

Burn assessment: A critical review on care, advances in burn healing and pre-clinical animal studies

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ABSTRACT: Burn, a severe skin injury due to electricity, radiation, chemicals, or friction, may lead to the death of affected skin cells. Burns are a painful and crucial problem which causes disabilities. Sometimes, burns may also associate with the mortality of burn-injured patients. First-degree, second-degree, and third-degree are three categories of burns. First-degree burns (superficial burns) create minor skin damage as it affects the only uppermost layer of skin, and domestic care is sufficient for the treatment. Second-degree burns have injuries beyond the upper layer of skin, and third-degree burns reach every layer of skin, including nerve injuries that require critical care in treatment. Burn injuries are not limited to local; they may also give systemic responses and cause serious problems. Microbial infection is the most severe challenge associated with second and third-degree burns injuries. The ultimate goal for treating burn injuries is re-epithelialization with minimum tissue scarring. Selection of the appropriate treatment will be based on the extremity of the burn injury. The most prevalent and effective treatment is topical agents containing mafenide acetate, silver sulfadiazine, silver nitrate, etc. Skin substitutes, negative pressure wound therapy, and skin grafting are advanced treatments for burn injuries. Burn treatment is also associated with complications such as infection, dehydration, low body temperature, and emotional problems. Animal studies for burn models are performed using rabbits, rats, and pigs. This may be an effective way to find out the new forms of burn treatment, including assessing newly developed formulations.

Keywords: Burn; burn healing; burn assessment; burn critical care

1. INTRODUCTION

Burns are identified as the death of the affected skin cells by brutal skin injury. Burns are the most severe and crucial health problem which causes disability and death [1]. Statistical Investigation has shown an estimated 70 lakh burn incidents in hospitals in India annually, and it ranks second in various injuries following road accidents. In 2010, The Indian Government initiated the National Programme for Prevention of Burn Injuries (NPPBI). This program targets reducing the burn mortality rate, managing burn injuries, and the foundation of the central burn registry. However, its impact is not yet apparent [2, 3]. One million people in India each year endure mild to severe burns [4]. In 2016, Around 70 lakh burn injuries were reported in India [2, 3]. According to the WHO factsheet, in march 2018, 1.95 hundred thousand deaths occur yearly due to burns. More severe burns need instant emergency therapeutic care to prevent serious health issues and death. First-degree, second-degree, and third-degree are the three main kinds of burns. The severity of injury to the skin expresses the degree of burn. First-degree burns are the most minor, and third-degree burns are the most severe. Describing every symptom and sign associated with a third-degree burn and reaching up to the bones is known as a fourth-degree burn. Burns have a diversity of Etiologic like scalding from hot and boiling liquids, burns due to chemicals and electricity, and fires such as flames from candles, matchsticks, and lighters) and immoderate exposure to the sun. First-degree burns to produce the minimum skin damage. Additionally, they are known as "superficial burns," as the uppermost skin layer is only affected. First-degree burns are generally handled

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with domestic care. Second-degree burn injuries are more severe as the injuries expand beyond the upper layer of the skin. Infection can be avoided by maintaining a clean wound and its bandaging. This may lead to a faster healing process. Third-degree burns create the most severe damage by reaching throughout each layer of the skin. Third-degree burns are commonly thought to be excruciating. However, these burns relate to nerve cell injury [5].

2. SKIN

The body's most significant organ is the skin [6], which protects the body and prevents foreign substances from entering the body (Figure 1). It has been roughly calculated that up to 1000–2000 million epidermal cells are shed, and most are replaced daily [6, 7]. The epidermis, dermis, and hypodermis are three layers of the skin [8, 9]. The function of the epidermis layer is to restrict the entry of dangerous microorganisms, maintain water content in the skin, and thus maintain body hydration. It is divided into five layers: Basal, lucidum, spinosum, corneum, and granulosum [8, 10]. A dermis layer is present in between the epidermis and hypodermis layer is comprised mainly of blood vessels, collagen protein, hair roots, sweat glands, nerve cells, mesenchymal stem cells (MSCs), and lymphatic vessels [8, 11, 12]. The dermis layer provides structural robustness to the skin. The hypodermis layer, the third layer of skin, comprises macrophages, adipocytes, vasculature, fibroblasts, and nerves. Aid and repair of the dermal and epidermal layers serve the hypodermis layer's purpose [8, 13].

