Chapter

Animal Models of Burn Wound Management

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Abstract

Burn injury is known as the most traumatic wound. In the clinical, most patients with burn injury suffer from extreme pain during wound management; hence, the effective treatment that involved advanced medication is needed. In the evaluation of burn wound care devices, the use of animal model is considered suitable as valuable tools to investigate the burn pathophysiology as well as the efficacy of treatment strategies due to the complexity and heterogeneous nature of the burn. This chapter aimed to review the preclinical small and large animal models of burn injury for translational applications and to highlight their benefits and limitations for the burn treatment design that are clinically applicable to humans.

Keywords: burn wound, treatment, animal model

1. Introduction

The skin is the largest organ of the body, and its destruction, especially caused by burn injuries, is sufficient to be life threatening. Burns are responsible for many pathophysiological changes, resulting in a severe form of trauma that initiates several complications such as an escalation in infection and mortality rates as well as prolonged hospitalization and time of inactivity [1, 2]. For affected large surface area, burns may turn into a systemic problem affecting a various range of organs [3]. Consequently, there will be an intense inflammatory process and prolonged hypermetabolism, coordinated by hormones, cytokines, and acute phase proteins, which are associated with delayed wound healing process, enormous catabolism, multi-organ failure, and death [4]. Further, burn patients also will associate with anxiety, sleep disturbances, social avoidance, depression, and a disruption in activities of daily living after physical rehabilitation [5].

In decades, many important advances have been made for the improvements of the burn care; however, more still needs to be undertaken. The comprehensive study of burn pathophysiology is vital for further improvements of the current treatment strategies. Numerous experimental models have been established and can be applied to address the systemic, cellular, or molecular responses that occur in burn injuries, particularly the development of animal models. The use of these burn animal models is crucial for burn research especially for investigating the properties of new medicines, as it is known that novel treatment strategies should be initially tested at the experimental level before the clinical use [6]. For accurately investigating any therapeutic approaches and relevantly translating to the clinical,

the utilization of animal models has to be reproducible and as close as possible to burn lesions occurring in humans. Nevertheless, each animal model has advantages and limitations that determine its translational significance for burn treatments. In addition, the selection of the model should consider the anatomical and physiological characteristics of interspecies that reflect the differences in how different types of wounds heal and analytical techniques be applied. This chapter will further discuss the common animal models of burn injury as well as provide researchers with a better understanding of their benefits and limitations for the burn treatment design that is proposed to be clinically applicable to humans.

2. Burn wound management

Burn injuries differ in their cause types and severity; hence, its treatment can be challenging to be managed. The first and second degrees of burn injuries usually are treated with the moisturizer, the topical agents, and/or an antimicrobial creams advised by the doctor [7]. This condition will typically heal within 2 weeks. On the other hand, because third degree of burn injuries destroys all of the skin layers, the majority of wound will tend to severely long-term consequences and cannot be managed by the primary healing process, so the additional surgical procedures, including skin grafting, skin substitutes, and the application of advanced wound dressing, are required [8, 9]. They act as filler to increase the dermal component of wound, improve the re-epithelization, and reduce the inhibitory factors and the inflammatory responses of wound healing, and therefore subsequent scarring [9, 10]. Numerous options for skin substitutes, dermal analogs, and advanced dressings existed, which can be broadly divided and utilized depending on the severity of burn injuries [11]. However, removing the eschar and covering the wound as early as possible are crucial since the main challenge in treating third degree of burn injuries is avoiding infection from any contaminations. In addition, appropriate deep burn care providing protection from physical damage and supporting the circulation of gas and moisture as well as a comfort to enhance the functional recovery should also be the priorities in severe burn wound care.

Advanced burn care has been associated with a deeper insight of the pathophysiology of burn wound healing as it demands the collaboration of many different tissues and cells that contribute to each phase of wound healing [12]. In severe burn, the phases of wound healing including inflammation, proliferation, matrix synthesis, and contraction, are dynamic and complex and tend to overlap [13]. Therefore, a better understanding of these phases is a key concept to continuously develop an advanced severe burn wound management.

