relative to the total skin area, the formula to calculate the body surface area of New Zealand white rabbit was: 100 x BSA = 11 x $(BW)^{\frac{2}{3}}$;

whereas: BSA: Body Surface area (m^2); BW: body weight (kg) [5].

Burn wounds were evaluated daily according to Table 1 and a token photograph with a scale using a digital camera until completely healed to calculate wound area through ImageJ software.

The wound contraction ratio was calculated using the formula: $\%D_t = \frac{SD_0 - SD_t}{SD_0} \times 100\%$. Whereas: SD_0 is the initial wound area, and SD_t is the wound area at the detailed time.

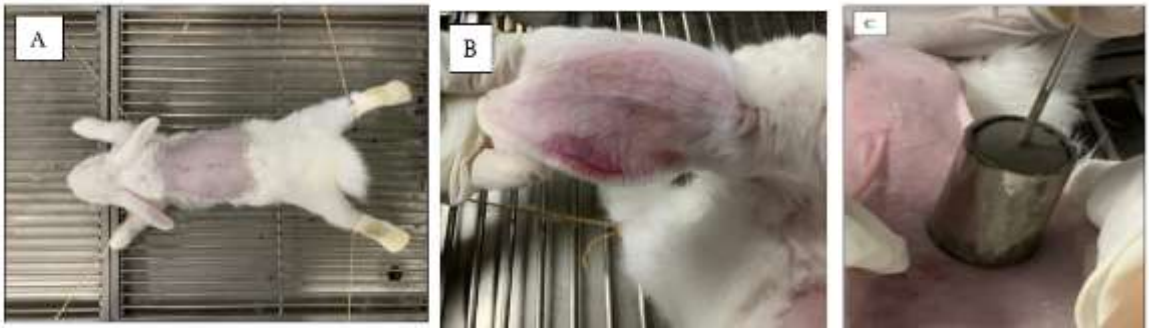


Figure 1. The burn induction process on the rabbit's skin
A: Rabbit immobilization; B: Intravenous injection into the rabbit's ear vein; C: Burn application process.

Table 1. The burn wound scoring criteria for burn wounds

Characteristic	Details	Score
Congestion	None	0
	Mild	1
	Moderate	2
	Severe	3
Swelling	None	0
	Mild	1
	Moderate	2
	Severe/blisters	3
Exudation	Dry wound	0
	Wet wound without pus	1
	Wet wound with yellow/green/white pus	2
Ulceration	No ulcer/scab/healed scar	0
	Superficial dry ulcer/no scab	1
	Superficial wet ulcer	2
	Deep wet ulcer	3
Burn area	Completely contracted	0
	Partially contracted	1
	Not contracted	2

On the 7th and 14th day after creating the burn wound, we took skin tissue samples from two burn sites in each group to analyze histopathological images. The cuts were covered with medical gauze to help stop bleeding and keep the wound clean. The collected tissue samples were fixed in 10% Formaldehyde solution. The satisfactory samples were

embedded in paraffin, cut into sections 3-5 µm thick, stained by the H&E method, and observed using the IX 73 microscope software system at magnifications of 4X and 10X.

Burn depth was classified into one of four degrees: First degree (damaged epidermis layer); Second degree (Extended into dermis); Third degree (Extended into the hypodermis); Fourth degree (Extended into the subcutaneous layer, into subcutaneous fat, muscle, and bone) [4]. Based on the physiological rabbit skin image, the microscopic lesions were scored according to Table 2.

Table 2. The histological evaluation criteria of burn wounds

Characteristic	Evaluation/Grade
Epidermal separation	No
	Yes
Epidermal structure fragmentation	No
	Yes
Epithelial structure deformation	No
	Yes
Hemorrhage/Congestion	No
	Mild (difficult to observe, under 20% of the microfield area)
	Moderate (about 20-40% of the microfield area)
	Severe (over 40% of the microfield area)
Inflammation (including capillary dilation, edema, and leukocyte infiltration)	No
	Mild (difficult to observe; under 20% of the microfield area)
	Moderate (about 20-40% of the microfield area)
	Severe (over 40% of the microfield area)

2.4. Statistical analysis

Wound area was calculated by ImageJ software version 1.8.0. Data was collected and analyzed by using Microsoft Excel 2016 and SPSS version 25.0. Quantitative variables were expressed as mean ± standard deviation ($\bar{x}$± SD) with the normal distribution data or median (min-max) with the non-normal distribution data. Comparison of the differences among groups was done by using appropriate statistical tests. The difference was considered statistically significant if the p-value was less than 0.05.