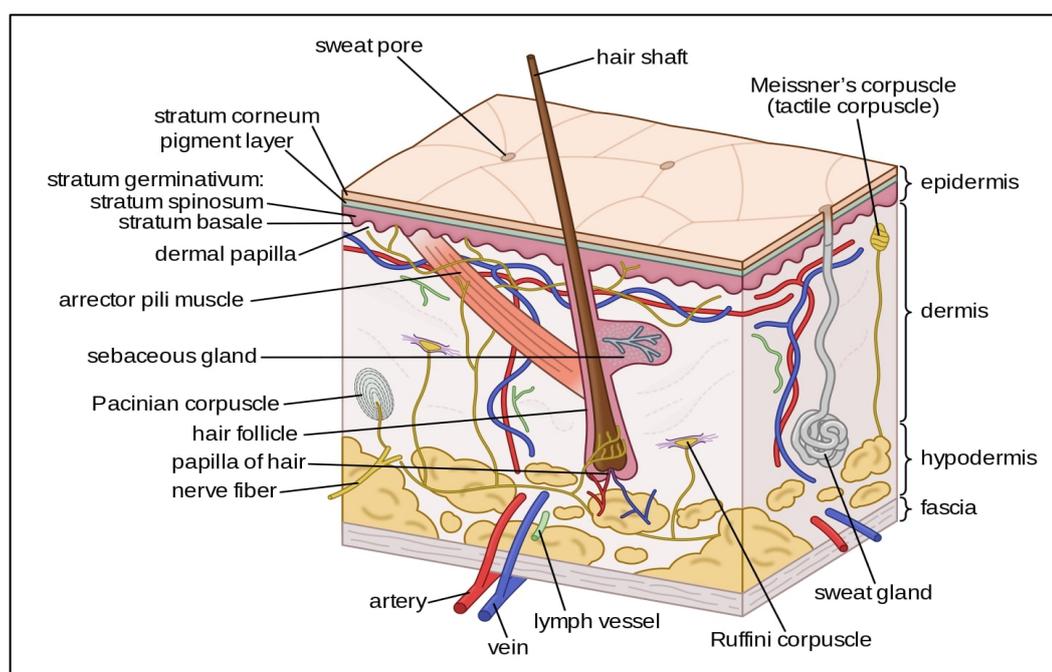


Figure 1. Anatomy of skin

3. THE VARIANCE BETWEEN BURNS AND WOUNDS

Burns have many generalized effects on the body. The skin has local damage in wounds, and generalized results are not seen [14]. Wounds are limited to the epidermal and dermal parts of the skin [15]. On the other hand, burns can affect much bigger surfaces, i.e., >20–30% TBSA (Total Body Surface Area), which shows significant burn injury. Burn injury caused due to electrical energy, radiation, chemicals, or abrasion results in the same damage as clinically the same as thermal injury [16].

3.1 Flame injuries:

These injuries are a usual form of burn. It generally occurs in women aged 16-35 and is more associated with a long time in cooking in loose-fitting clothing [17-19]. Flame injuries are of any depth; they may be of the total thickness or a portion thereof. Flame Injuries:

- 1) Scalds: Injuries occur due to hot liquids and steams. Scalds create superficial burns and may associate with a large skin area [19].
- 2) Contact burn occurs when the skin comes in contact with a great degree of a hot object or less hot object for a more extended period. Common causes of contact burns include irons, oven doors, radiators, the glass fronts of gas fires, and vitro-ceramic cooking stations. Contact burns, which frequently result in deep cutaneous or full-thickness burns, can cause fatal harm [19].

3.2 Electric injuries:

Here, electric current proceeds from one point to another throughout the body and generate "entry" and "exit" points. The electrical current can potentially harm the tissues in this area [20]. Electric injuries are split up into high voltage, which is more than 1000 V, and low voltage, which is below 1000 V. Their extremity is based on Current Contact time, Current type (AC vs. DC), and Voltage [21]. Low voltage burns create minor deep-thickness burns; meanwhile, high voltage burns create immense deep tissue burns and cause limb loss. High voltage burns, also known as flash burns as high voltage burns current, do not enter the human body, but high-temperature energy creates apparent burns to visible body parts like hands, upper limbs, face, and neck. Burned clothing may cause deeper burns. The cardiac cycle gets affected by electrical burns and may cause arrhythmia. Treatment should include cardiac monitoring.

3.3 Chemical injuries:

These injuries may occur because many different chemicals (Table 1) are used daily. The extremity of the caustic burn is based on the amount of the agent, depth of penetration, exposure duration, the concentration of the agent, and the mechanism of injuries [22-24].

Table 1. Different chemical substances and their mechanism of injuries

Sr. No.	Class of chemicals	Tissue injuries mechanism
1	Acidic Substances	Coagulation-induced cell death in live tissue
2	Alkali Substances	Liquefaction-induced cell death in live tissue, Dreadful burns due to extensive penetration
3	Solutions of organic substances	Lipidic membrane gets dissolve
4	Solutions to inorganic substances	Formation of salt due to direct binding

4. PATHOPHYSIOLOGICAL CONDITION AND HEALING PROCESS OF BURNS

A burn injury creates local responses as well as systemic responses. Local reactions to burning damage are identified by increased capillary permeability and hydrostatic pressure [14, 25, 26]. Figure 2 shows zones of coagulation, stasis, and hyperaemia as distinct areas of the burn injury. This bifurcation is based on the extremity of the wound. The necrotic area of the burn is named the zone of coagulation. It minimizes progressive damage to burning skin. When an injury occurs, tissue around the necrotic area is permanently destroyed. The area near the injured zone is known as the zone of stasis, with a gentle degree of injury with lower tissue perfusion. It is also connected with vascular damage, where inflammation occurs. Hyperaemia's periphery (distinguished by inflammatory vasodilation due to high blood flow) will initiate the healing process and generally avoid further necrosis [21, 23, 27]. The systemic response comes into the picture after burn injury reaches 30% of TBSA (Figure 3).