Experimental model is essential when studying on the burns and its underlying mechanisms. Many animal models of burn injuries using mice, rats, rabbits, dogs, and pigs are reported. They have been widely used to examine the burn wound pathology, the effect of systemic drug application, local therapy, and the effect of burn trauma on the entire organism [14–16]. The use of animal models is considered suitable as valuable tools to examine the burn pathophysiology instead of *in vitro* experiment due to the heterogeneous nature of the burns and its similarity to the characteristics of the human skin. The accurate animal model that closely mimics the overlapping phases of severe burn wound would enable the researchers to investigate the potential of novel treatments and study each phase more precisely. However, each animal model of burn has its own advantages and limitations, so the evaluation of several models of burn wound in animals is important and will be further described below.

3. Animal models in burn wound studies

The use of animals as experimental models in various biological researches for transposition into human physiology was initially provoked by Bernard in 1865 [17]. Over time, the notable similarities of anatomy and physiology between humans and animals have further encouraged many researchers to investigate a large range of mechanisms and therapies in the animal models before translating their findings to humans. In burn studies, there are some common techniques for producing wound burns in the animal model including hot water, hot metal tools, electricity, and heated paraffin [18–20]. In these methods, the back of the animal is shaved, and a heated material is executed to the skin to induce the desired burn surface area. The specific parameters such as raised temperatures and duration of exposure are required in each different burn models [21–23]. Furthermore, the integral planning for the burn animal model experiment is also crucial to be estimated. The most significant difference in the skin histology between human and animal is the density of hair. The rapidity of reepithelialization and the morphology of hair follicle are extremely influenced by the hair cycle; it would affect the planimetry area of wound and the microscope data of observable skin biopsy [24–26]. For instance, the hair cycle of rodents is short (approximately around 23–28 days). In order to avoid their hair cycle effects for the evaluation of the wound, rodents with a similar birth date should be used. Because different animals possess different hair cycles, the specific time consideration of each animal model is necessary to be highlighted. In addition, the hair might reduce the heat transfer, and some serious infections source could be hiding in the hair; thus the animal hair needs to be thoroughly depilated. Shaving by hair clipper and then applying with hair removal cream can remove the hair entirely. However, the hair removal cream might induce contact dermatitis so its administration time should be carefully controlled. Last but not least, appropriate post-operation care is needed to be considered too in order to elevate the survival rate of animal. The rational use of antibiotics can prevent wound infections, and the proper administration of analgesics can improve the appetite and self-harm of the animal [27, 28]. Moreover, large areas of burns can also cause severe loss of body fluids; therefore, intensive monitoring and handling for the dehydration of animals are necessary.

The right choice of method of burn induction and its maintenance in animal models are important as this impacts the burn outcome and determines how the wounds are treated. There is diversity among the species in the structure and anatomy of the skin along with their pros and cons as an experimental burn injury model. In this section, several animal models of burn in literature will be evaluated.

3.1 Mouse

As a research model, mouse contains the major layers of the human skin (e.g., epidermis, dermis) and provides the main insights of the signaling pathways associated in the healing process due to the variety of mouse-specific reagents and transgenic feasibility in mouse. Mouse also shares several physiological and pathological features with human, including cardiovascular, musculoskeletal, and other internal organ systems [29]. Additionally, the morbidity of mouse in research is relatively low owing to an extensively reduced healing time and superior immune system [30, 31].

In burn, mouse animal models are usually used to understand the burn wound healing process and have a reproducible model. Recently, Lateef et al. demonstrated a highly reproducible partial-thickness injury in mouse that mimics the key aspects