3. Results

Rabbits during the study period had stable health status and weight, and were similar among the three groups. During the burn process, the rabbit's health was closely monitored, and no complications occurred after the anesthesia process.

Table 3. Evaluation of burn scores on the rabbit's skin

Burn scores	Day 1 Median (Min-Max)	Day 7 Median (Min-Max)	Day 14 Median (Min-Max)	Day 21 Median (Min-Max)
Group 1: 10 seconds (n=8)	3.0 (3-6)	6.0 (5-6)	3.0 (1-4)	0.5 (0-1)
Group 2: 20 seconds (n=8)	4.0 (3-5)	6.0 (4-6)	3.5 (2-5)	1.5 (0-2)
Group 3: 30 seconds (n=8)	4.0 (3-5)	5.0 (4-6)	4.0 (3-5)	2.0 (1-3)
p^*	0.980	0.184	0.034	0.008
(* Kruskal-Wallis H test)				

After causing the burns, the injuries were monitored, evaluated, and scored daily depending on criteria such as congestion, edema, exudation, ulceration, and burn size. The results of burn scoring are shown in Table 3.

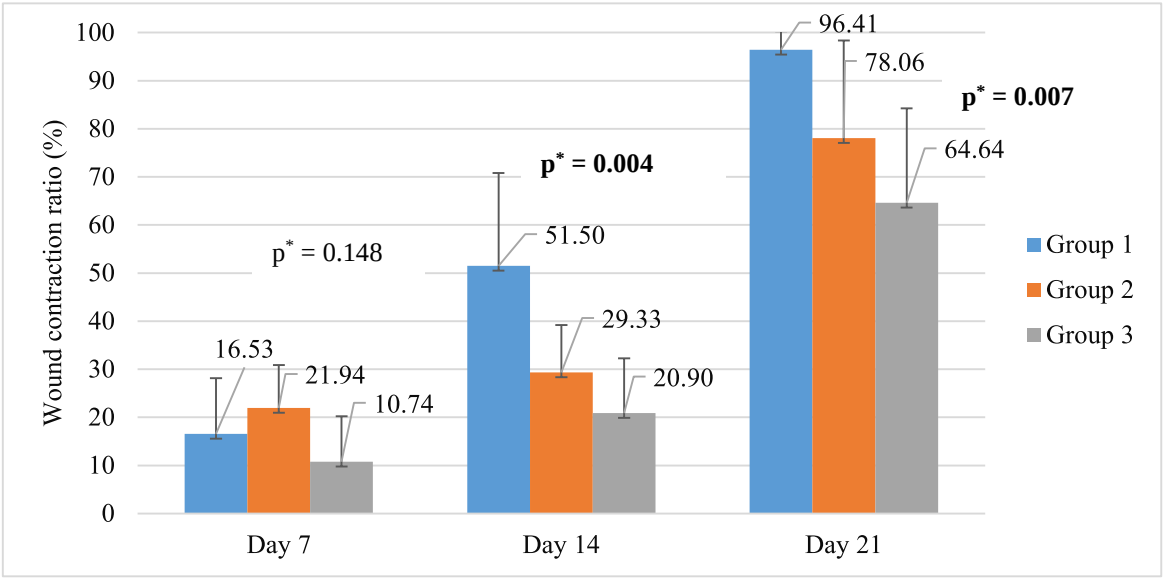
On the first day after burn induction, the burn scores were similar among all groups with burn timings of 10 seconds, 20 seconds, and 30 seconds (p>0.05). After one week post-burn, the burn scores for all groups increased because of exudation and edema, and there was no difference among the groups (p>0.05). Over the next two and three weeks, the burn wounds gradually healed, and the burn scores had a significant difference among the three groups (p<0.05). Hence, the longer the burn timing was, the more severe the burn injury was.

The burn area occupied approximately 1.39-1.44% of the rabbit skin surface area. The ratios of burn area to rabbit skin surface area were similar among groups (p=0.842). The results of the burn area are presented in Table 4.