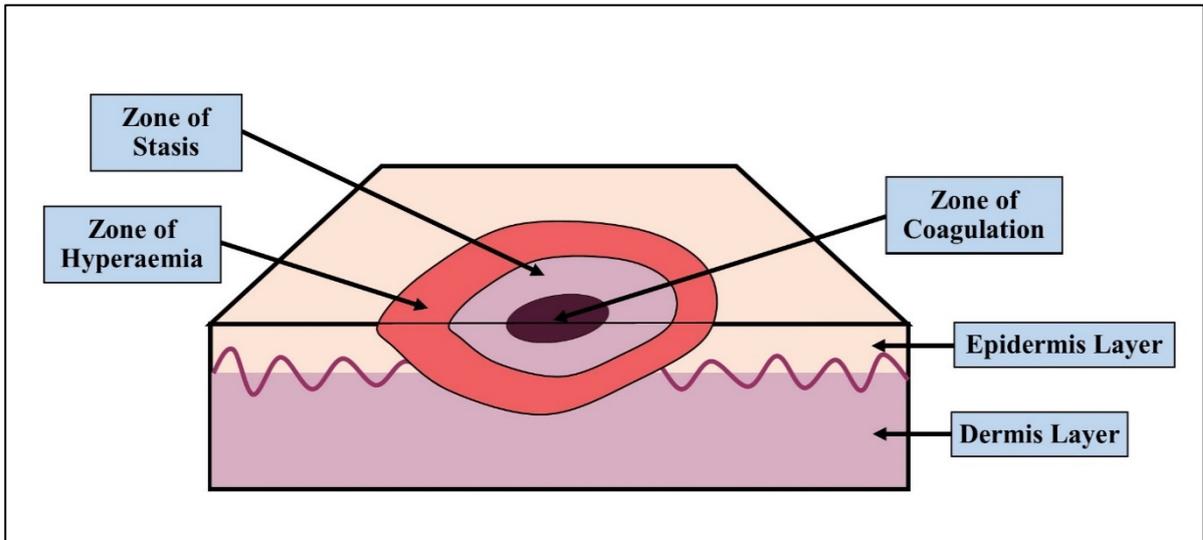


Figure 2. Structure of Skin and its layers

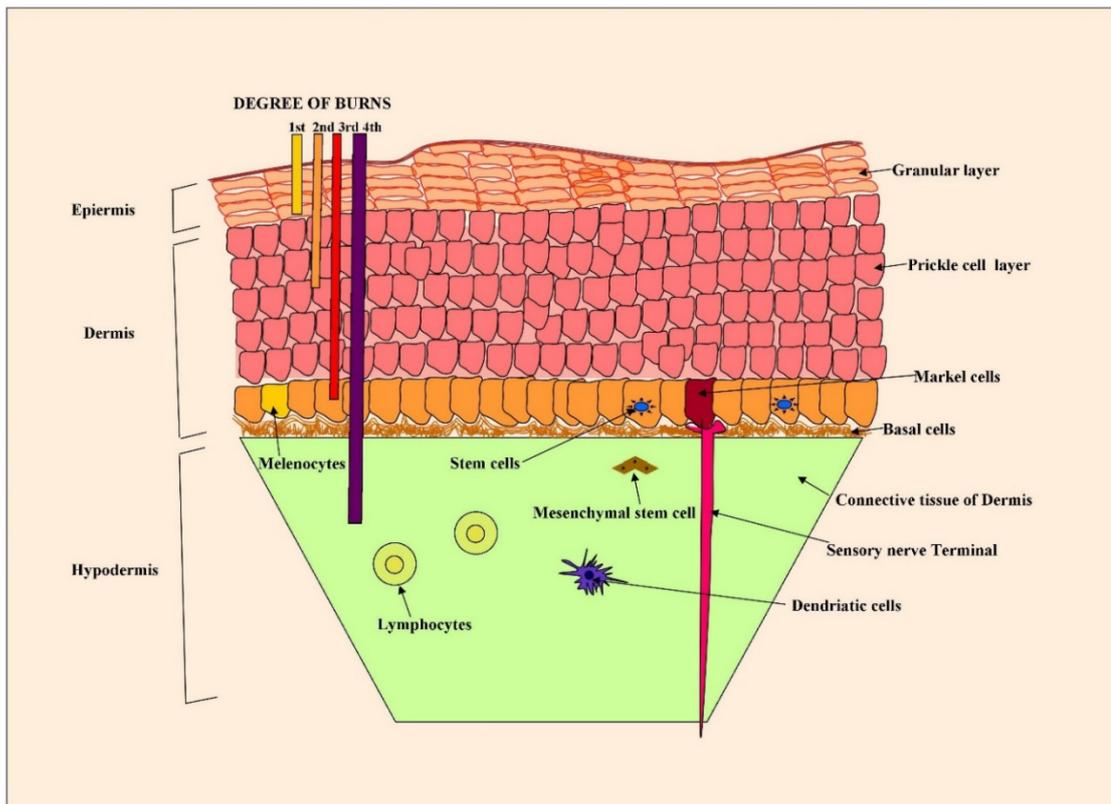


Figure 3. Different zones of burn injury

Cardiovascular response: Capillary permeability is augmented due to the loss of fluids and intravascular proteins into the interstitial compartment. Additionally, when the tumour necrosis factor is released, it lessens myocardial contractility. It also causes fluid loss from the burn wound. Respiratory response: bronchoconstriction occurs owing to the release of inflammatory mediators, and in intense burns, respiratory distress syndrome may happen. Metabolic response: Basal metabolic rate (BMR) increases up to three times compared to the standard rate. Immunological response: The body's defence mechanism is reduced [19, 20]. The ultimate goal in burn damage is re-epithelialization with the most negligible scarring. Phases of burn wound healing include four stages (Table 2), which are associated with biological processes, i.e., phases of hemostasis, inflammation, proliferation, and remodelling (Figure 4) [8, 28]. The hemostasis phase and

inflammation phase first give a response to injury and prevent blood loss at a specific site by clotting. Here, fibrin formation occurs through several events, such as augmentation of platelet, activation of the immune system, clotting of blood, and complement system activation [6, 12, 28-30].

