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Animal Models For Evaluation of Wound Healing

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Abstract

Wound healing and repair is an age old complex process that has been studied extensively. Acute wounds are less severe and its healing process is achieved under normal sequence leading to proper restoration of the affected area. Chronic wound occurs when wounds have not proceeded through orderly manner. It is a active process that begins immediately after an injury occurs. A number of animal models have been developed for studying all the components of the healing process. Animal models have been used extensively to evaluate the mechanisms involved in wound healing. Animals like rats and mice have been used in evaluating the wound healing potential and efficacy of therapeutic drugs. In this review, the existing animal models for wound healing process are discussed along with the significance of the models.

Keywords: Wound healing, animal models, acute wound, Chronic wound

Introduction

Among all the organs of the human body, the skin is regarded as the largest, accounting for 15% of the total body weight [1]. Skin functions to protect vertebrates from environmental, physical and chemical stress. It also protects the body from the antigenic and thermal injury [2]. The skin consists of many nerve endings that are able to receive environmental signals and send this information to the central nervous system [3].Wound injury is the disturbance of the anatomical, cellular and functional integrity of the tissue. Wound occurs due to chemical, physical, microbial, thermal, immunological infections to the tissue [4].Physiologically, wounds are of two types as Acute and Chronic wound. In acute wounds healing is done in normal sequence leading to proper restoration of the affected area. Examples of acute wounds include

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surgical wounds, bites, burn, minor cuts, abrasions, traumatic wounds like lacerations and also gunshot injuries and are caused by external skin injury. Acute wound that failed to heal will ultimately become the chronic wound [5]. As per the Healing Society (WHS) wound can be categorized as chronic wounds into four categories based on their etiologies: pressure ulcer, diabetic ulcer, venous ulcer and arterial insufficiency ulcer [6]. Animal models have become indispensable tools for researchers in scientific study [7]. Animal models hold an importantrole in pre-clinical testing of new medicines and pharmaceuticals. With the introduction of animal models, a well comprehensive wound healing process has been achieved. The animal model is essential in evaluating therapeutic agents that can be used at treat wounds and wounds infections.In this review available animal wound healing models are discussed in detail.

Pathophysiology of wound Healing

Wound healing begins immediately after an injury and continues depending of the intensity of injury[8]. A proper healing of wound is essential for the restoration and functional status of the damaged tissue or skin [9].There are four continuous, well automated phases of wound i.e. hemostasis, inflammation, proliferation and remodeling[10]. Constriction of the vessels and clot formation initiates the process of hemostasis. Pro-inflammatory cytokines and growth factors such as TGF-β, platelet-derived growth factor (PDGF), fibroblast and epidermal growth factor (FGF, EGF) are generally released during the process. These growth hormones target the inflammatory cells and promote migration into the wound [11]. Infiltration of inflammatory mediators (neutrophils, macrophages and lymphocytes) are the key factors responsible for initiation of inflammatory phase reaction. The neutrophils act by removing help invadingof microbes and cellular debris in the wounded area. In the early stages, macrophages discharge cytokines which enhances the inflammatory response clearance of apoptotic cells. Apoptotic cells are cleared and that results in a transition to a repairing stage that releases keratinocytes, fibroblasts. It also promotes angiogenesis to initiate tissue regeneration. T-lymphocytes drift into wounds followed by the macrophages and other inflammatory cells, and are accumulated in maximum amounts at the late-proliferative/ early-remodeling stage. In a recent study it is reported that the mice deficient in T- and B-cells show that the scar formation is reduced in the absence of lymphocytes [12]. In proliferative phase, there is epithelial proliferation and migration over the provisional matrix within the wound. Inside the wound bed, fibroblasts

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stimulate collagen as well as glycosaminoglycans and proteoglycans, which are major constituents of the extracellular matrix (ECM) [11].The wound finally undergoes physical contraction, vascular maturation and regression takes place and a new skin is formed [13]. Figure one represents the stages involved in wound infection.