Table 4. Changes in burn area during the experiment

Burn area (cm^2)	Day 1 ($\bar{x}$ ± SD)	Day 7 ($\bar{x}$ ± SD)	Day 14 ($\bar{x}$ ± SD)	Day 21 ($\bar{x}$ ± SD)
Group 1: 10 seconds (n=8)	7.82±0.84	6.54±1.28	3.75±1.43	0.29± 0.31
Group 2: 20 seconds (n=8)	7.63±1.26	5.96±1.26	5.33±0.75	1.58 ±1.53
Group 3: 30 seconds (n=8)	7.28±0.85	6.51±1.11	5.77±1.17	2.57±1.36
p*	0.641	0.552	0.045	0.007
(* Kruskal-Wallis H test)				

The results of the burn scar contraction ratio over time are depicted in Figure 2.



(*Kruskal- wallis H test)

Figure 2. Changes in the burn scar contraction ratio over time.

The burn scar contraction ratio in Group 1 (10 seconds) increased rapidly from the second week and healed faster than the other two groups. Group 2 (20 seconds) and Group 3 (30 seconds) showed the accelerated increases in burn scar contraction ratio from the third week.

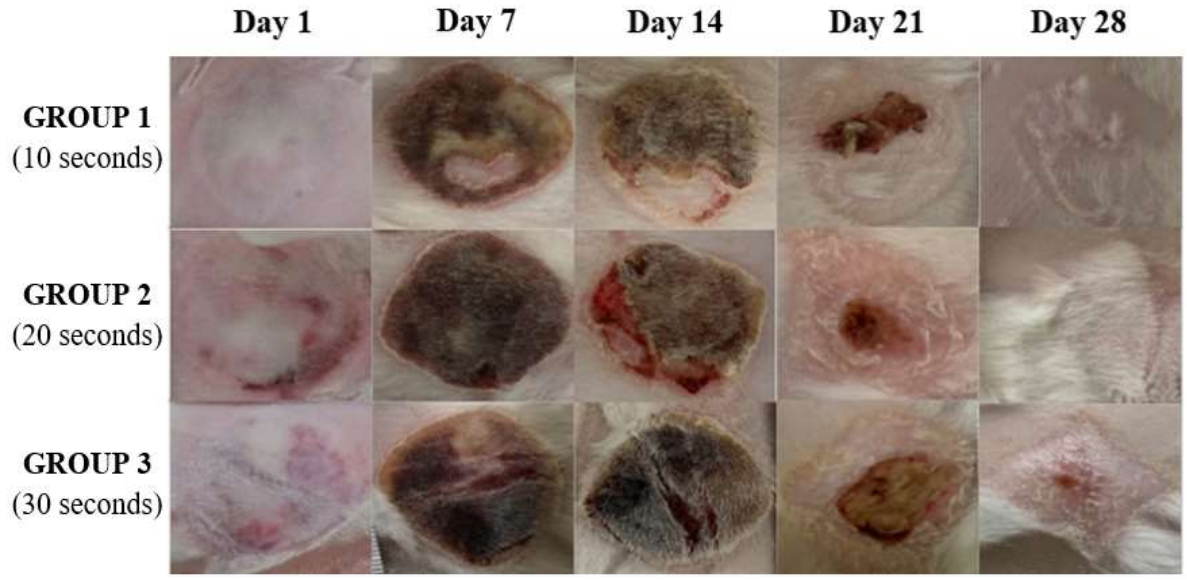


Figure 3. Images of burn wounds over time.