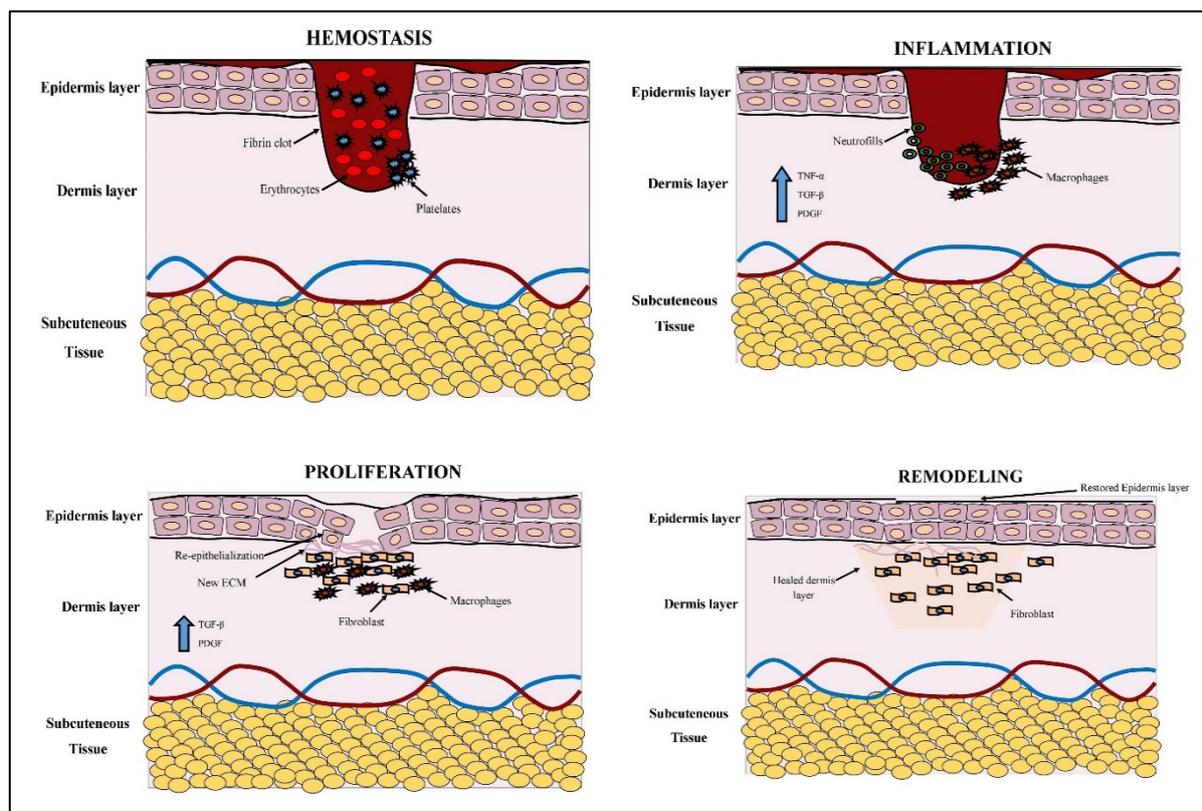


Figure 4. Burn wound healing process of each step

The inflammation phase prevents infection in wounds. At the site of injury, inflammatory cells are attracted by cytokines and transform tumour necrosis factor (TNF- α), platelet-derived growth factor (PDGF), and growth factor-beta (TGF- β) [28, 29]. TGF- β and other growth factors are also released by macrophages, which regulate the relocation of fibroblasts and epithelial cells into the wound [30]. The proliferation phase starts then onwards.

Triggering of keratinocytes and fibroblasts by cytokines and growth factors indicates the starting of the proliferative phase [31]. Keratinocytes move towards the wound, which leads to the restoration of the vascular network [32, 33], and the re-epithelialization process starts [34]. The dermis layer is restored in the proliferating phase. Accumulated fibroblasts and myofibroblasts produce ECM (extracellular matrix) proteins, namely fibronectin and growth factors like TGF- β [12, 35, 36]. The final steps induce the manufacturing of granulation tissue with fibroblasts, granulocytes, and macrophages. The last step of this phase is collagen formation, which fibroblasts initiate.

Scar from a burn wound yields more fibroblasts, collagen, and elastin. Here, myofibroblast initiates the contraction of the wound [34, 37]. Ending the response to the injury is crucially dependent on keratinocytes and inflammatory cells like macrophages and T cells dying [12, 36], which gives the final appearance of a burn wound.

Table 2. Different chemical substances and their mechanism of injuries

Phases of Burns	Description	Key responsible factors and their effect
Hemostasis [6, 12]	Platelet cluster, activation of defence mechanism, clotting of blood, and complement system-induced	Fibrin: Augmentation of platelet, activation of the immune system, clotting of blood
Inflammation [14]	Neutrophils and Macrophages remove foreign materials, control fibroblast, and epithelial cells in wounds	Neutrophils: Dilatation of blood vessels, Monocytes: leakage of fluid from the blood vessel into the surrounding tissue, Macrophages: swelling
Proliferation [31]	Re-constitution of an epithelial layer; restoration of the vascular network, fibroblasts induced collagen formation.	Keratinocytes: closing of the wound, Fibroblasts: restoration of the vascular network
Remodelling [12,14]	Collagen and elastin were produced; end of the response to the injury by the formation of myofibroblast	Collagen: tight cross-links between the collagen (Maturation of wound) Elastin: Induces cell activities like the migration of cells, synthesis of the matrix, and protease formation [38] Fibroblasts/myofibroblasts: contraction of the wound (Breaking of a fibrin clot, creation of new ECM and collagen structure) [39]

5. TREATMENT AVAILABLE FOR BURN SCARS

The healing of burn scars will depend on the depth of the wound. Superficial burns are generally treated easily without scarring, while severe burn needs special care for faster healing [34, 40]. Surgical and non-surgical treatments are available for burn injuries, which are selected based on the severity of the burn injury (Table 3).