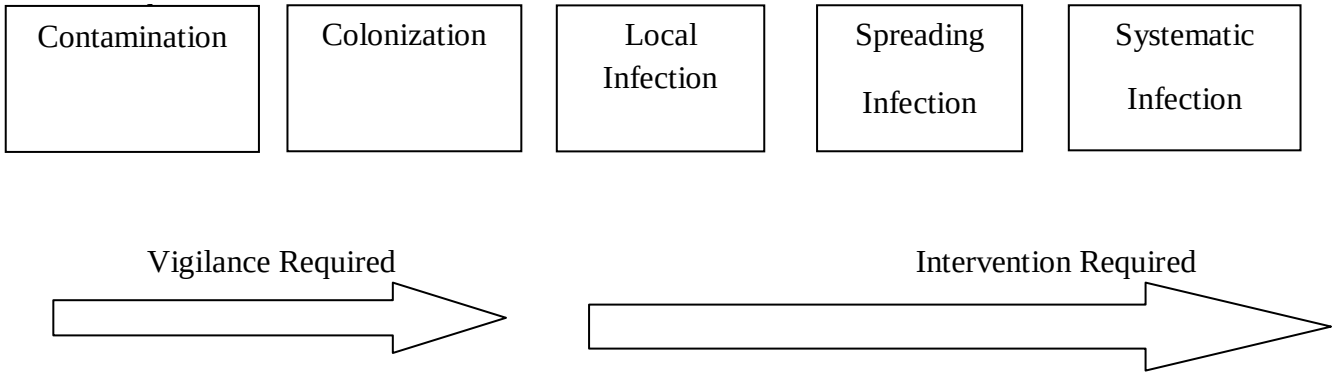
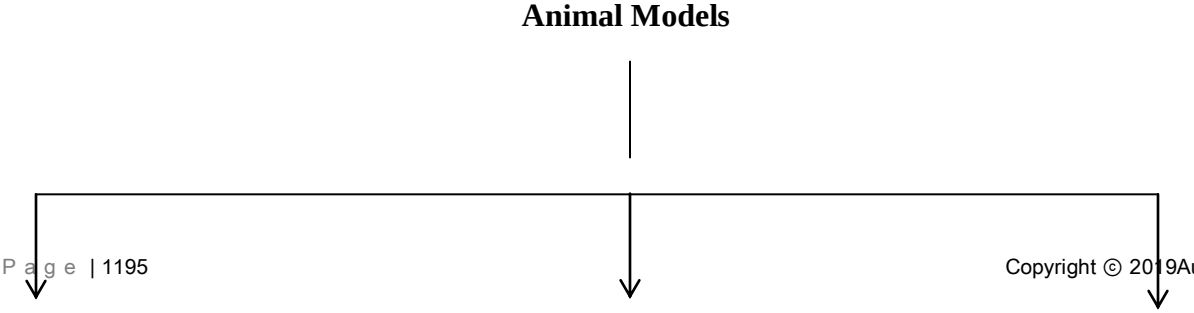


Figure 1- Stages involved in Wound Infection [14]

Animal Models for Wound Healing Activity

Following type of animal models are being used for wound healing studies. They are broadly classified into 3 categories.



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Acute	Chronic	Miscellaneous
Excision model	Vascular ulcers	Ear wound models
Incision model	Diabetic ulcers	Dead space models
Burn model	Pressure ulcers (PUs)	Scarless models

Animal models for acute wounds

Acute wounds are flesh injuries that restore through a proper sequence of physiological events and results in restoration of anatomic and functional integrity. Its healing occurs generally less than 8 weeks. The most important pathogen in this type of wound is *Staphylococcus aureus*[13].Acute wound models are further categorized into 3 sub-categories; Excision model, Incision model, Burn model.

Excision model:

In this case, circular wound is formed with about 2 cm in diameter and is formed on thoracic region of the rats under the specific conditions [14-15]. Wound area is to becheckedimmediately with a transparent polythene graph paper on the wounded area. This is termed as the initial reading;this is followed by application of test and standard drug. Results are observed post wounding by measuring the area. The percentage area of the wound is then calculated[15].The effect of phenytoin on the collagen formation in excision wounds and tensile strength in incision wounds.It was concluded that phenytoin helps in the shortening of wound healing time and also progresses the quality of the healing tissue[16]. In 2010 YusufOzayreported the effects of *Equisetum arvense* ointment on dermal wound healing on albino Wister male rats, using the excision wound model. After inducing the excision, the treatment group was given 1:1 mixture of Vaseline and lanolin ointment.It was observed that with the use of *Equisetum arvense* ointment, the wound closure ratio was 95.26% and 99.96% [17]. In a study the wound healing potential of Polyherbal ointment of *Centella asiatica Rubia cordifoia, Terminalia belerica, Withania somnifera*and *Plumbago Zeylanica*by excision wound model. Effect of the ointment

