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ANIMAL MODELS FOR THE EVALUATION OF WOUND HEALING ACTIVITY

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SUMMARY

Wound is defined as the loss of breaking cellular and functional continuity of the living tissues and management of wounds is frequently encountered with different problems. Drug resistance and toxicity hindered the development of synthetic antimicrobial agents with wound healing activity. Many factors should be considered before selecting a wound healing model for a specific study. A wide variety of models have been developed for examining different aspects of the repair response thus many animal models are used for the evaluation of wound healing activities. Rats and mice have been widely used in the study of skin wound healing and efficacy of different treatment modalities. These particular species are mostly selected because of its availability, low cost and small size. In this review, we discussed about the wound and types of wound models that can be used along with the topics like wound location, where it is feasible to create the wound, wound size, strain and sex of rat, weight and age range as well as anaesthetics and analgesics and analytical measures that are used in wound healing studies. The present review will be helpful for the evaluation of drugs having potential for wound healing activity.

KEYWORDS

Animal models, Excision model, Incision model, Wound healing.

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INTRODUCTION

The wound may be defined as a loss or breaking of cellular and anatomical or functional continuity of living tissues. Wound healing is a biological process that is initiated by trauma and often terminated by scar formation. Thus healing is essentially a survival mechanism and represents an attempt to maintain normal anatomical structure and function[1].

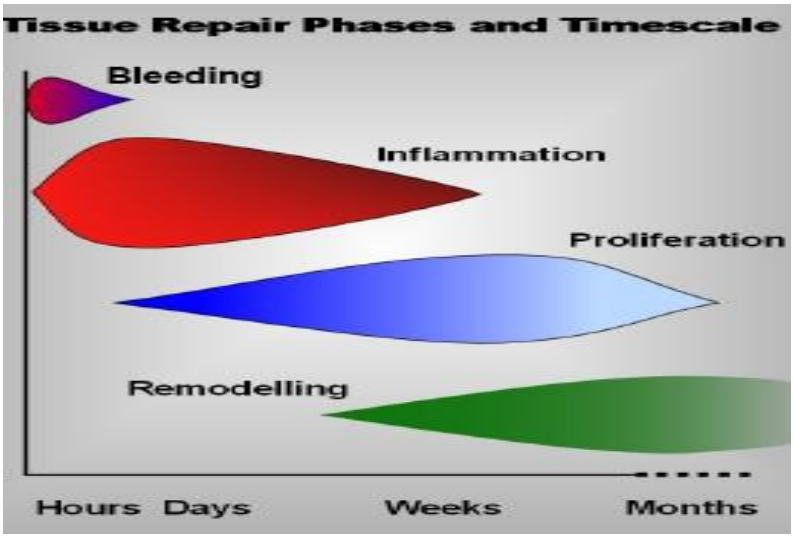
In a normal state wound healed by various process, which is fundamentally a connective tissue response, initial stage of this process involves an acute inflammatory phase followed by synthesis of collagen and extracellular macromolecules which on later remodelled to form a scar[2].

The process of wound healing occurs in different phases such as coagulation, epithelisation, granulation, collagen formation and tissue remodelling. Animal wound healing models are important biological tools to understand basic process of tissue repair and to develop and validate strategies for treatment of wounds. Wound healing in human beings have many unique aspects that related to physiology, age, environmental factors, etc. but the opportunities to carry clinical experiments. To understand mechanism and to formulate therapy for wound healing are limited[3].

The process of wound healing also gets affected by other disease such as diabetes etc, antineoplastic drugs and antibiotics may also interfere. Although animal wound healing models are imperfect reflection of wound healing processes in human beings and its clinical challenges, these models continue to be crucial tools for the development of new strategies and approaches for therapy of wound healing[3].

General process of wound repair

Wound healing is a process by which tissue regeneration occurs. It is a simple, dynamic process of restoring integrity and tissue layer, which involves an array of inter related and concomitant events[4]. The process of wound repair differs little from one type to another and is generally independent of the form of injury. Although the different steps in the wound healing process occurs in a continuous, integrated manner, it is convenient to divide the overall process into three overlapping phases and several natural components for descriptive purposes.