Table 3. Treatment for burns

Types of burn	Symptoms	Generalized Treatments for burn Injury [5, 41]	
			Available treatment
1 st degree	Redness, minor inflammation, pain, dry and peeling skin		Apply cold water on the burn wound for three to five minutes or lengthier; apply analgesics for relief of pain, application of gel or cream containing herbal or appropriate ingredients along with local anaesthetics to calm the skin, and protect the affected area by applying antibiotic ointment and loose gauze
2 nd degree	Redness, splotchy skin, pain, swelling, blisters, scarring		Apply a thin layer of antibiotic ointment to the burn for healing; protect the burn with sterile, non-sticky cotton gauze may prevent infection and assist the skin's recovery.
3 rd degree	Burned areas might be charred black or white, leathery skin, destroyed nerves, numbness, difficulty breathing, carbon monoxide poisoning		Wear compression garments, skin grafting, surgery, physical therapist

5.1 Pharmacological treatments of burns

Analgesics, commonly referred to as painkillers, are essential in treating burns. Potential side effects and drug interactions must be avoided to treat pain in burn patients effectively. Acetaminophen and oral NSAIDs are mild analgesics that have a ceiling effect in their dose-response relationship. These constraints make these substances inappropriate for managing typical, severe burn pain. Minor burns can be successfully treated with acetaminophen and oral NSAIDs, typically in an outpatient setting. A reliable and efficient method of delivering adaptable analgesia to burn injury patients is patient-controlled analgesia (PCA) with

intravenous opioids. Patients with burn injuries frequently experience anxiety, which may be strongly related to pain. Anxiety can worsen pain by increasing background pain and the expectation of procedural discomfort. When treating burn pain, anxiolytic medications are frequently used with opioids. Benzodiazepines have been demonstrated to lessen procedural pain in patients with high levels and background discomfort when given as an opioid adjunct [42]. Systemic antibiotics are also essential in the treatment of burn injuries. Using antibiotics to treat underlying illnesses can lower morbidity and prevent a fatality. Once an infection has been identified, antibiotics should be given per the French Society for Burn Injuries recommendations. Topical antimicrobial therapy is frequently used as a complement to surgical treatment or systemic antibiotics to cure or prevent infections. Medicaments should not promote drug resistance and should complement the type of bacteria inducing the infection. Antibiotic use in burn patients is more challenging than in patients with other illnesses. This is because the burn victim's pharmacokinetic characteristics are altered, and there are significant individual differences. The key alteration is scanty tissue concentration of antibiotics, which immediately causes therapy non-fulfillment and drug resistance; as a result, drug resistance lowers the efficacy of standard doses of antibiotics. Furthermore, the effectiveness of medications like ciprofloxacin or penicillin-resistant to the enzyme penicillinase has decreased for *Staphylococcus aureus*. There is variability regarding the potency of new products that have become commercially available in the present day. Therefore, antibiotic adaptation, delivery and treatment duration are more challenging to establish in burn patients, and practitioners should examine all risk factors for morbidity and fatality when considering their use [43].

One of the most effective ways to control microbes in a disease-ridden wound is the ideal use of topical medications. Topical agents reduce the sepsis and mortality rate; each topical agent has advantages and disadvantages. The effectiveness of topical agents is identified by in vitro bacterial growth and reduced colony counts in vivo [44]. Some of the topical antimicrobial medications used in burns treatments are listed below.

Silver nitrate

It is a non-toxic, 0.5% solution and is very effective against *P. aeruginosa*, *S. aureus*, and *E. coli*. A mesh dressing is placed on the wound, and the silver nitrate solution is introduced. Silver nitrate has limited penetration capacity, the same as silver sulfadiazine because silver ions are very quickly bound to the natural chemical substances of the body. During treatment with silver nitrate, serum electrolytes monitoring is necessary. 0.5% silver nitrate solution is light-sensitive. After drying, the solution turns black when it is in contact with Cl-containing compounds and tissues. An adverse reaction such as high fever is also reported if the solution of silver nitrate becomes dry [45, 46].

Silver sulfadiazine (SSD)

SSD, a 1% water-soluble cream, is an amalgam of silver and sulfadiazine. The silver ion attaches to the organism's DNA and releases sulfonamide, and further, it interacts with the metabolism of the organism [44]. The advantage of this drug is its ability to reduce pain. Penetrating power is solely confined to the epidermal layer. It does not, however, relate to pulmonary fluid overload or acid-base disturbances, as it is found with mafenide acetate [46]. The side effect of this drug may be a reduction in granulocyte, but it is an argumentative statement [45, 46].

Mafenide acetate

It is presented as an 8.5% water-soluble cream base and a 5% aqueous solution. It has been of utmost effectiveness to the vast spectrum of microorganisms, predominantly for use with all strains of *Clostridium* and *P. aeruginosa*. The cream is employed at least twice daily and should be re-applied if removed from the wound. Moreover, Mafenide acetate has the maximum capability to breach burn eschar and prevent the growth of a microorganism colony in the necrotic area. Besides cream formulation, the 5% mafenide acetate solution is also helpful for the burn wound. The highest antimicrobial effect may be achieved by keeping the dressing wet with the mafenide acetate 5% solution. At every 8 hours, the dressings may be changed. This solution has some adverse effects, such as a carbonic anhydrase inhibitor, and it may cause acid-base imbalance when applied to a vast burn surface area. It may also cause respiratory acidosis.

Povidone-iodine

The topical usage of povidone-iodine is painful. Some recent studies state that the absorption of iodine in burn wounds is very high, leading to iodine toxicity, kidney failure, and excess acid formation. It can cause damage to fibroblasts by cytotoxic effect [45-48]. It is also used as a topical disinfectant.

Gentamicin sulfate

The 0.1% gentamicin sulfate-containing cream formulation is available on the market. It is similar to other aminoglycoside antibiotics, such as neomycin and kanamycin. Rapid resistance development is the major drawback owing to its excessive use as a topical antimicrobial medication [44].

