Review Article

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A review on burn and burn models in animals

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Abstract

Burns can be defined as tissue damage caused by a variety of agents such as scald, fire, flammable liquids etc, which results in skin damage. Based on the destruction of skin layers, burns are classified as first degree burn, second degree burn, third degree burn and fourth degree burn. The depth of the burn depends on the time and exposure of agent to the skin. The wound healing processes consist of 3 steps, namely, inflammatory phase, proliferative phase and remodelling phase. The first aid treatment involves application of cool or cold water for a definite period of time. There are various topical agents used in the treatment of superficial burns such as silver sulfadiazine, sucralfate, mafenide acetate etc., and many herbal medicines such as *Allium cepa*, *Aloe ferox* etc. Pre-clinical study requires the animals to be initially anesthetized using various anesthetic agents or their combinations (e.g. ketamine or combination of ketamine and xylazine or ketamine and diazepam). There are various methods of inducing burns in experimental animals and their parameters for evaluation are wound contraction, reepithelization and histopathological examination.

Key words: Burn, Models, Wounds, Silver sulfadiazine, Reepithelization

1. Introduction

The skin is one of the largest organs in the body that performs copious vital functions including fluid homeostasis, thermoregulation, immunologic, neurosensory and metabolic functions [1]. The primary function of skin is to serve as a protective barrier against the environment. When this barrier is damaged, pathogens infiltrate the body resulting in an infection or a wound [2]. A wound is described as a break in the continuity of tissue from violence or trauma and is regarded as healed if there is restoration of wound site or inflamed tissue to normal condition [3]. Cutaneous wound repair comprise of an orderly progression of events that establish the integrity of damaged tissue. The sequence of events that repairs the damage is categorized into three overlapping phases: inflammation, proliferation, and tissue remodeling. Impaired wound healing may be a consequence of pathologic state associated with diabetes, immune disorders, ischemia, venous stasis, and injuries such as burn, frost-bite and gun-shot wounds [4].

Burn can be defined as tissue damage caused by variety of agents such as heat, chemicals, electricity, radiation etc. The most common are burns caused by scalds (lesions produced by moist heat), fire, flammable liquids etc. Burn injuries to skin result in loss of its protective function and act as a barrier for microorganisms leading to high risk of infection [5]. Burns are one of the most widespread injuries all over the world. In the United States, more than 1 million burn victims need medical attention every year, but only 4.5% of them require hospitalization [6]. Similar situations exist in United Kingdom, where burns comprise 1% of work load in emergency wards as well as 0.014% of hospitalization. Thus, most burns are not severe and could be managed outside the hospital [7]. According to World Health Organization (WHO), around 300,000 deaths are estimated per year worldwide due to burns [8].

Burns are one of the most common and devastating forms of trauma. Healing impairment in burn injury is characterized by improved free-radicals mediated damage, delayed granulation tissue formation, decreased angiogenesis and reduced collagen reformation leading to chronic wound healing [9].

Burns are responsible for many pathophysiological changes [10], expressing a severe form of trauma which may result in severe complications such as: a rise in infection rate, an increase in hospital stay, prolonged time of inactivity and also greater mortality rate. Among other changes concerning some physiological changes are also observed such as post-traumatic stress syndrome in case of victims of extensive burns [10, 11, 12].

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Table 1. Characteristics of various types of burn injuries

S. No	Depth of Burn	Layer/s destroyed	Cause	Surface	Colour	Pain Sensation	References
1	1st degree burn (superficial burn)	Epidermis	Sun or minor flash	Dry with no blisters	Erythmatus	Painful	[17]
2	2nd degree burn (superficial partial thickness)	Entire Epidermis and layer of dermis	Flash or hot liquids	Blisters with moist	Motted red	Painful	[18]
3	3rd degree burn (full thickness)	Extends into subcutaneous tissue	Flame	Dry, Leathery	Partly white or charred	Little pain	[18]
4	4th degree burn	Extends through entire skin and into underlying fat, muscles, bones, tendons, and bones.	Prolonged exposure to flame or from electric injury	Dry	----	Painless	[19]

2. Types of burns

According to the destruction of skin layer, burns are classified as first degree burn, second degree burn, third degree burn, and fourth degree burn. The 1st and 2nd degree burns are known collectively as partial thickness burns. The 2nd degree burn is the deeper injury than 1st degree burn. It involves all the epidermis and corium [13]. Most 2nd degree burns are characterized by blister. They are usually accompanied by considerable subcutaneous oedema. The rate of healing is dependent upon the depth of skin destruction and presence of infection [14].

