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Research Article

A Mouse Model of Scald Wounds

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Abstract

Background: There were many mouse models of scald wounds have been created in the previous study. However, expensive materials, complicated and dangerous operation and poor repeatability were all disadvantages of most of these models. The purpose of this study was to establish a mouse model of scald wounds with controllable depth and area using some common consumables in the laboratory.

Materials and Methods: A homemade scald-producing device was produced in our laboratory by using some common consumables, including 50ml centrifuge tube, water bath kettle, thermometer etc. Twelve male C57BL/6 mice were randomly divided into two groups (n=6 in each group). Mice in control group were not burned. Mice in experiment group were scalded with hot water. More specifically, the mice in experiment group were fixed into the home-made fixture device, allowing the shaved dorsum expose to 90°C water for 9s. 24h later, skin samples of all mice in two groups were harvested for histopathological examination (H&E), masson staining and CD31 Immunohistochemistry assay. Terminal-Deoxynucleoitidyl Transferase Mediated Nick End Labeling (TUNEL) assay was performed to detect the apoptosis of scald wound.

Results: This mouse model of full-thickness scald injury could be produced successfully by scalding with 90°C hot water for 9s. At 24h after scald, the skin tissues of mice in the experiment group exhibited the fractured and necrotic epidermal layer away from dermal layer, with almost unviable dermal hair follicles. Amounts of collagen degeneration, blood vessels necrosis and lots of increased apoptotic cells were demonstrated in scald skin. In addition, compared with the control group, coagulation necrosis and homogenization change of partial subcutaneous adipose tissue were observed in the scald skin. And even the superficial portion of some intradermal muscle layer was injured. The above experiments were repeated three times. The mouse model of scald wounds was successfully produced every time. The depth and area of burn were controllable. The model possessed a great repeatability.

Conclusions: These results indicated that the homemade scald-producing device could be successfully and conveniently used for producing the mouse model of scald wounds. Simple structures, low cost, transparent fixture device for easy observation and safe operation are all its advantages. Much more important, the depth and area are controllable through precise temperature control and standardized animal positioning.

Keywords: Mouse; Model; Scald wounds; CD31; Apoptosis

Introduction

Burns are one of the most general and fatal forms of trauma. Globally, the incidence of burn is still very high in modern life, especially in the developing countries. Immediate and specialized treatment was usually needed for patients with severe burn injury [1]. Over the past several decades, major advances have been made in the treatment of burns, and the survival rates for burn patients have improved dramatically. However, burn wounds still lead to serious mortality and morbidity, especially in children and adolescents [2]. Burn wounds often result in immune dysfunction, the delayed systemic inflammatory reaction, shock, sepsis, and some other severe complications [3-5]. Burns can also cause disability, disfigurement, lasting psychological and large economical burden to patients [6]. It is still necessary and urgent to keep going on studying on the treatment and pathogenesis of burn wounds.

Animal models of burn wound have been crucial to the advancement of the therapeutic methods of scald wound, which have made great contributions to the scientific research of cutaneous wound healing and the associated systemic effects. Because of the anatomical similarity to human's skin, large animals were used firstly for burn models [7]. However, on account of ease of handling and using of a large number of animals for getting statistical significance, mouse models of thermal injury have been payed more and more attention.

Nowadays, the most frequently-used models are the contact burn and the scalding burn. The scalding model usually employs a template with an aperture, through which part of the body is immersed into a water bath with a controlled temperature for a specific period of time [8]. Most of these methods of making such a model are costly and complex. Expensive materials, dangerous operation and poor

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repeatability are all defects in the process of producing such burn models. So a feasible and convenient mouse model of scald burns is needed in the process of medical related animal experiments. Based on these requirements, we focused on creating a mouse model of scald injury with the following characteristics: (1) transparent fixture for easy observation, (2) low cost, (3) safe operation, (4) great repeatability, (5) simple structure, and (6) ease of handling.

Materials and Methods

Animals and materials

Twelve 20±3g male C57BL/6 mice, aged from 6 to 8 weeks, were provided by the Experimental Animal Center, Second Military Medical University, Shanghai. The mice were housed in cages under constant temperature (23-27°C), relative humidity (50%-70%) and 12h light/dark cycles. A certified diet and free access to water were provided to the mice. All animal experiment procedures were approved by the Ethics Committee of Animal Experiments of the Second Military Medical University (Shanghai, China), which was consistent with the Guide for Care and Use of Laboratory Animals published by the US NIH. Anti-CD31 was purchased from Abcam, Cambridge, Massachusetts. The TUNEL Apoptosis Detection Kit was purchased from Roche. And Masson's Trichrome Stain Kit was provided by Sigma-Aldrich (St. Louis, MO, USA).