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was compared with Soframycin cream. The percentage closure of excision injury was assessed. The Polyherbal formulation was observed to promote healing of wounds in animals [18]. Wound healing potential of ointment formulation of *Milicia excelsa* (Welw) was evaluated by exicision wound model. It was observed that the wound contraction and epithelialization were enhanced after treatment with the ointemnt. Shorter time for epithelialization was also observed [19]. Wound healing potential of*Typha elephantina*was evaluated by excision and incision wound models in rats. Epithelization time, hydroxyproline and hexosamine content (wounded tissue), wound contraction, ascorbic acid and malondialdehyde content (plasma) in plasma were determined.Increased levels were observed in treatment group. malondialdehyde content was significantly reduced in treatment group animals. Potent wound healing activity was reported [20].In 2015, S Mageswari et al., evaluated the wound restorative activity of the *Carmona retusa*in mice. The ointment was prepared and it'swound contracting ability and closure time was compared with a standard drub Nitrofurazone (0.2%) ointment. Study was conducted on six animals using the excision model. An increase in wound healing and of epithelization was observed. Significant improvement in wound closure rate was observed[21].A. Pandurangan reported the wound healing activity of leaves of *Chromolaena odorata*using excision model inrats. The wounds were treated with the prepared ointment for 14 days. It was observed that the ointment possesses a significant cutaneous wound dressing activity [22]. Research study on the protective effect of *Lygodium flexuosum* against incision, excision, and dead space wound model in rats was conducted. Increase in the percentage of wound contraction time was observed on concluding day. The epithelization time was decreased [23].Kim JY in 2015 conducted a research study on chitosan loaded tyrothricin to evaluate its wound healing efficacy. The formulation covered the wound area completely with the aid of chitosan.The effect of the film-forming gel was compared to that of no fill-forming gel (negative treatment) and was further compared with commercially available sodium fusidate ointment and TYR gel (positive control treatments). Significant wound healing potential was reported when compared to the already marketed treatments [24].

Incision model

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The tested animal's skin is shaved and anesthetized, and two long incision of 6cm lengthmade through the paravertebral skin and the cutaneous muscles at a distance of about 1.5 cm from the midline of the back of animal. After incisions, the parted skin is stitched at 0.5cm intervals tightly using a suture thread and a curved needle. The sutures were removed on the 7^{th} day followed by drug administration up to day 9 and the tensile strength was measured on day 1 [25].Wound management activity of *Sesamum indicum* seed and oil in rats was evaluated using incision wound model. Improvement in the breaking strength was witnessed in incision wound model.Both thesewere found to be effective in managing the incision wound when applied topically [26]. Wound healing potential of methanolic extract of *Trichosanthes dioica* (fruits) was reported in rats using incision wound model.Epithelialization period, wound contraction, hydroxyproline content, tensile strength and histopathological studies showed promising results as compared to control [27-28].Wound healing potential of *Carapa Guianensis* bark extract was reported [29]. Promising results were seen in wound contraction; improved tensile strength and epithelization were also observed. The extract was administered orally after mixing in the drinking water. Granulation tissue weight, skin breaking strength, and hydoxyproline content were observed after the treatment period. On the 15^{th} day, the treated animals showed 99% reduction in the wound area when compared to controls (93%). Faster epithelization and improved skin breaking strength were seen in extract-treated animals. *Pinus brutia* bark extract and Pycnogenol were evaluated for wound healing potential by incision model in rats [30]. A histopathological analysisrevealed significant activity of the formulation in as increased vascularization, degeneration of hair roots, and a decrease in necrotic area.

Burn model

For the extensive studies of burn many animals has been used: dogs, sheep, rabbits, guinea pigs, hamsters, and pigs [31-33]. The most commonly used species for burn model study is rats and mice. Partial and full thickness burns can be made by various means like scalding the skin, heating of a weighted conductive object, flame, and microwave radiation [34]. The first burn wound model was developed in 1968. The method used was the scalding method (the use of hot water). Refined and validated method was later suggested by George Winter in 1978.He usedtemperature range of 60^0C for 60seconds, which produced a partial thickness burn (300um deep) whereas with increase in temperature up to 80^0 C for 15-20secondsproduced a full