Polymyxin/Bacitracin

Polymyxin and Bacitracin are used as ointment formulations. Since these topical treatments are non-toxic, many medical professionals recommend them for skin graft covering. At the same time, they are not effective in controlling the infection.

Nitrofurantoin

Nitrofurantoin has effectiveness against all gram-negative bacteria except *P. aeruginosa* bacteria. Nitrofurantoin is effective up to 75% compared to the 21% effectiveness of Polymyxin/Bacitracin [44].

Mupirocin

It is produced in the fermentation carried out by *P. fluorescens*. Antimicrobial activity is obtained from protein synthesis inhibition in the bacterial cell [49]. It is beneficial in methicillin resistance treatment in burn wound infections [50].

Nystatin

Nystatin, derived from *Streptomyces noursei*, is an antifungal medicine. It is generally used in an oral dosage form. However, topical use of nystatin powder at a dosage of 60,00,000 U/g has successfully treated burn sites with fungal infection. Nystatin powder may be given in combination with mafenide acetate (5% aqueous solution) and SSD (1% cream) to avoid the growth of fungi and yeast at the necrotic site [51, 52].

5.1.1. Marketed products

Sulfamylon (Mafenide acetate), Nilstat (Nystatin), Polysporin (Bacitracin/polymyxin), Furacin (Nitrofurantoin), Silvadene (Silver sulfadiazine), Bactroban (Mupirocin), Betadine (Povidone-iodine) and Garamycin (Gentamicin sulfate) are the few marketed products used in the treatment of burns.

5.2. Clinically advanced treatment available for burns

5.2.1. Skin grafting

When a burn injury completely demolishes all layers of skin, and a primary healing mechanism cannot heal the wound, the surgical procedure should be adopted [53]. Skin grafting is suggested during complete and partial-thickness burn injuries. The removal of the necrotic portion is followed by analogous skin grafting. Skin grafting transfers healthy skin from a patient's beneficial site to their dead or dying site. The skin grafting technique has its limitation. Unfortunately, less than 50% of TBSA is treated by this treatment [54, 55]. Over time, this issue can be conquered by recurrent skin transplantation from the donor sites. But the healing of the donor site is minimized, and skin disorders may rise [56, 57]. And sometimes, this technique also makes severe scarring possible [58, 59].

5.2.2. Split-thickness Skin Graft (STSG)

Unlike a full-thickness skin graft, an STSG only contains the epidermis and dermis components. It is classified according to the thickness of STSG. In STSG, the thickness of the graft (Thin: 0.01 to 0.03 cm, Medium: 0.03 to 0.04 cm, Thick: 0.04 to 0.07 cm) can be varied based on the patient as per their requirements. It is limited to the dermis layers that can increase to form a new epidermis layer [60,61].

5.2.3. Skin substitute

It is generally used when there is restricted availability of donor skin, but the burn wound is vast. Replacements that are either biological, synthetic, or both are the classes of skin substitutes. Biological skin alternatives have similar constituents as natural skin [59]. Unlike biological reserves, synthetic materials provide greater structural integrity but are less bioactive than biological substitutes, and artificial substitutes

have no disease transmission risk. Currently, limited synthetic skin substitutes are available in the market [62]. Different skin substitutes are classified in Table 4.

Table 4. Different types of skin substitute and their valuable composition in a burn injury

Sr. No.	Skin substitute	Composition
1	Karo skin [63]	Dermal and epidermal cells containing human skin
2	GraftJacket® [63, 64]	Human dermis layer
3	StrataGraft™ [65]	Keratinocytes derived from Human dermal fibroblasts and stratified epidermis
4	OrCel® [66]	Fibroblast and keratinocytes containing type I collagen matrix (Bilayered)
5	Dermagraft® [67]	Absorbable polyglactin Frame matrix containing Neonatal fibroblasts
6	Laserskin® [68, 69]	The hyaluronic acid membrane containing fibroblasts and keratinocytes
7	MySkin™ [63, 70]	Surface coating of autologous keratinocytes supported by silicone
8	Pelnac®	Silicone film assembled from porcine tendon and atelocollagen type I sponge layer

5.3 Wound Dressing

Wound dressings are beneficial for wound coverage and re-epithelialization. It is also a skin desiccant that prevents contamination and further skin damage. Wound dressing acts as a support for wounds and enhances the healing process of the injured area. Various types of dressing are available in Markets. Dressing selection depends on factors such as the wound bed's state, burn depth, wound site, moisture retention and drainage amount, the requisite regularity of dressing changes, and cost. The ideal dressing characteristics offer protection against contaminants and physical harm, enabling gas exchange and moisture retention and ensuring comfort for accelerated operative recovery [71, 72]. Significant classes of wound dressings [33] are classified in Table 5 with their Marketed name and Manufacturer name.

Table 5. Different types of skin wound dressing

Sr. No.	Class	Name of Brand	Name of manufacturer
1	Alginate Wound Dressing	Aquacel	ConvaTec, Bridgewater, NJ, USA [73]
		Comfeel	Coloplast, Minneapolis, USA [71]
		Sorbsan	Mylan, Morgantown, USA [71]
2	Antimicrobial Dressing	Acticoat	Smith & Nephew, UK [73]
		Silverlon	Argentum, Geneva, USA [74]
3	Collagen-based Wound Dressing	Fibracol	Johnson & Johnson, NJ [75]
		Puracol	Medline, Mundelein, IL, USA [76]
4	Hydrocolloid Dressing	Duoderm	ConvaTec, Bridgewater, USA [73]
		Granuflex	ConvaTec, Bridgewater, NJ, USA [77]
		Tegaderm	3M, Maplewood, USA [71]
5	Hydrogel-based Wound Dressing	Dermagel	Maximilian Zenho & Co, Belgium [78]
		SilvaSorb	Medline, Mundelein, USA [79]
6	Polyurethane foam	Allevyn	Smith & Nephew, UK [73]
		Lyofoa	Molnyscke, Sweden [71]