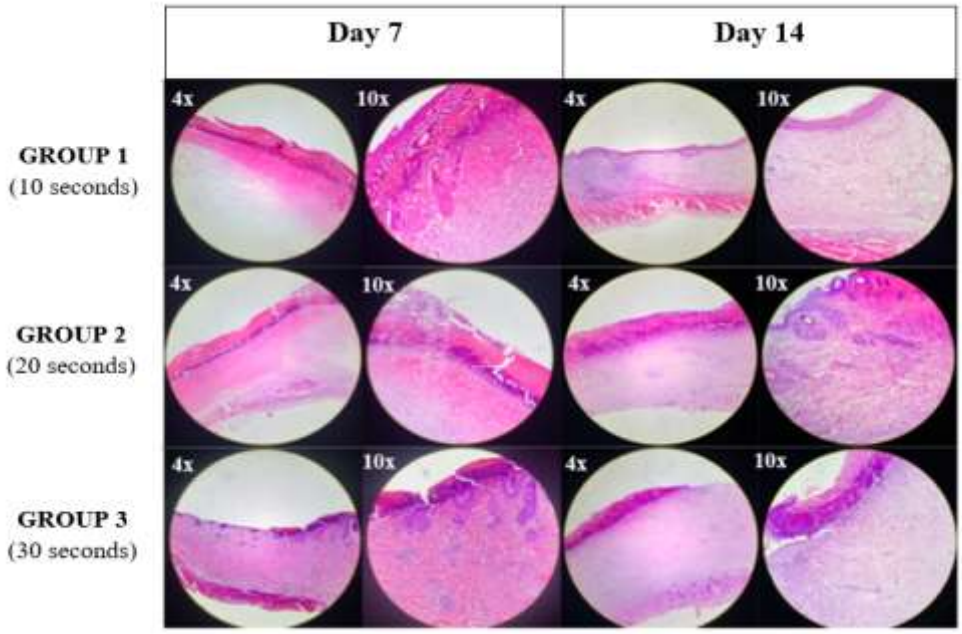


Figure 4. The histopathological images of burn wounds.

The burn wounds in Group 1 (10 seconds) completely epithelialized starting from the 17th day post-burning. Meanwhile, in Group 2 (20 seconds), the burn wounds of rabbits in this group only began to completely epithelialize from the 24th day. Group 3 (30 seconds) had the longest time for complete epithelialization, beginning from the 28th day. After 41 days of induced burning, all burn wounds in three rabbit groups have completely epithelialized. Hence, the longer the burn exposure duration, the longer the time for epithelialization. The longer the burn duration, the longer the epithelialization time was (Figure 3).

Rabbit skin samples were taken on the 7th and 14th day, then fixed and stained with the H&E method. The histopathological images of rabbit skin were presented in Figure 4.

From the histopathological images, it can be observed that in Groups 1 and 2, the epidermal layers were damaged, but the basal membrane layers were still intact. Therefore, with the burn timing of 10 seconds and 20 seconds, the burns were classified as second-degree burns. For a burn timing of 30 seconds, the injury extended to the dermis, resulting in a third-degree burn. The results of histopathological scoring for burn wounds are presented in Table 5.

Table 5. The histopathological scoring results during the experiment

		Epidermal separation	Epidermal structure fragmentation	Epithelial structure deformation	Hemorrhage/ Congestion	Inflammation
Group 1 (10 seconds)	Day 7	No	Yes	Yes	No	Mild
	Day 14	No	Yes	Yes	No	Mild
Group 2 (20 seconds)	Day 7	No	Yes	Yes	Mild	Moderate
	Day 14	No	Yes	Yes	Mild	Mild
Group 3 (30 seconds)	Day 7	No	Yes	Yes	Severe	Severe
	Day 14	No	Yes	Yes	Mild	Moderate

From the histopathological results, it can be observed that the conditions of epidermal layer separation, disruption of epidermal tissue structure, and deformation of epidermal tissue structure were similar among groups. On the 7th day post-burn induction, no hemorrhage condition was observed in Group 1 (10 seconds), while this condition increased in Group 2 (20 seconds) and was most severe in Group 3 (30 seconds). Additionally, the inflammation was more pronounced in groups with longer burn exposure duration. By the 14th day post-burn induction, both hemorrhage and inflammation had decreased in the three groups. Specifically, Group 1 (10 seconds) showed no signs of either condition.

4. Discussion

Currently, the burn models in experimental animals have been carried out in many studies with diverse methods on different animal species such as rodents, rabbits, or pigs. The animals used in this study were the New Zealand white rabbit, as rabbit skin has physiologic characteristics closely related to human skin. Furthermore, rabbits and humans share similarities in metabolic characteristics. Specifically, severe burn wounds cause systemic pathological changes and increased metabolism that are similar in both rabbits and humans [6]. In contrast, the skin structure of rodents is elastic and not tightly attached to the subcutaneous structure [7]. Additionally, rodents have a thinner epidermis and dermis compared to humans [7, 8]. Moreover, wound healing in rodents primarily occurs through wound contraction, whereas in humans, it involves epithelial regeneration and granulation tissue formation [9].

There are various causes of burn injury. Approximately 86% of burns are caused by thermal injury, while about 4% of those are

caused by electrical agents and 3% are caused by chemical agents. Flame and scald burns are the most common causes of burns in children and adults. Hence, in this study, we chose the wet thermal method to create burn wounds on rabbit skin [10]. Our research successfully established a wet thermal burn model on rabbit skin. After inducing burns, rabbits in all groups maintained stable conditions with pink skin, smooth white fur, quick mobility, normal digestion, and excretion. It can be observed that the burn method did not adversely affect the overall health of rabbits.