Tissue Repair Phases and Time Scale

Inflammatory Phase (Day 0-5)

The inflammatory response is initiated at the moment of injury. Surgical or traumatic wounds disrupt the tissue shape and architecture and cause hemorrhage. Initially, blood fills the wound and exposure of this blood to collagen in the wound leads to platelet deregulation and activation of Hageman factor. This is turn sets into motion a number of biological amplification system including the complement kinin and clotting cascades and plasmin generation. This condition serves to amplify the original injury signal and lead not only to clot formation, which unites the wound edges, but also to the accumulation of a number of mitogens and chemo attractants at the site of wound. Production of both kinins and prostaglandins leads to vasodilatation and increased small vessel permeability in the region of the wound. This results in edema in the area of the injury. Within 6 hours, circulating immune cells start to appear in the wound. Polymorphonuclear neutrophils (PMN) are the first blood leucocytes to enter the wound sites. Their main functions appear to be phagocytes of the bacteria, which have been introduced into the wound during injury. In the absence of infection, PMNs have a relatively short life span in the wound and their numbers decrease rapidly after the third day. The next cellular, immune component enter to the wound is macrophages. These macrophages have a much longer life span than the PMN and persist in the wound until healing is complete[5].

Proliferative Phase (3-14 days)

In the absence of significant infection or contamination, the inflammatory phase is short, and after the wound has been successfully cleared of devitalized and unwanted material it gives away to the proliferative phase of healing. Granulation tissue consists of a combination of cellular elements, including fibroblasts and inflammatory cells. Fibroblasts first appear in significant numbers in the wound on the third day post-injury and achieve peak numbers on the seventh day. This rapid expansion in the fibroblast population at the wound site occurs via a combination of proliferation and migration. Fibroblasts are the primary synthetic element in the repair process and are responsible for production of the majority of structural proteins used during tissue reconstruction. In particular, fibroblasts produce large quantities of collagen, a family of triple-chain glycoprotein, which forms the main constituent of the extracellular wound matrix and which are ultimately responsible for imparting tensile strength to the scar. Capillary buds sprout from blood vessels adjacent to the wound and extend into the wound space. While these events are proceeding deep in the wound, restoration of epithelial integrity is taking place at the wound surface. Re-epithiliazation is complete in less than 48 hours in the case of approximated incised wound, but may take substantially longer time in the case of larger wounds where there is a significant tissue defect [4].

Maturation Phase (Day 7 to 1 year)

Collagen remodeling during the maturation phase depend on continuous collagen synthesis. Some of the growth factors that stimulate the synthesis of collagen and other connective tissue molecules also modulate the synthesis of activation of metalloproteinase, enzymes that serve to degrade these epithelial cell migrations (ECM) components. The net result of ECM synthesis verses degradation result in remodeling of the connective tissue framework-an important feature of both chronic inflammation and wound healing. Collagen rapidly becomes the predominant constituents of the matrix. The initially randomly distributed

collagen fibers become cross linked and aggregated into fibrillar bundles, which gradually provide the healing tissue with increasing stiffness and tensile strength. After a 5-day lag period, which corresponds to early granulation tissue formation and a matrix largely composed of fibronectin and hyaluronic acid; their remodeling during scar formation is dependent on both continued collagen synthesis and collagenase enzymes. The high rate of collagen synthesis within the wound returns to normal tissue levels by 6-12 months, while active remodeling of the scar continues for up to 1 year after injury[4].

Animal Models for Evaluation of Wound Healing Activity

Generally these types of wound models are used to study the wound healing property.

1. Excision wound model
2. Incision wound
3. Burn wound model
4. Dead space wound model
5. Ear wound models
6. Superficial wound models

Excision Wound Model[6, 7, 8]

In this type of model circular wounds of about 2 cm are made on depilated dorsal thoracic region of rats under aseptic conditions and should be observed throughout the study. The area of wounds should be measured immediately by placing a transparent polythene graph paper over the wound and then tracing the area of the wound on it. This is taken as initial wound area reading. Drugs are to be applied test and standard as well and observations are to be taken on the alternate post wounding days by tracing the wound area and percentage area of wound closure is calculated.

Incision Wound Model[9]

In this model cuts are made in the skin of the animal after giving anesthesia with anesthetic ether. Two para-vertebral long incision of 6 cm length made through the skin and cutaneous muscles at distance about 1.5 cm from the midline on each side of the depilated back of the rats. After the skin incision made, the parted skin kept together and should be stitched at 0.5 cm intervals continuously and tightly by using suture thread (No. 000) and a curved needle (No. 11). When the wounds get cured thoroughly, the sutures are to be removed on day 9 and tensile strength of the healed wound should be measured on day 10 by continuous and constant water flow technique.