5.3.1 Negative pressure wound therapy (NPWT)

Here, the application of a vacuum to the burn wound area via a foam dressing stimulates the granulation of tissue, raises blood perfusion, and decreases the rate of colonization of bacteria [6, 80]. NPWT is currently used in burn wound care [60]. In humans, NPWT reduces injury development [81], and an optimized NPWT environment increases the healing process [60]. Research has also indicated that NPWT enhances wound healing by combining split skin grafts with Matiderm® or Pelnac® skin replacement [59, 82, 83]. NPWT leads to improved grafting conditions, decreases the chance of infection in burn injury, and reduces sepsis progression and bacterial proliferation [84, 85].

6. COMPLICATIONS DUE TO BURNING INJURY

Burn patients lose their skin, a primary barrier, so they have a more significant risk of complications following complications generally occurring in the burn patient.

Infection: The most severe challenge in burn care management is infectious, and they are the principal reason for burn injury fatalities [86-88]. A few antibiotics are helpful in a burn injury, and bacteria's growth is mainly responsible for the infection. Bacteria are generally of two types, Gram-positive and gram-negative bacteria. *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Enterobacteriaceae* are common gram-negative bacteria that cause infection in a burn injury. Piperacillin-tazobactam, carbapenems, and cephalosporins antibiotic treatments are used respectively for particular Gram-negative bacteria. Penicillin treatment is indicated for Gram-positive bacteria like *Staphylococcus aureus*, *Streptococcus*, and *Enterococcus* [59]. Infection can lead to sepsis, which causes hypotension and reduced perfusion of organs, and it also reduces the skin healing process, leading to multi-organ failure [89-91]. Other than infections, the following complications may occur [5].

Dehydration: Burns cause fluid loss, so blood volume may become insufficient to supply blood to the entire body.

Low body temperature: Skin takes place in adjusting body temperature; however, during burn injury, the body can lose heat faster than in average conditions, which may lead to hypothermia.

Contractures: When a scar forms, it can tighten the skin leading to difficulty in the movement of bones or joints.

Emotional problems: If the damage is on the face or on other visible areas, this may lead to emotional issues.

7. ANIMAL MODELS USED IN BURN RESEARCH

Burn injury is not associated with a single pathophysiological condition but involves multiple organs that cause structural and functional abnormalities. *In vitro* experiments are unable to predict the complexity nor address the pathophysiology. Hence, the *in-vivo* study is essential. The *in-vivo* analysis can be performed to mimic the post-burn pathological mechanisms and also applied to evaluate the novel therapeutic approaches. Wound healing experiments are generally performed on animal species such as mice, rats, rabbits, and pigs (Table 6).

Mouse

In the burn healing process, mice are the most frequently used animals in experimental work. Mice are used due to a superior immune system and a low morbidity rate, and they have reduced healing time. Mouse models of burn injury also have drawbacks, such as mice cannot develop the same wound-healing conditions as humans. The wound healing process is rapid in mice due to wound contractions compared to humans.

In contrast, in humans, the re-epithelialization process occurs, which is a bit slower than the mice [92-94]. Mouse skin is covered with hairs that are denser compared to human hairs. Generally, 6 to 8 weeks old and healthy mice are used for the mouse burn model. The mice are anaesthetized by administering ketamine/xylazine through an intraperitoneal route or other anaesthetics. In some cases, a 1 mL saline solution is also given as it acts as a cushion to the spinal cord [95].

Rat

Rats' skin is composed of similar composition as of humans, and it also has the same layers, such as the epidermis and dermis layers. The rats' skin is looser; hence it has more elasticity than human skin. Rats are also used more frequently due to the low cost. The wound healing mechanism of a rat is the same as a mouse so it won't conduct similar healing conditions as humans. Rats have fewer chances of systemic sepsis [94-96]. The burn model of the rats is identical to the burn model of the mice. Rats have higher tolerance as they can hold up to 60% TBSA.

Guinea pig

Guinea pigs have numerous potential advantages, including a high pain tolerance, being lightweight, and being easy to handle. Their anatomy resembles those of humans, and their skin is sensitive enough to be burned easily. According to some researchers, the metabolic reaction to acute burn injury in guinea pigs is remarkably indistinguishable from humans' postburn metabolic response [97]. The stage of the hair development cycle affects the burn depth in small rodents like rats and mice. However, this is not the case with guinea pigs. In addition, the guinea pigs' epidermis and dermis are around the same thickness as human skin and have the least amount of thickness variability compared to rat and rabbit skin [98].

Pig

Skin structure similarities in anatomy and physiology enable the pig as an appropriate model for studying human skin. The epidermis and dermis layers of the pig are thicker than in humans. In mice and

rats, layers were thinner, so it could not mimic similar healing conditions, while in pigs, it is closer. Pigs have many more similarities, such as the density of hairs, orientation and distribution of blood vessels, epidermal enzyme patterns, and lipid film of the skin. Pigs' healing processes also have similar stages, such as inflammation, proliferation, re-epithelialization, and remodelling, which are also seen in humans [92, 94, 99].

Rabbit

The high cost of the pig model is addressed using the rabbit model, as well as it also has similar metabolic relevance to humans. The rabbit model creates circumstances to evaluate the systemic effect of burns and allows the investigation of dynamic changes [100]. Hypermetabolism response was also assessed easily in rabbits.