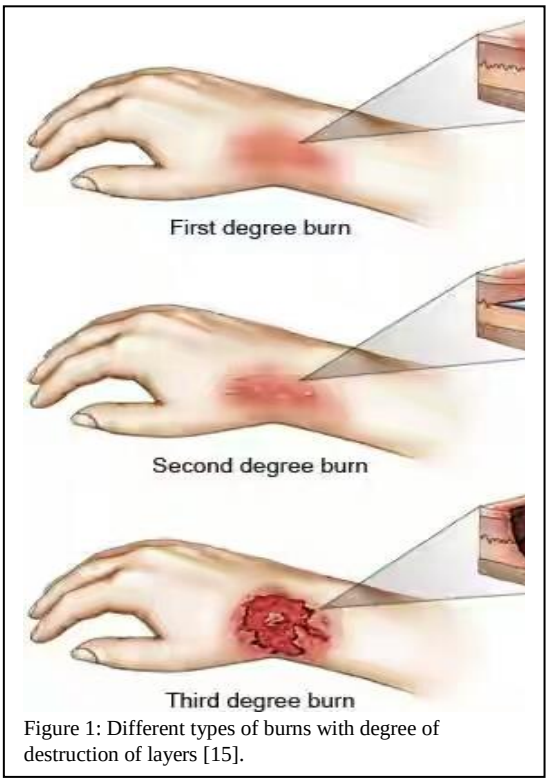

Figure 1: Different types of burns with degree of destruction of layers [15].

3. Classification of burn depth

Superficial burn: These usually heal within 14 days and leave minimal scarring. Burn erythema may be described as skin redness and pain. First and second degree burn are collectively called as superficial burns [16].

Deep burn: These take prolong time to heal and does with severe scarring. Third degree burn is a kind of deep burn [16, 17].

4. Causes of burn

There are various causes of burns as described by Alex Benson et al. 2006 [20]. Flame, scald and flash are the main causes of thermal injuries. In electrical injury, the depth of burn depends on exposure of a person to electrical volt. Acids and alkalis are the main causes of chemical injury.

5. Phases of wound healing

5.1. Wounding

Initially wound gets cleared off devitalized tissue and foreign materials that set a platform for further healing stages and regeneration process. It involves a brief and transient period of intense vasoconstriction and hemostasis [20, 21].

5.2. Inflammatory phase

Clinical inflammation, the second stage of wound healing presents as erythema, swelling, and warmth. It increases vascular permeability, resulting in migration of neutrophils and monocytes into surrounding tissues [21].

5.3. Proliferative phase

It is dominated by the formation of granulation tissue and epithelization. Chemotactic and growth factors released from platelets and macrophages stimulate the migration and

activation of wound fibroblasts that produce a variety of substances essential to wound repair [21, 22].

5.4. Wound remodelling

During wound remodelling mature scars develop as a collagen, forming a more organized lattice structure that progressively continues to increase tensile strength [22].

Table 2. Summary of wound healing phases

S. No.	Phase	Characteristics	Cells involved in phase	References
1	Inflammatory	Vasodilation Fluid extravasation Edema	Neutrophils Monocytes Macrophages	[21]
2	Proliferative	Wound Closure Revascularization	Keratinocytes Fibroblasts Macrophages Lymphocytes	[21]
3	Remodelling	Wound Maturation Scarring	Collagen Elastin Fibroblast/Myofibroblast	[22]

6. Protocol for managing burn injuries

Benson Alex [20] described the protocol for managing burn injuries.

A = Airway control
B = Breathing and ventilation
C = Circulation and Cervical spine control
D = Disability
E = Exposure and environmental control
F = Fluid resuscitation
H = Haemorrhage control

7. Treatment for burns

7.1. First aid treatment for burn injuries

Various regulatory authorities have recommended different methods regarding the first aid treatment of burn injuries. The recommendations all advocate the application of cold or cool tap water, however in most cases they are ambiguous or conflicting with regards to specific temperature, durations of treatment and delay after which treatment is still effective. So some recommendations suggest using ice or ice water while some do not recommend it [23, 24].

Alex Benson described the “drop and roll” procedure as a first aid treatment for burn in which the person needs to drop to the ground and roll over to extinguish flames from burning clothings by using a wet blanket [24].