of the inflammatory and hypertrophic scarring responses observed in humans [32]. Further, Calum et al. have established a 6% third-degree burn injury mouse model with a hot air blower [33]. This model resembles the clinical situation and provides an opportunity to examine or develop new strategies such as new antibiotics and immune therapy for handling burn wound. Moreover, a 25% third-degree burn injury was demonstrated by exposure to boiling water for examining the efficacy of new formula-based traditional medicine [34]. Although burn mouse model has its specific advantages, evidently this model fails to completely mimic the wound healing process of humans. Mouse wound healing occurs mainly through wound contraction and the presence of enriched progenitor cells from their dense skin's hair, which facilitates rapid skin healing and keratinization [30, 35]. In order to alleviate the wound contraction issue, the splinting strategy (performing mechanical fixation of the skin by using devices or splints) has been developed [36]. This method could maintain the wound volume to remain relatively constant, so it allows the histomorphometric or biomolecular quantification of the cellular response under well-controlled, experimental conditions. Another issue is the differences of chemokines and chemokine receptor system between human and mouse including chemokines IL-8, neutrophil-activating peptide-2, inducible T cell chemoattractant, and monocyte chemoattractant, which is critical for wound repair as they contribute to the inflammatory events and reparative processes [31, 37]. Because management strategies for burn injuries are advancing, it becomes essential to consider the potential limitations when assessing the translational accuracy from mouse to humans.

3.2 Rat

Rat is one of the most widely used animal models in burn studies and mainly shares similar features with mouse burn model. Both of them have the cheapest cost in terms of housing, maintenance, and reproduction. Compared with the mouse, rat possesses a larger body size and also is easier to handle as well as less easily stressed by human contact. Despite their popularity, the rapid wound healing mechanics in rats are opposed to the wound healing process seen in humans. This limitation is because rodents (rat, mouse) own a subcutaneous panniculus carnosus muscle that facilitates skin healing by both wound contraction and collagen formation [30, 38]. However, this rapid wound contraction enables the researchers to quickly study the comprehensive mechanics of wound healing to develop advanced treatment strategies.

Motamed et al. have demonstrated third-degree burn rat animal model to investigate the efficacy of amniotic membrane combined with adipose-derived stem cell treatment. The burn wound was fabricated using a hot bar (boiled in water) suppression on the dorsal site for 30 seconds [39]. In our previous study, we have developed a similar model using the implementation of 190°C brass block onto the rats' backs parallel to the midline for 20 seconds [40]. This model was used to evaluate the medical dressing's treatment on severe burn wound as well as its inflammatory responses and healing mechanisms. Recently, a rat model of polytrauma (the combination of severe burns, bone fracture, and blunt force trauma) was established to investigate the abnormal immune response leading to inadequate healing and resolution [41]. This model is proposed to create a useful model of battlefield injuries or severe traumatic injuries in a civilian population for evaluating the interventional strategies to enhance wound healing outcomes. Nevertheless, while the rat burn model is relatively simple, it loses significance when it purposes to learning the complex post-burn etiology of hypermetabolism. In the early post-burn phase with high total body surface area in humans, hyperglycemia will occur

and initiate an overall increase of glucose and lactate [42]. As the burn wound of greater than 60% of total body surface area in rats results in reduced survivability and is not maintainable for the experimentation [14], therefore, it needs to be considered to have a burn injury model with high total body surface area to recapture the hypermetabolism observed in human burns.

3.3 Pig

It is well known that the pig's skin characteristics such as structure, function, and cellular components most closely resemble that of humans. The epidermis and dermis of the pig are thick just like the case in humans, and their epidermis ranges from 30 to 140 μm and from 50 to 120 μm, respectively [16, 43]. Physiologically, the pig's skin responds as the human skin does to various growth factors and cytokines and displays the reepithelialization rather than contraction during the wound healing process, similar to that observed in humans. In addition, they also share important similarities such as epidermal enzyme forms, epidermal tissue turnover time, the keratinous proteins, and the composition of the lipid film of the skin surface [16]. Based on those aforementioned great anatomical and physiological similarities between pig and human, pig then has been extensively used as the experimental burn models than nearly every other animal model.