Table 6. Animal models used in burn/wound injury

Sr. No.	Animal Model	Key findings
1	Mouse	Interferon-gamma has a crucial influence on the recovery process of the burn wound. It inhibits Collagen (A key factor for re-epithelialization) and the synthesis of fibroblasts, which takes longer to heal burn wounds. The local examination was done after inducing severe burns in interferon-gamma deficient or sufficient mice. The authors' findings suggested inhibiting gamma interferon speeds up the healing process after a burning injury [101]. Authors have tried to characterize the expression of an actin filament protein known as gelsolin, present in the brain in mice subjected to burn injury. Gelsolin affects the motility of cells, glueyness, and apoptosis. Gelsolin activates monocytes, and astroglia cells, thereby playing an essential role in further apoptosis of neurons induced by swelling following burn injury [102]. The research findings concluded that intestinal immunity was improved due to significantly enhancing the level of IgA in burn-injured mice supplemented with enteral nutrition and glutamine compared to conventional enteral nutrition only [103]. Burn injury complication that is both severe and common is an infection, which is directly associated with the size of the burn. Cases of sepsis are higher in a burn if TBSA is more than 30% here; the authors' findings suggested that the treatment using simvastatin in burn-injured mice helps reduce the raised level of interleukin-6 further helps to reduce mortality in mice [104].
2	Rat	The experiment was performed to evaluate the effects of carbachol on rat enteral recovery from burn shock in terms of intestinal absorption rate and blood flow to the intestine's mucosa. The findings showed that carbachol increases intestinal water absorption rate as mucosal blood flow improves [105]. The study was focused on observing the morphological changes of the muscle fibres away from the site of thermal burn injury in rats. A noticeable morphological difference in muscle was found at the place of thermal burn injury, which covers 45% of the total body surface area [106]. The purpose of the study was to determine how ulinastatin affects fluid vasopermeability and responds to inflammation. Ulinastatin helps prevent systemic inflammatory reactions and fluid leaking into tissue after a catastrophic burn [107]. The investigation was done to determine the modulating effect of anaesthesia, analgesia, and euthanasia techniques on the inflammation profile in the rat burn model. Results concluded that understanding such effects is necessary for a study to examine the pathophysiology of the inflammation process in animal models with burn injury [108].
3	Pig	Various methods were developed to evaluate the healing process of wounds. This comprises tensiometry, immunohistochemistry, electron microscopy, granulation tissue depth analysis, and digital photography analysis. The results suggest that the hypertrophic burn scar in domestic pig appear similar to the hypertrophic scar in humans. The study's developed model helps evaluate and contrast various burn injury treatments [109]. The analysis was performed to classify the different methods of burn treatment in a standardized animal model of the pig through the histopathological assessment of scalds and contact burns [110]. The pig was used as an animal of illustration of the experimental burn wound. A stainless-steel round bar, previously heated to 50-110°C, was used to insert the burn wound using the push-pull force technique. Saline dressing and lidocaine HCl gel were used to heal burn wounds. The

authors concluded the examination by applying a pig as an animal model and using therapeutic formulation to heal burn wounds [111].

- 4 Guinea-pig The study evolved the comprehension of the healing process for burn wounds in the guinea pig after inducing a burn. The burn was produced on depilated dorsal skin using a round aluminium template previously heated to 75°C and was applied to the skin for 5 seconds. The study was performed to understand the mechanism of the burn wound healing process, i.e., epithelialization, contraction, and scar formation [112].
- 5 Rabbit To treat wounds, ulcers, and burns, therapeutic herbal remedies have been utilized for a very long time. The study was focused on the exploration of alcoholic extract of the yarrow plant for the treatment of experimentally induced burn wounds in New Zealand white rabbits as an animal model. The study concluded that the yarrow extract was the potential to enhance burn wound healing and lessen the microbial load of the wound [113]. Alkali burn injury was produced in New Zealand white rabbits. A cross-linked, chemically altered hyaluronan derivative was used to treat experimentally induced burn wounds.
Contrary to the control group, the treated group's rate of wound closure was higher, as the burn wound healing process improved in the treated group [114]. The study's objective was to evaluate the efficacy of quince seed mucilage, traditional preparation used in treating burns and skin wounds. A wound was created in Iranian male rabbits, and a cream containing quince seed mucilage (5%, 10% & 20%) was administered twice daily to the wound. The study concluded that 10-20% of quince seed mucilage-containing formulations showed an excellent prospect in the healing of wound injury [115].

8. SUMMARY AND CONCLUSION

The skin comprises the major part of the human body. It serves as an obstacle and safeguards the body from the environment. A zone of coagulation, a zone of stasis, and a zone of hyperemia characterize burn injury. Systemic responses come into the picture when burn injury extends more than 30% TBSA. It alters cardiovascular responses and immunological responses. The homeostasis and inflammatory phases are the first events of the burn healing process wherein the immune system will be activated and cause oedema formation. Revascularisation is part of the proliferative (the second phase of the burn injury) phase that leads to re-epithelialization. Topical agents are one of the most preferable and effective ways to treat a burn wound. Reduction in the chance of sepsis and mortality are the critical benefits of topical treatments for burns. Silvadene, Sulfamylon, Betadine, and Furacin are some marketed topical products helpful in treating burn injury. Some advanced treatments are also available such as skin and split skin grafting. These techniques are used to treat full-thickness burn injuries. Karo skin, GraftJacket®, OrCel®, and MySkin™ are examples of skin substitutes. The effectiveness of topical agents were evaluated by *in vivo* studies. Different animals are being used, such as rats, mice, rabbits, and pigs. Novel therapeutic formulations can also be evaluated using various animal models. This kind of animal model involved research paves the way for developing newer medicines and dosage forms to improve burn treatments.

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