7.2. Topical treatment

The most prevalent topical treatment for partial thickness burns is 1% silver sulfadiazine (SSD). SSD is a topical agent of choice for severe burns and is used almost universally today in preference to compounds such as silver nitrate and mafenide acetate [26, 27]. Silver sulfadiazine in spite of being effective, causes some systemic side effects consisting of neutropenia, erythema multiforme, crystalluria and methemoglobinemia [28]. Topical agents which are used only as antimicrobials include silver nitrate, sulfamylon and a combination of sulfonamide and SSD. Sulfamylon has broad spectrum activities, but is easily absorbed systemically and can lead to toxic complications. SSD has become the standard topical treatment for burn wounds [27, 28]. One of the potential burn dressings is sucralfate. Sucralfate is basic aluminium complex of sucrose sulfate and is a cytoprotective agent. There are many other topical antimicrobial agents which are used to treat superficial burns such as pirfenidone [29], chlorhexidine, mafenide acetate, povidone-iodine ointment, phenytoin, minoxidil gel, gentamicin sulfate, bismuth impregnated petroleum gauze, honey, Dakin’s solution (0.025% sodium hypochlorite) [30].

Table 3. Recommendations from some organizations concerning first aid treatment of burn injuries [25].

S. No.	Organization	Recommendation
1	Red cross	Lots of cool water for 10 minutes
2	St John Australian first aid	Cold running water for 10 minutes or until it returns to normal temperature.
3	International Liaison Committee on Resuscitation (ILCOR)	Cool with cold water as soon as possible, avoid ice or ice water for > 10 minutes especially if burns are large.
4	Australian Resuscitation Council	Immediately cool the burn area with cool water for upto 20 minutes. Do not use ice. Do not apply lotions.
5	British Burn association	Cold (tap) water for 20 minutes, no more to minimize risk of Hypothermia especially in large burns.

Silver sulfadiazine 1% cream is soft, white, water miscible cream containing the antimicrobial agent silver sulfadiazine in micronized form. Each gram of 1% silver sulfadiazine cream contains 10 mg of micronized silver sulfadiazine. The cream vehicle consists of white petroleum, stearyl alcohol, isopropyl myristate, sorbiton monooleate, polyoxy 40 stearate, propylene glycol and water, with methyl paraben 0.3% as preservative. Silver sulfadiazine 1% cream spread easily and can be washed off readily with water [31].

8. Pathogenesis of burn wound repair

Wound repair depends on neoangiogenesis, the activation of local immune response and in the presence of growth factors which include epidermal growth factor (EGF), transforming growth factor β (TGF-β) and basic fibroblast growth factor (b-FGF). Silver sulfadiazine (SSD) and sucralfate are known to have multiple beneficial effects on wound healing. SSD causes rapid healing through stimulation of re-epithelialization, formation of granulation tissue and increase in fibroblasts [16]. The drug induces the proliferation of dermal fibroblast and keratinocytes *in vitro* and inhibits the release of interleukin-2 and interferon-α from damaged skin cells. The physiological effect of the drug is to diminish inflammatory reaction and improve mucosal healing. Limiting the inflammation might decrease fibrosis and structure formation and EGF expression as well as the expression of other factors involved in tissue repair process. SSD and sucralfate stimulate the effects on vascular factors such as angiogenesis, which play an important role in tissue repair [32-36].

9. Animal model for burns

Some animals such as rats, mice, hamsters, rabbits, pigs, sheep and dogs, have been used as the study models for understanding the stages of healing. The use of these animals is not universal. Some studies have the advantage of presenting physiological and pathological characteristics similar to those of humans, considering stimulus to nervous, cardiovascular, endocrine and immunological systems [37]. The rat has many advantages in that it is small, allowing ease of handling. They are also cheap and have a high reproductive rate. However, their disadvantage includes those of their differences when compared to human i.e. size, metabolic characteristics and anatomy. Rats have been used in several studies [38]. The pig is the animal which is closest to humans in terms of some characteristics such as metabolism and structure of skin. Besides these advantages, they have great risk of infection, required greater care and expenditure. But the cost benefit shows that they are more demanding in terms of investments [39].

10. Location of burn

Dealing with the location of wound, the back is the choice in most cases, because it is difficult for animal to provoke further injuries to wound by licking or scratching the back. Some other location or a combination of locations that included the back are scapular, temporal extremities, lumbar and abdomen [40].