To create wet thermal burns, this research used a cylindrical metal block weighing 200 grams, with a diameter of 3cm, and about 7 cm^2 in surface area. The metal block was immersed in boiling water at 100 °C until the stabilized temperature. The burn application times for groups 1, 2, and 3 were 10 seconds, 20 seconds, and 30 seconds, respectively. Different degrees of burns were achieved depending on varying application times. However, the longer the metal block remained in the air, the more its temperature decreased due to heat dissipation, potentially affecting the study's results. In microscopic images of skin, burn injuries extended from the epidermis to the hypodermis, with burn severity ranging from 2 to 3. Rabbits' skins in group 1 (10 seconds) and group 2 (20 seconds) were broken into the epidermis, which was similar to a second-degree burn in humans. While as with a burn application time of 30 seconds, the injury extended into the dermis, leading to a third-degree burn. The depth of burn injury is a major factor in the speed and duration of the injury. To compare the healing differences among rabbits burned for 10 seconds, 20 seconds, and 30 seconds, we evaluated wound area, healing time, wound contraction rate, and wound scoring over time. Rabbits in group 1 (10 seconds) began to fully heal from day 17 post-burn, while groups 2 (20 seconds) and 3 (30 seconds) required longer healing times. With the third-degree burn in Group 3, it took from 28 to 41 days to completely heal on rabbit skin. Additionally, our results showed that the wound contraction rate in group 1 (10 seconds) was over 50% after 2 weeks, whereas it took until the third week for the other two groups to reach this rate. The prolonged injury duration was suitable for monitoring clear and effective burn treatment outcomes.

Currently, various experimental animal burn models have been studied worldwide using different methods. Kulyar et al. used a metal plate (10 mm in diameter) heated to 100 °C on a Bunsen burner flame with equal pressure for 40 seconds to create full-thickness burn wounds on rabbit skin, which was equivalent to a third-degree burn in humans [11]. The mean healing time of burn wounds in his study was 36 ± 2 days for the full-thickness burns. This result is similar to the burn timing for 30 seconds in our study.

In another model by Djerrou et al., six male New Zealand rabbits were burned using a 200-gram metal cylinder (3 cm in diameter) heated in boiling water for 3 minutes and applied to the skin for 15 seconds [12]. This method created a third-degree burn on rabbit skin and required the average healing time of 37 ± 3 days. Hatibie et al., induced second-degree burns on rabbit skin using direct flame for 5-7 seconds [13]. This study evaluated histopathological conditions and burn degree, but did not mention the healing time.

In a study in Vietnam, a simple burn model on rabbit skin was developed by using a 250 g aluminum block (2 cm x 2 cm) heated in boiling water with different forces (0 N and 5 N) and burning times (5 seconds, 10 seconds, and 15 seconds) [14]. In this model, the burn wounds were healed after 6 days, which is not suitable for other experiments with a long burning duration. This study demonstrated specific burn induction procedures and methods, but did not evaluate burn severity, burn area, or histopathological analysis.

Another benefit of our research was to supply a detailed histologic assessment scale for inflammation, hemorrhage/congestion, and epidermal/epithelial structures. This helped in evaluating histopathological images more easily. Consequently, epidermal separation was not

observed in any group, and epidermal structures were fragmented with epithelial structure deformation. Hemorrhage/ congestion ranged from none to severe, and inflammation varied from mild to severe on the 7th day. By the 14th day post-burn, both conditions had reduced in all groups. Notably, the third-degree burns in Group 3, the hemorrhage/congestion and inflammation conditions, were severely presented on the 7th day post-burning.

Our study provided a method and procedure for experimental burn induction and evaluated various parameters such as burn area, wound contraction rate, healing time, histological image analysis, and burn injury severity on rabbit skin. Our research contributed to the diversity of experimental burn models with varying degrees of burns, laying the foundation for future burn treatment studies depending on the burn depth.

5. Conclusion

The study successfully established a burn model on rabbits with different burn degrees depending on the time of burn application, laying the groundwork for new experimental burn treatment studies.

Using the hot thermal burn method for application times ranging from 10 seconds to 20 seconds with a metal block weighing 200 grams, the second-degree burns or partial-thickness burns were induced. With a burning time of 30 seconds, a third-degree burn model was induced with full-thickness damage and required a healing time of more than four weeks.

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Conflicts of interest

None declared.

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