Burn Wound Model[10, 11]

Partial thickness burn wounds are created on overnight starved animals. Under anesthesia, pentobarbitone (30 mg/kg, i.p.), hot molten wax at 80^0C is poured into a cylinder of 300 mm^2 circular opening placed on the shaven back of the animal until wax get solidified. Solidification of wax normally takes 10-12 minutes. Cylinder is now removed that leave the demarked partial thickness circular burn model.

Dead Space Wound Model[11,12, 13, 14]

In this type of model the physical changes in the granuloma tissue. The subcutaneous dead space wounds are to be created in the region of axilla and groin by making a pouch through a

small nick in the skin. The cylindrical grass piths measuring 2.5 cm in length and 0.3 cm in the diameter are introduced in to the pouch. Each animal receive 2 grass piths in different locations. Implantations of grass pith induce granuloma formation. The wounds are sutured and mopped with an alcoholic swab. Granulomas surrounding the grass piths were excised and slit open. The tensile strength of tissue piece (obtained by trimming the rectangular strip of granular tissue) measuring about 15 mm in length and 8 mm width was determined on 10^{th} post wounding day by adopting continuous water flow technique.

Ear Wound Models

In cases where human healing occurs entirely by reepithelialisation and granulation formation without contraction, the ear wound model may be more suitable because it heals without contraction and has a vascular cartilage wound bed[15].

The Hairless Mouse

The hairless mouse looks like a nude mouse but has a thymus gland, and therefore it has an intact cellular immune system. The homozygous hairless mouse ear was first described for studying dermal microcirculation[16]. The model has been used in burn studies,[17] reperfusion injuries and flap necrosis[18, 19, 20]. The model consists of full-thickness dermal wounds created on the dorsal aspect of the ears. In order to visualize wound epitheliazation and neo-vascularisation, the anesthetized animal is placed with the ear/wound trans-illuminated in a trinocular compound microscope[21, 22].

The advantages of this model discussed here is that virtually all healing observed in this model is attributable to epitheliazation and subsequent granulation accompanying the neovascularisation based on the structure of the mouse ear. The bed of the full-thickness dermal wound consists of cartilage. This layer prevents healing by contraction[23] and prevents vessels from nourishing the wound from the bed. This allows investigation of the effect of different topical agents on epitheliazation and vascularisation in a clinically relevant way. However, it must be emphasized that the architecture of the hairless mouse skin is different from human skin in many ways. The epithelial layer of hairless mouse skin consists of only a few cell layers, of which even the uppermost layer displays distinct nuclei[20].

Rabbit Ear Model

This model was introduced in 1991, the rabbit ear wound model,[24] which is an analog to the hairless mouse ear wound model. This model has been used as an ischemic model as well as a model for investigating hypergranulation. In this model, several wounds on each ear can serve as treated groups or controls. Tissue explants of the new tissue can be labelled in culture for new collagen, protein, glycosaminoglycan or DNA synthesis. The rabbit ear has the additional advantage of a very constant anatomy of the three major vascular pedicles. When two of these are divided, the ear is rendered reproducibly ischemic, and yet with complete survival[15]. This allows an examination of different agents during ischemic conditions with impaired healing.

Blister Wound Model

This model can be used both in animals and human for evaluation of epidermal regeneration and the influence of different compounds and drugs. Blisters are produced by suction using different types of devices[25,26] producing standardized, small epidermal blebs. Transdermal

invasion is therefore avoided. Often, several blisters are produced in the same anatomical area or different locations on the body. A total rebuild of acanthotic epidermis is in the hairless hamster found 120 hours after formation of suction blisters[27]. In humans the blisters can be raised in midvolar forearms by a vacuum of 20 cm Hg[28]. Normal human skin will, under these conditions, begin to blister after 35–55 minutes. A scalpel blade can then remove blister roofs. This procedure creates identical superficial wounds of similar diameter and uniform depth[29]. Several types of tests can be performed using this model as absorption of drugs or compounds in different solutions or molecular weight. These results can be used to investigate a wide range of drugs by passive diffusion and provide a short route for short-term delivery of otherwise poorly absorbed peptide and protein drugs[30]. The absorption effect can also be evaluated under occlusive and semi occlusive conditions. Transepidermal water loss (TEWL) can be measured daily and used as a measure for the effectiveness of the barrier function of the epidermis[31].