11. Size and thickness of burns

The size of burn relative to total body surface area (TBSA) presented a wide variation with values ranging from 1% up to 60% of total body surface area. The depth of burn is dependent on the amount of heat energy applied to the tissues and the time of exposure to tissue [41].

12. Anesthetics

Use of anesthetics is compulsory in animal burn model, in accordance with the requirements of Directive 86/609 of the Council of European Communities. Various anesthetics and its combinations employed in rat burn model are depicted in Table 4 and Table 5.

Table 4. Various anesthetics employed in rat burn model

S. No.	Anesthetic	Dose	References
1	Ketamine	100 mg/kg, i.p.	[41]
2	Xylazine	5 mg/kg, i.p.	[42]
3	Pentobarbitone	30 mg/kg, i.p.	[43]
4	Diazepam	3-5 mg/kg, i.p.	[47]
5	Midazolam	5 mg/kg, i.p.	[44]
6	Thiopental	40 mg/kg, i.v. 20-40 mg/kg, i.p.	[45]

Combinations of anesthetics are also employed to produce surgical level of anesthesia for 15-30 min and sedation for 1-2 hr.

Table 5. Combinations of anesthetics employed in rat burn model

S. No.	Combinations	Dose and Route	References
1	Ketamine-xylazine mixture	100 mg/kg, i.p. + 5 mg/kg, i.p.	[46]
2	Ketamine-xylazine-acetylpromazine mixture	50 mg/kg + 2.5 mg/kg + 0.75 mg/kg, i.m.	[44]
3	Ketamine-diazepam	100 mg/kg, i.p. + 5 mg/kg, i.p.	[47]
4	α-Chloralose-urethane	55 mg/kg , i.p. + 1000 mg/kg, i.p.	[44]

13. Laboratory methods applied for inducing burns

Regarding the techniques for producing burns, the hot water model was used the most. Hot water is easy to use for

an animal experiments [48]. Some techniques for inducing burns in animals are as follows:

- In some studies, rods are used to induce burns on rats. Rods are usually made up of brass, aluminum, or stainless steel rod may be used. The material of rod influences how rapidly heat is conducted from rod to skin, and subsequently the depth of burn. Metal rod with high thermal conductivity would cause a deeper level of burn compared to metal rod with lower thermal conductivity, when exposed to skin for same duration. Using a metal rod with low thermal conductivity would allow greater control over depth of burn infliction [48].
- Thermal lesion on the back of the animal is produced as follows: After anaesthetizing animal with suitable anesthetics, electric shaver/depilatory cream is used to expose a cutaneous surface on the back; the rat is then placed on its back in a mouldable metal wire cage. Once the animal is securely immobilized in the metal cage, the shaved dorsal area shall be submerged in water at 105 °C for 5 sec [49] or for 12 sec at 70 °C [50]. This model inflicts a deep dermal burn in entire cutaneous area exposed [51].
- Another method of dermal burn is to produce lesion by direct contact. The back of animal is shaved and a copper disc (diameter 4 cm), heated to 250 °C or aluminium metal rod heated to 80 °C is applied to skin as many times as necessary to burn desired surface area [52].
- Another method of inducing burn is based on skin contact with glass chamber through which water circulates at a predetermined temperature at a constant pressure of 10 g/cm^2 [53].
- Burn wound may be created by pouring hot (80 °C) molten wax (2 gm) in the metal cylinder, placed on shaven back of animal at the nape of neck [54].

Most commonly used techniques for producing burns is by using hot water. The hot liquid is usually the cause of burns in children, mainly those under five years of age. Hot water is also easy to use for an animal experiment. The most ubiquitous agent for producing burn in Brazil is liquid alcohol but in different countries alcohol is not used as model for producing burns in rats because of its issue of controlling the thickness and size of burn [55].

14. Laboratory techniques applied to study burn samples

Burn area can be analysed by various laboratory techniques such as microscopy, immunohistochemistry, ELISA, western-Blot, PCR, and electrophoresis. Microscopy is widely used technique to study burn sample [56].

15. Analgesia during postoperative period

Various analgesics employed during postoperative period are buprenorphine, dolantine, dimenidrinate and tenoxicam etc [56].

16. Nutritional requirements

Some essential nutrients required for wound healing are vitamin A, vitamin C, zinc, carbohydrates, glucosamine, amino acids such as arginine, glutamine etc. In addition to amino acids and vitamins, administration of insulin has been shown to decrease healing time by reducing protein catabolism and increasing skeletal muscle protein synthesis [57].