The structure of the scald apparatus

50ml centrifuge tube (Corning Incorporated, New York, USA), thermometer, water bath kettle, heat sources, and some other laboratory consumables were used for making scald apparatus. The apparatus mainly consisted of the fixture and heating device (Figure 2). Fixing device, made from 50ml centrifuge tube, included the handle (1), ventilation (2), skin contact window (3) and the cap (4). The ventilation could guarantee that the mouse in the tube could breathe smoothly, and through which the mouse tail was fixed out of the tube to avoid the scald of tail vein in the study. The handle was set in the upper of the tube, with which the operator could control the location and the time dipping in the hot water. The size of the skin contact window was designed according to the need of the scald area. The cap can prevent the hot water from entrying into the tube. The heating device mainly contains water bath kettle (5), thermometer (6), heat source (7) and some other common lab consumables.

Groups and scalds

A total of 12 mice were randomly divided into two groups (n=6 in each group) as follows: control group (unburned group) and experimental group (scald group). After weighed, all mice of two groups were anesthetized with an intraperitoneal injection of 1.5% pentobarbital in sterile saline (0.13ml/20g). The dorsum of the mouse was shaved carefully from the base of the tail to the neck including the two flanks by using an electric shaver (Panasonic, Osaka, Japan), to achieve an uniform burn which would be hindered by the hair. Before scald infliction, the shaved skins were sterilized with some alcohol swabs. After cleaning, the moue was left for 2min to dry and keep consistent with the ambient temperature to standardize the temperature of the skin before scald. After these, experiment group (n=6), mice were inserted headfirst into the fixing device to make sure that the shaved dorsum was centered by the skin contact window. The mouse tail was fixed out of the tube through the ventilation. And

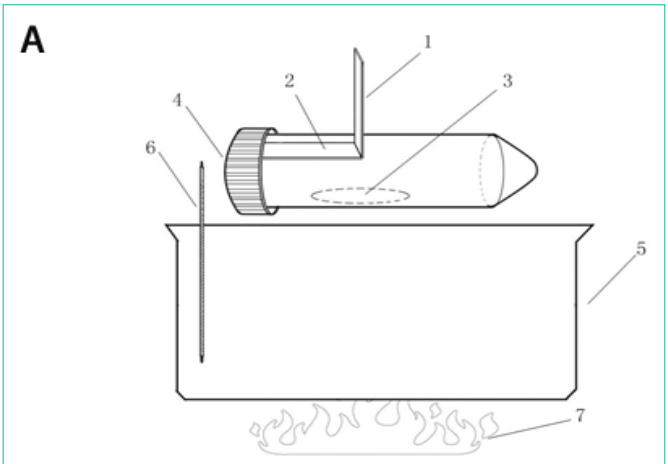

Figure 1: Scald apparatus: Scald-producing device was composed by the fixture and heating device. Fixture device, made from 50ml centrifuge tube, included the handle (1), ventilation (2), skin contact window (3) and the cap (4). The heating device contained the water bath kettle (5), thermometer (6), and heat source (7).

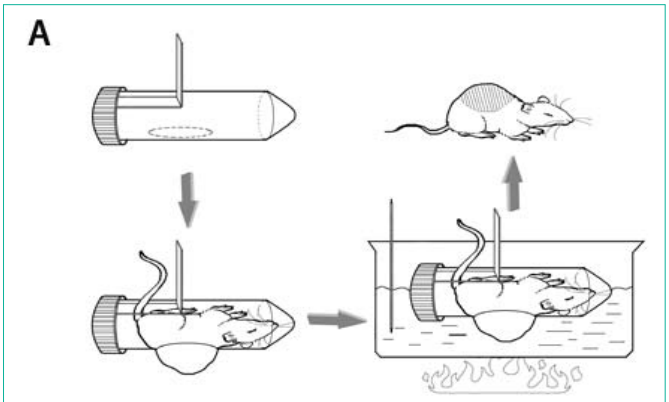

Figure 2: The procedure of scalds: After anesthesia and shaved of the dorsum, the mouse was placed into the fixture and then exposed the shaved dorsum to 90°C water for 9s. The mouse model of scald wound was finished.

then the cap was covered. With the handle, the centrifugal tube was vertically put into the water bath to expose the shaved dorsum skin to the 90°C water for 9s. Then intraperitoneal injection of 1ml 0.9% Normal Saline (NS) per mouse was appplied for fluid resuscitation. 24h later, 6 mice of experiment group were killed and the scalded area with a 0.3cm surrounding margin of unburned skin was removed to detect histopathologic changes. And at the same time, 6 unburned mice of sham group were also killed and the dorsum skin tissue was acquired for histopathologic evaluation (Figure 1).