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thickness burn [35].Effect of Botox was studied in burn wound model. Second degree burn was inflicted in animals and they were observed for 28 days. Trans epidermal water loss (TEWL), transforming growth factor beta (TGF-beta1, histological alterations, and tumor necrosis factor alpha (TNF-alpha) were analyzed at the end of the study. TNF-alpha had a little increase of 21-fold followed by decrease in the level. Increase in levels of TGF-beta and TNF-alpha, levels were observed on day 3; highest increase on 8^{th} day and declined until complete healing. It was concluded that with the introduction of Botox, wound healing process and cosmetic value increased [36].The effect of immediate burn excision on the course of further injury was evaluated in pigs. It was assumed that immediate excision of burn will prevent or reduces spread of necrosis in adjacent areas. Four burn sites separated by three interfaces of unburned skin were produced. Two full-thickness excisional wounds per pigs were used as controls. Injury was microscopically assessed and reported as the percentage of unburninterfaces that progressed to necrosis tissue after 7 days of injury. It was observed that the unburned interfaces of both comb burns and excised comb burns had undergone progressive injury and were 100% necrotic. However, the interfaces of the control excisional wounds were intact, no necrosis were found. It was then concluded that immediate burn excision neither prevents nor limits burn injury progression[37-38]. Burn induced immunosuppression was evaluated in animals by using standard burn model. Third degree burn upto an extent of 6% was inflicted in the animals with the help of a hot air blower.A decrease in concentration of leucocytes at the peripheral blood was observed in 48 hours. Polymorphonuclear neutrophilic granulocytes dominated inflammation was seen in the histopathological analysis. It was concluded that the burn mouse model is a potent wound model and provides an opportunity to study and develop novel strategies in treating burn wound patients [39].

Animal Models for Chronic Wounds.

A chronic wound occurs when a wound is not healed in the usual phases of wound healing in a proper and systematic manner. Although there might be difference in the causes at the molecular level from that of other types of wound, they share similar features like; excessive levels of proteases , cytokines, ROS, and senescent cells, persistent infection, and a lack of stem cells (Frykberg et al 2015). Common features associated with these wounds are, persistent infections,

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prolonged inflammation, drug-resistant forming biofilms, inactivation of both epidermal and dermal cells [40-41].

Types of Chronic wounds

These are categorized as vascular ulcers (e.g., arterial and venous ulcers), diabetic ulcers, and pressure ulcers (PUs)[41]

Diabetic Ulcer:

Most of the leg and footamputations in US are due to diabetic ulcers having an incidence of 2% per year. Pathogenesis of the diabetic ulcer occurs due to a neuropathic impairment of musculoskeletal balance, also due to an immune compromise from the leukocyte dysfunction and peripheral disease [42].

Pressure Ulcer: pressure ulcer generally results from ischemia due to the extended compression on a bony area. They usually are prominent in paralyzed or comatose patients due to non-periodic repositioning of the area [43]

Venous stasis ulcer: this type of chronic wound results from congestion through the venous area around the lower extremity that results in hypoxia. These may also occur due to the leukocytes migration through capillaries slowly than the normal, even occluding them, becoming triggered, and causes damages to the vascular endothelium.

Animal models for chronic wounds are not available. Most of the animal models are centered around hypoxic injury by creating an acute wound followed by ischemic, pressure, reperfusion damage or diabetic conditions. Rodents and large animals are most commonly used for wound healing activity. Chronic wounds are done by the infliction of a structural damage of the skin layer. Some methods include acute surgical wounds, abrasion, punch wounds, extrinsic pressure, and burn wounds. Acute wounds subjected to clinical situations like ischemia, diabetes are the foundation of the wound chronicity

Miscellaneous models

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Apart from the acute and chronic wound model, there are some other animal models used for research. This model is used when a specific study is required. Other animal models are; ear wound models, Dead space models, superficial models.

6.1. Ear wound model:When healing of wound occurs by re-epithelialization, and granulation formation without contraction, the ear model is most suitable. Healing on the ear occurs without contraction [44].

6.2. Dead space model: used for the granulation and reparative tissue growth study. In this study an artificial tissue space is created which is infused with plasma that results in fibrin clot and granulation tissue formation [45].

1. Conclusion:

The advancements in area of wound management are largely dependent upon the availability of suitable animal models. It is assumed that all the animal wound models reflect human wound healing process. As the major processes for wound development and repair are already studied.The clinical strategies can be developed to control wound repair mechanisms. Clinical similarities are easier to achieve in acute models rather than for the chronic wound. The animal models not only give an insight into the process of wound but are a valuable tool to get directions for the mechanism of action of various drugs. As a number of animal models are available with specific advantages there is a need to develop the future models for chronic wounds like bed sores, diabetic foot and other chronic conditions. Animal models can be used in future research for understanding the exact mechanism and to find the strategies to manipulate the normal wound healing processes

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