17. Assessment of burn wound healing

17.1. In vivo parameters

The healing is assessed based on physical parameters, epithelization period, wound contraction and histopathological examination [58].

17.1.1. Wound contraction

Wound contraction is the centripetal or concentric reduction in size of an open wound. It is noted by following the progressive changes in wound area planimetrically, excluding the day of the wounding. The size of wound will be traced on a transparent paper on 3rd, 7th, 14th and 21st day. The tracing are then transferred to 1 mm^2 graph sheets, from which wound surface area is calculated. The evaluated surface area is then employed to calculate the percentage of wound contraction by following formula [58, 59].

$$\text{Wound contraction (\%)} = \frac{\text{Initial wound size} - \text{specific day wound size}}{\text{Initial wound size}} \times 100$$

Percentage of Healing = (100 – Percentage of wound contraction)

17.1.2. Epithelization period

It is monitored by noting the number of days required for eschar to fall off from the burn wound surface without leaving a raw wound behind [58, 59].

17.1.3. Degree of hair growth

Hair growth rate on days 3, 7, and 14 after initiating the study is assayed with 10 fold magnification in both groups considering the score [42] as below:

1) Low hair growth: hair growth on burn area between 0 and 30 numbers

2) Medium hair growth: hair growth on burn area between 30 and 70 numbers
3) Low hair growth: hair growth on burn area more than 70 numbers

Table 6. Plants used in burn treatment [68-77]

S. No.	Plant (family)	Part used	Extract	Chemical constituents	Animal model	References
1	*Allium cepa* Linn (Liliaceae)	Bulb	Chloroform, alcohol	Kaemferol, sitosterol, ferulic acid, prostaglandins	Excision and incision on rats	[69]
2	*Kaemperia galanqal* (Zingiberaceae)	Rhizomes	Alcohol	Mainly flavonoids	Rats	[70]
3	*Aloe ferox* (Asphodelaceae)	Leaves	Juice	Vit C, vit E, and amino acids	Excision model on mice	[71]
4	Rubus species	Aerial parts	Methanol	Flavonoids such as kaempferol, quercetin	Rats, mice	[68]
5	*Ficus religiosa* (Moraceae)	Leaves	Hydroalcoholic	Saponins	Excision	[72]
6	*Hyptis suaveolens* (Lamiaceae)	Leaves	Chloroform, petroleum ether	Tannins, saponins and triterpenoids	Excision and incision on rats	[69]
7	*Thespia populnea* (Malvaceae)	Fruits	Aqueous	Terpenoids	Excision Incision	[74]
8	*Morinda citrifolia* (Rubiaceae)	Leaves	Ethanolic	Tannin content	Excision Incision	[75]
9	*Memecylon edule* (Melastomataceae)	Leaves	Methanolic	Triterpenes, tannins and flavonoids	Excision (mainly rat model)	[76]
10	*Trigonella foenum-graecum* Linn (Fabaceae)	Seeds	Aqueous	Alkaloids such as neurin, trigonelline, and gentianine	Excision Incision	[77]

17.1.4. Histopathological examination:

Histopathological examination of re-epithelized skin tissue of rats, are preserved in 10% formalin solution for histopathological examination. While performing histopathological study, tissues are embedded in paraffin wax, cut into fine thin sections of 3-5µm thickness and were stained with hematoxylene-eosin and observed for histological changes under 10X or 40X magnification [60, 61].

17.2. In vitro parameters

The granulation tissue excised on eighth postwounding day is used to analyze the biochemical parameters like antioxidant analysis including superoxide dismutase, catalase, glutathione S-transferase activities, hydroxyproline content, vitamin C content and total protein content [62, 63].

18. Future directions for superficial burns treatment

More research is needed to augment insulin delivery, which can decrease healing time by reducing protein catabolism and increasing skeletal muscle protein synthesis [64]. Anabolic agents such as oxandrolone also have shown to have an overall effect on wound healing, thus in future it can be useful to treat superficial burns [65]. Resuscitation, wound coverage and grafting are the future research areas on burn patient care. Research in inflammation, infection, stem cell grafting, biomarkers, inflammation control and rehabilitation will continue to improve individualized care and create new treatment options [66].

19. Role of Ayurveda in treatment of burns

In Ayurveda (an ancient Indian system of medicine), treatment of burn involves the use of various herbal medicines. Herbal medicines include herbs, herbal materials, herbal preparations and finished herbal products that contain an active ingredient, parts of plant, or other plant or combinations [67].

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