H&E staining

H&E staining was conducted to show the histopathologic changes of scald and unburned wounds. The excised full thickness wound tissues were fixed immediately in 10% paraformaldehyde, dehydrated with an increasing series of alcohol concentration, paraffin embedded and cut into 4-μm-thick sections. For H&E staining, after deparaffinized, rehydrated, washed and dried, slices were dyed using hematoxylin for 8-10min. Then the slices were immersed in 0.5% HCl alcohol solution for 10s, followed by 0.5% eosin for 30s. Then 95% and 100% ethanol were applied for dehydration. The slices were

transparentized in dimethylbenzene [10]. Two blinded observers examined final results microscopically by using a light microscopy (Nikon Eclipse 80i, Japan).

Masson staining

The skin tissue samples of 12 mice were excised and fixed immediately in 10% paraformaldehyde, followed by routine histological processing paraffin embedding and microtomy with 4μm cuts. For Masson's trichrome staining [11], after deparaffinized, rehydrated, incubated in hematoxyliniron solution, and washed by hydrochloric acid alcohol, then slices were disposed by acid fuchsin solution and 1% phosphomolybdic acid. After these, slices were incubated with aniline blue, and 1% glacial acetic acid was used, then dehydrated in 95% and 100% ethanol and transparentized in dimethylbenzene. Slices were examined under a light microscope.

Immunohistochemistry

Anti-CD31 immunostaining were also performed to highlight blood vessels injury in each section. After fixed in 10% paraformaldehyde, embedded in paraffin, and sectioned by a slicing machine, skin tissues sample were treated with primary antibody against CD31. After rinsed with PBS, the sections were incubated with secondary antibody [12]. And after some other steps, the sections were observed under a light microscope to evaluate the vascular injury of scald mouse. The number of CD31+ blood vessels within five 50 × fields of each biopsy was calculated.

Tunel staining

TUNEL staining was performed to investigate the apoptosis level of scald wounds. A TUNEL Apoptosis Detection Kit was used according to the manufacturer's protocol [13]. Briefly, after disposed using ethanol and acetone, sections were incubated in a terminal deoxynucleotidyl transferase enzyme mixture. Subsequently, the sections were incubated with antidigoxigen in fluorescein. Some other steps later, slices were analyzed under a light microscope. Five fields of each biopsy were evaluated under a light microscope at a 50X magnification for TUNEL-positive cells.

Statistical analysis

Data was presented as mean + standard deviation. SPSS 17.0 (IBM company, NY, USA) was used for the statistical tests. Difference between two groups was calculated with Student t-test. $P<0.05$ was considered statistically significant.

Results

The epidermis and dermal hair follicles were damaged severely in scald mouse skin

Histologic sections of unburned and scald wounds were showed in Figure 3. The acquisition of full-thickness burns was confirmed (Figure 3B and 3D). The epidermis (e) of scalded mouse skin was fractured and discontinuous, away from the dermal layer (d). The dermal Hair Follicles (hf) were damaged severely, which were almost unviable after scald (Figure 3B and 3D). In addition, coagulation necrosis and homogenization change of some subcutaneous adipose tissue and the damage of superficial intradermal muscle layer were also detected. The histological structure of unburned mouse skin was exhibited (Figure 3A and 3C). The epidermis layer was connective and lots of hair follicles were abounding in the dermal layer.

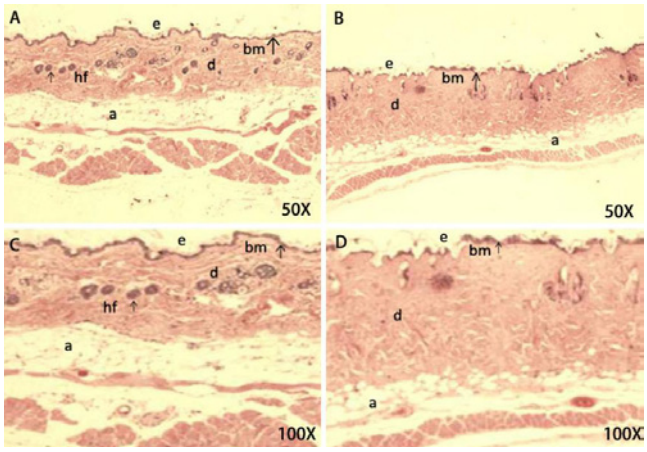

Figure 3: H&E appearance of unburned and scald mouse skin: (A and C) Cross section of unburned mouse skin showed continuous and contacted epidermal cell layer (e), normal dermal (d) layers and subcutaneous adipose tissue (a). The dermis was in the middle of the epidermal basement membrane layer (bm) and the subcutaneous adipose tissue (a). (B and D) Photomicrograph of injury wound tissue demonstrated fractured epidermal cell layer, discontinuous basement membrane layer (bm) and almost unviable dermal hair follicles.