Severe burn injuries cause hypertrophic scarring that generates the painful permanent hard, red, and raised scars. With great similar skin characteristics to human, pig appears to produce scarring most identical to human hypertrophic scarring. Cuttle et al. have demonstrated a pig model of hypertrophic scarring after burns using a glass bottle containing water at 92°C to the skin of a large white pig for 14 seconds to create the partial-thickness burn wound [44]. This model of hypertrophic scarring after deep dermal partial-thickness burn injury can be used to further understand the pathophysiology of burn wound healing and scar formation as well as for the testing of various agents which could potentially improve the outcome of the burn wound. Another report demonstrated the reproducible burn hypertrophic scar model using the Bama miniature pig by applying a homemade heating device for 35 seconds followed by debridement surgery [45]. This model has displayed a similar macroscopic, histologic, and biologic criteria of burn wound compared to the human hypertrophic scars. As some burn characteristics in human can be practically well mimicked, hence, the examination of various treatment strategies for severe burn injuries can be specifically applied to gain a comprehensive understanding of the mechanisms of burn healing.

Several studies developed the severe burn pig model in order to evaluate the advanced strategy for the reconstruction of burn injuries. Our laboratory has demonstrated a severe pig burn model using a minimally invasive surgical technique with an easy-to-learn, cost-effective, and reproducible method [46]. This model provides crucial tools for the evaluation of any clinical dressings and uncovers the pathophysiology of burn wound healing. Recently, full-thickness burn wounds in pig model were utilized to evaluate the effect of fractional CO_2 laser therapy on objectively measured scar outcomes including scar area, pigmentation, erythema, roughness, histology, and biomechanics [47]. This model offers a powerful platform to examine the efficacy of laser therapy as a function of many treatment parameters such as the timing of therapy initiation, energy, and laser density. The use of pig as a large animal model provides the standardized location of burn injury and the therapy investigation in greater depth of wound via noninvasive and invasive analyses. Further, Singer et al. established a partial-thickness burn in pig model to investigate the efficacy of topical nitric oxide application to the burn wound [48]. They found that topical

application of a nitric oxide-releasing agent accelerated wound reepithelialization and angiogenesis in this model. As there are similarities in skin anatomy and physiology between pig and human, therefore, this treatment can be considered as alternative burn care in patients. However, future studies should discover other approaches to deliver nitric oxide to burn wounds and improve long-term outcomes.

Besides those advantages to capture most pig burn model can be quite challenging to implement due to its risk of infection and high expense of housing with the greatest care.

3.4 Rabbit

Severe burn injuries are known to induce analogous hypermetabolic and pathological systemic alterations in rabbits and humans [49]. Hence, due to their remarkable similarity in metabolic characteristics, rabbit was considered as a promising animal model for burn research. Rabbit is also a cost-effective choice as burn animal model compared to the use of pig.

Rabbit model provides facilities to conduct the systemic effects of burn injury such as dynamic changes in whole-body amino acid and substrate metabolism [49]. It has also been revealed that rabbits present a high level of resting energy expenditure after a thermal injury that indicates the same evidence in burn patients [49]. Moreover, rabbit model has proven to demonstrate the involvement of leucine as an important amino acid in muscle anabolism that shows the similar kinetics and pattern of change post-thermal injury in human patients [50]. Recently, Friedrich et al. have demonstrated a quantifiable deep partial-thickness burn model in the rabbit ear using a dry-heated brass rod for 10 and 20 seconds at 90°C, resulting in a measurable burn progression and minimization of burn healing by contraction [51]. This animal model could be an important new tool to guide the treatment strategies of burn hypertrophic scarring.

3.5 Dog

Instead of several animal models that have been developed in early research, dog can be performed as a mature model for burn-blast combined injury studies. Hu et al. have established the Beagle dogs in the development of a stabilized, controllable, and repeatable animal model that can mimic the actual site of the burn-blast combined injury using explosion and napalm burns [52]. The hemodynamic changes in the early shock stage of burn were successfully investigated in this model, and it also can be used as a good research platform on the mechanisms of fluid resuscitation during burn-blast combined injury shock. Another dog burn-blast combined injury model was established including blast injury caused by explosion immediately followed by seawater immersion that is known to induce the hemodynamic changes and metabolic acidosis [53]. This model supports the investigation of the early symptoms and unique pathophysiology of the blast-burn combined injury that will be valuable in defining the suitable management of such patients. However, the use of dog burn animal model for examining the comprehensive of wound healing process needs to be more considered due to the ethical regulations, limited standardized reagents, and its looser skin over the body/trunk which results in a wound that heals primarily by contraction. Rapid contraction is a common feature of animals with loose skin, while in the tight-skinned species (human, porcine), the wound closure occurs principally as the result of reepithelialization.