Tape Stripping Model

The skin barrier is located in the lowest part of the stratum corneum[32]. In this model, successive stripping of the epidermis using adhesive tape is able to disintegrate this barrier. This disintegration can be estimated by an evaporimeter measuring TEWL[31]. Twenty successive stripping procedures using adhesive tape will normally produce a humid skin surface. This method is less damaging for the epidermis than the blister model, leading to changes in physiological processes like TEWL, which is increased compared to the blister wound.

Partial Thickness Excision Wound Model

This type of wound is produced by a hand-held or electrical dermatome[33,34,35,36,37]. The depth of the wound can be adjusted to the desired type of wound. The model can be used for evaluation of local environmental factors and topical agents. The advantage of this model is small or no wound contraction, the model can be used in animals as well as humans, the surface area can be calculated exactly, and the wound is stabile and easy to handle. The main disadvantage is that it is a surface wound with no possibility to measure matrix and collagen development. Donor sites are widely used as one of these models in humans.

Wound Location

About 80% of the reviewed studies were identified that had wounds were made on the dorsum of the rats[38,39,40,41,42,43,44]. Also studies overlapped and shred the data in support of categorizing the groups according to wound placement. Wounds were made in different areas within the same study; for example, wounds were made on the dorsum and the ventral portion of rats[45].

Exact statements as to locations and depths of wounds were not always readily available, leaving the reader to draw assumptions from both the text and figures [46]. Because an important variable in governing the rate of wound contraction is how tightly the skin is adherent to the underlying tissue, wound location is highly significant.

Wound Size (Depth and Dimensions)

It was found that partial-thickness wounds were used in the studies with burn models [44,47,48]. Conclusions of this review agree with those of that incisions were typically full-thickness

wounds made through the panniculus carnosus. Partial-thickness wounds made with devices such as a dermatome may have inherent problems for the study if performed in haired animals such as the typical Sprague Dawley and Wistar rat. The high hair density causes an exaggeration in the rate of reepithelialisation in the rodent model[49]. The advantages and disadvantages of the use of depilatory agents with or without shaving in haired rats need to be considered with each study. Variations were found in the dimensions of the wounds. Only a few studies used the same size wound in the same location with the same wounding technique, which was a 4-cm incision wound on the dorsum of the rats[50, 51, 52, 53]. Because the numbers of studies using similar techniques is so small, no meaningful statements can be made to illustrate consistent findings in the data. In studies that used round wounds, the sizes varied such as 6-mm diameter punches[54], 15-mm diameter wounds[55, 56], 1.5-cm diameter circles[57], and up to 2-cm diameter wounds. Incisions varied from 5 mm[58] to 8 cm long[59]. Examples of the burn wounds included round nitrogen burns approximately 6 mm in diameter,[60] a scalding wound covering 15–20 % of total body surface area on shaved dorsums[61], and burns caused by a metal rod 1.5 cm in diameter[62]. Size is a key consideration in the study of wounds[63, 64].

Strain and Sex of Rats

In various studies Sprague-Dawley strain of rat is being used by the researchers[55,65]. The second most popular was the Wistar strain which was reported to be used in several studies[66,67]. On the other hand other types of strain was used include nude/hairless which comes third on the part of strain being used[68,69]. Dark Agouti strain[70], Lewis strain[60,71,72], and Brown ACI strain[73] are also reported to be used by the researchers. Male rats were used more than females in the reviewed research groups. Several studies used both sexes[74,75], while several others used only females[76,77]. Various studies did not state the sexes of their rats[40,78]. Also few articles provided rationales for their selections of strains or sexes of rats used. One article stated that Sprague Dawley rats have been studied extensively for wound healing and cited articles that used them for the wound healing models they wished to study[40].

Weight and Age Range

The majority of the studies used rats weighing from 250 to 300 gm. The age of rats used in the studies varied from newborns 19 to 36 months[79]. The study that used newborns did so as a result of speculation that newborns may have a diminished inflammatory reaction to injected recombinant adenoviruses, thus facilitating more efficient gene transfer[58]. Thirty-six month-old rats were used to study aging effects on healing. Sizes in the studies varied from the weight of newborn rats up to 500 gm[80]. Because rat ages and body weights can be correlated[81] and aging has been reported to result in prolonged or poor wound healing[63,79], the age and/or weight of the animal may present an additional variable in wound healing analysis.