Damage of lots of collagen fiber was detected in scalded skin

Masson staining is a great indicator of burn wound depth and range of the tissue necrosis boundary, which is often used to detect the distribution and morphological changes of collagen fiber after scald. A normal characteristic of masson staining of unburned mouse skin was demonstrated (Figure 4A and 4C). A large number of collagen deposited and arranged neatly in unburned mouse skin. However, after scald, collagen fiber was destroyed severely, arranged disorderly and sparsely (Figure 4B and 4D). And fewer dermal hair follicles were observed in the scald skin, which was rich in the unburned mouse skin.

The number of CD31+ blood vessels was decreased obviously after scald

CD31 immunostaining is a highly specific marker for vascular endothelial cells [14,15]. Vascular morphometry was conducted on sections of both groups by immunohistochemical staining for CD31. Most of the blood vessels in the dermis were destroyed after scald, and the basement membrane and smooth muscle layer were discontinuous and even absent. While amounts of normal blood vessels were abounded in the unburned dermis layer (Figure 5A). Data analysis showed the number of CD31+ blood vessels decreased evidently after scald (Figure 5C).

More cells were undergoing apoptosis in the scalded epidermis and dermis

TUNEL staining was performed to investigate the apoptosis level of scald wounds [16]. In this study, compared with control group (Figure 6A), much more TUNEL-positive cells were observed in scald epidermis and dermis after 24h of scald (Figure 6B). The number of TUNEL-positive cells increased prominently, especially in the scalded epidermis and epithelium of hair follicles and blood vessels (Figure 6C).

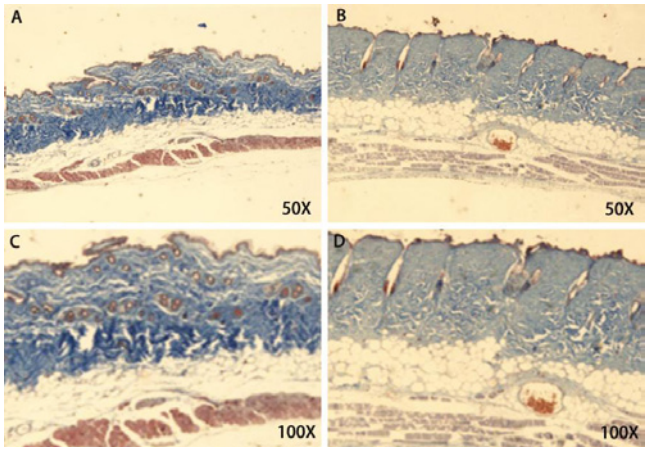

Figure 4: Masson staining of the unburned and scald wound. (B and D): much more severe collagen denaturation was observed, which arranged disorderly and sparsely, and some superficial portion of intradermal muscle layer was damaged; (A and C): a normal characteristic of unburned wound.

Discussion

Over the past few decades, although treatment technology for burn patients has improved dramatically, burns injuries are still considered to be the leading cause of accidental death in the world [17]. A large number of cases of burns occur every year, especially in the developing countries. Burn wounds often lead to a devastating effect both functionally and cosmetically, involving severe pain, decrease of life quality and high cost of treatment for lots of patients worldwide [18]. More and more attention has been payed on the scientific research of burn wounds.

Animal models of burn wounds have made great contributions to the development of scientific research in novel therapies and diagnostics. A better understanding of the potential mechanisms of burn progression and healing has been acquired through animal models of burn wounds [19]. Because of the anatomical similarity, large mammal became preferred subjects for related research firstly [19]. However, the large size and high cost had limited it for the practical use. Although anatomical differences between the rodent and human skin still exist, mice have been used widely for animal models of burn wounds due to some following advantages: easy to handle, little life space and the cheapest cost in terms of housing and maintenance [22-24]. Much more important, we can conduct experiments on a large number of mice at any point and acquire large amounts of results.