4. Clinical advantages of animal models in burn research

In clinical purposes, animal research models should be determined by maximizing their translational relevance to humans. Besides that each animal model has the unique strengths and limitations (summarized in **Table 1**), its most important value is the capability to represent the nature of disease and accurately evaluate the outcomes. There are several reasons the treatment strategies are considerably tested on animal models: (1) animals offer a degree of environmental and genetic manipulations that are rarely feasible in humans as well as unique insights into the pathophysiology and etiology of disease that frequently reveal novel targets for directed treatments; (2) if preliminary testing on animals shows their not clinically useful results, it may not be essential to test on humans; and (3) the authorities concerned with public protection have to ensure the toxicity and safety of the treatment strategies through animal testing [54].

Progress has been made in the area of assessment and measurement, either the comprehensive evaluation of burn pathological mechanisms or novel therapeutic approaches, by involving the animal models of burn. As we have discussed before, there are numerous animal models of burn established to disclose these issues. The ultimate goal of these animal studies is to examine a safe and effective test condition

Species	Advantages	Disadvantages	References
Mouse	• Shares several physiological and pathological features with human (e.g., the skin, cardiovascular, musculoskeletal, other internal organ systems) • Superior immune system • Provides various mouse-specific reagents and transgenic feasibility • Low morbidity • Cost-effective • Easy handling	• Rapid healing along with wound contraction issue • Different chemokines and chemokine receptors system • Looser skin with dense hair structure	[29–31, 35, 37]
Rat	• Similar to mouse but possesses a larger body size and is less easily stressed by human contact	• Similar to mouse	
Pig	• Possesses great anatomical and physiological similarities with human	• Risks of infection and morbidity • High expense of housing and care	[16, 43]
Rabbit	• Shares remarkable similarity in metabolic and pathological alterations of burn with human • Lower cost than pig	• Risks of infection and morbidity	[49]
Dog	• Similar environment to human • Can mimic the actual site of the burn-blast combined injury so it can be used as a good research platform on the mechanisms of fluid resuscitation during burn-blast combined injury shock as well as its early symptoms and unique pathophysiology	• Ethical regulations • Limited standardized reagents • Cost hurdles • Looser skin over the body/trunk	[52, 53]

Table 1.
Comparison of the advantages and disadvantages of burn animal model.

for clinical trials in humans with burn injuries. Generally, the choice of animals for burn model is mainly based on cost and ethics and further is based on which species will give the best correlation to human trials.

Several substantial advancements have been made in burn patient care such as controlling wound healing, developing novel graft and coverage preferences, optimizing dietary needs, and testing unique pharmacological interventions, resulting in an improved patient's survival and decreased hospitalization period [11]. For example, Wang et al. established a clinical scar in a pig burn model that is found to greatly correlate with scar histology, wound size, and reepithelialization data [55]. This clinical scar scale demonstrated a reliable and independent tool for assessing the burn wound healing outcomes without using other healing and scar measuring systems. Clinically, scar appearance and function are the major concerns to both burn victims and their carers, so its minimization is one of the ultimate goals of burn care, which relies on the appropriate evaluation of the scars.

5. Conclusion

Burn injury is one of the most severe forms of trauma that is related with significant pain and various physical, psychological, and social diminishments; therefore, the exploration of advanced treatment strategies in order to obviously heal and reduce the lifelong burn wound recovery phases is demanding. Burn animal models have been proposed as valuable tools that provide considerable insights into the burn pathophysiology as well as for investigating the properties of new medicines before the clinical use. Accordingly, the standardization of animal models is crucial for all scientific research, and it can merely be achieved with a comprehensive description of the experimental techniques along with their advantages and limitations. With a better understanding of burn underlying phenomenon as animal models paved the road to its mechanisms, progressive research is expected to continuously identify novel treatment strategies to improve the quality of life for burn patients.

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Conflict of interest

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