Anaesthetics and Analgesics

Injectable anaesthetics such as sodium pentobarbital or mixtures of ketamine and xylazine were used more often than inhalation agents in the reviewed study groups. Ether was used in several studies groups[67,82,83] and halothane was also used[84]. Some used combinations of injectable and inhalation agents such as ketamine with methoxyfurane[85], and sodium pentobarbital with isoflurane[86]. In choosing anaesthetic agents, the cost, availability, and the anaesthetist's familiarity with the drugs must be considered. Personnel and the institution

must be compliant with governmental narcotics regulations. This usually requires additional documentation and added security measures.

Analytical Measures

A major advantage in the use of animal models is the ability to provide harvested samples. Wound healing investigators have used a combination of macroscopic and histological observations, biochemical and biomechanical measurements, and measurements of wound healing markers, often in combination with analyses of cellular and immunologic responses to evaluate the progress of wound repair[87]. Various testing methods have been used to determine the success or failure of treatment modalities. The purpose of wound healing is to restore tissue continuity and resistance to externally applied forces[88]. In animal models, the progress of healing can be assessed by measuring the development of the mechanical strength of the wound[88]. In the majority of the studies reviewed, evaluation of the skin models was accomplished by testing both the tensile and breaking strength of the wounded area with a tensiometer. Areas of contraction and reepithelialisation were documented with tracings and area grid techniques, often in conjunction with computer analysis. Electron microscopy, dye testing of vasculature patency, immune-histochemical testing, and genetic level investigation are a few examples of the other types of testing employed to evaluate outcomes. Varying methods used in reporting results only add to the difficulties encountered in the attempt for direct comparison between studies[63].

DISCUSSION

Characteristics such as a short gestation, short life span, docile behaviour, and ready availability of animals with well-defined health and genetic backgrounds are keys in the decision to use the rat as the choice of a research animal[89]. Rats fall into two basic groups: inbred and outbred[90]. Inbred strains are developed through at least 20 generations of brother–sister matings[90]. Outbred rats have less than 1 % inbreeding per generation and have been maintained in a closed colony for at least four generations[90]. Of the two most commonly used outbred stocks, the Sprague-Dawley is generally larger than the Wistar, but both are considered docile and serve well as general purpose models[89]. Fisher rats are smaller than the Sprague-Dawley and the Wistar[89]. Fisher and Norway rats are identified as inbred strains and the Fishers are also described as being a general purpose model, whereas the Norways are listed as appropriate for such studies as aging and kidney research[81]. Nude rats, which are mutants, have been used in immunology-based research[81]. Lewis rats, an inbred strain, have been used for transplantation studies, endocrinology, multiple sclerosis, and experimentally induced autoimmune diseases[89]. The ACI, another inbred strain, have been used for research dealing with congenital genitourinary anomalies and prostatic adenocarcinomas[89]. In general, preference for the models is most likely a result of long-term experience, known breeding characteristics, cost, genetic stability, and access. Currently, there is no literature indicating the superiority of one strain over another for wound healing studies. Developing an animal model that has all the complexity of human chronic wounds may be an unattainable goal, because non healing and delayed healing wounds in humans are often the result of combinations of impaired circulation, inadequate nutrition, age, limited physical activity, and/or chronic physiological imbalance[49]. Models need to be developed that show these impairments are comparable. This would permit a higher level of confidence in animal data. In this overview, concrete comparisons between wound healing study groups were difficult, as each study group had its own agenda. Differences were found in types of models, locations and sizes of wounds, as well as choices of rats, anesthetics, and parameters measured. Results of wound contraction coefficients show that choices of strain, sexes, and

ages of rats are variables that somehow must be standardized in the search for reproducibility[63].

Having a model that is merely reproducible is not enough to investigate modifications to the wound healing process[63]. The model must also have the capacity to allow the detection of the effects of potential treatments or procedures[63]. Experimental models with standardization of techniques and reproducibility need to be explored so that results from different treatment modalities can be compared in a scientific manner. Accurate comparisons between studies regarding the ultimate efficacy of treatments would add to the knowledge base of wound healing. Attempts to compare studies for the advancement of wound healing knowledge are being hampered by the differences found between the studies. Standardization in reporting could facilitate comparisons and may instigate additional research that favours the inevitable comparisons between the studies. This increased knowledge base would be vital in transferring animal-derived data to human clinical situations.

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