Lots of experimental mouse models of burn wounds have been established and used in previous studies by contact with a heated brass block [25] or hot water [26]. However, most mouse models of burn wounds were costly, complicated to operate and difficult to observe the burn procedure. In our work, we presented a mouse scald model with some common laboratory consumables, such as the centrifuge tube, water bath kettle etc. Indeed, major advantages of the mouse model of burn wounds are its transparent fixture device for easy observation and low cost, which is almost impossible in most of other animal models.

In addition, our experimental model was established to standardize burn wounds with controllable size and depth through the precise temperature control and standardized animal positioning. The scald wound healing is a slow process with high risk of infection and scar formation, which mainly depends on the depth and area of the injury [27]. The size of burn wounds was decided by the calculated dimension of the skin contact window of the fixture device, and upper limit was the size of the animal's back. The director could get different burn area by changing the size of the skin contact window as needed.

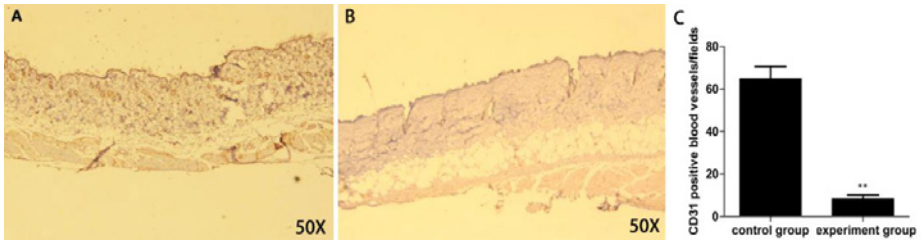

Figure 5: CD31 immunostaining highlighted blood vessels of unburned and scald mouse skin. Most blood vessels were destroyed obviously in the scald wound (B), while amount of CD31+ blood vessels were rich in the unburned mouse skin (A). (C) Analysis of CD31+ blood vessels. Data showed that blood vessels decreased sharply after scald. Data are showed as mean + standard deviation (n=30 fields in each group; **p<0.01 versus control group).

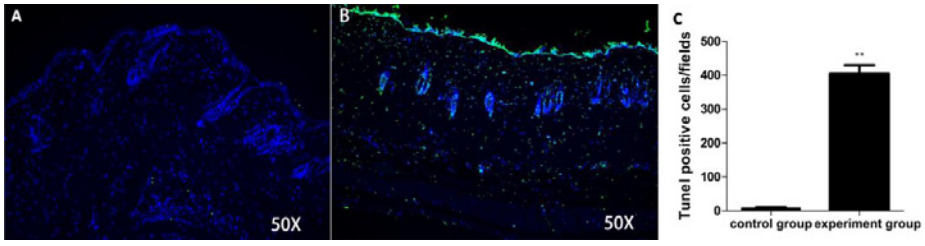

Figure 6: Cells apoptosis in the epidermis and dermis of unburned and scald skin. Scald and unburned skin tissue slides were subjected to TUNEL assay. Much more apoptotic cells were observed in scald wounds (B) than unburned wounds (A). (C) Analysis of the Tunel-positive cells. Data showed that apoptosis of the scald skin increased sharply compared to the unburned group, especially in the epidermis and epithelium of hair follicles. Data was presented as mean + standard deviation (n=30 fields in each group;**p<0.01 versus control group). TUNEL = terminal-deoxynucleoitidyl transferase mediated nick end labeling.

For most animal models of scald, burn depth was usually defined by three different elements – temperature, time of exposure, and contact pressure, and the scald depth would be stable 24h later [28]. Our experimental model was established to standardize burn wounds with controllable size and depth. The size of the wound was decided by the calculated dimension of the aperture. The director could acquire different burn area by changing the size of the skin contact window in the body of centrifuge tube. The upper limit was the size of the animal's back. Different burn depth can be acquired by changing the scalding time and the water temperature in the water kettle bath.

In our work, we have confirmed the acquirement of full-thickness burn wounds through H&E staining, masson staining, CD31 immunostaining, and tunel- staining, by exposing the shaved skin in 90˚C water for 9s using our homemade scald-producing device. The process of making the scald-producing device was successful and convenient.

Conclusion

In summary, the home-made mouse model of scald wounds was successfully designed, which was constructed and implemented using some common experimental materials in the laboratory. Transparent fixture device for easy observation, simple structures, low cost, and safe operation are the major advantages. Much more important we could acquire different area and depth trough the precise temperature control and standardized animal positioning. Therefore, this mouse model of scald could be used widely for scientific studies of burn wound in the future scientific research.

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Authors' contributions: F.Z. and F.Z. participated in the design and manufacture of scalding-producing device. Y.X. and F.Z. performed the animal experiments and analyzed the results. Y.X. wrote the article. F.Z. was also in charge of the final revision of the article.

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