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YARA İYİLEŞMESİ ARAŞTIRMALARINDA DENEYSEL HAYVAN MODELLERİ: MEKANİZMALARI, UYGULAMALARI VE SINIRLILIKLARI

Nazli KARİMİ AHMADİ[1,a]
Okan ARIHAN[1,b]

[1]Hacettepe University, Faculty of Medicine, Department of Physiology, Ankara

ORCID[a]**:** 0000-0002-3534-6621
ORCID[b]**:** 0000-0001-6201-7383

***Sorumlu Yazar:** Nazli KARİMİ AHMADİ
E-Posta: nkarimi@hacettepe.edu.tr

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ÖZET. Deri, insan vücudunun en büyük organıdır ve su kaybı ile patojenler gibi dış tehditlere karşı alttaki dokuları koruyan kritik bir bariyer görevi görür. Ancak bu görevi yerine getirirken, sıklıkla mekanik travma, termal yanıklar, kimyasal maruziyet, iskemi ve enfeksiyonlar gibi çok çeşitli yaralanmalara maruz kalır. Bu farklı yaralanma türlerinin hayvan çalışmalarında modellenmesi, yara iyileşmesi mekanizmalarının anlaşılmasını yardım etmektedir. Bu makale, yara iyileşmesi araştırmalarında yaygın olarak kullanılan deneysel hayvan modellerine odaklanmış bir genel bakış sunmakta, bu modellerin mekanizmalarını, uygulama alanlarını ve sınırlılıklarını özetlemektedir. Kemirgenler, domuzlar ve insan dışı primatlar, çeşitli cilt yaralanmalarını taklit etmek için sıkça kullanılmakta ve doku onarımında rol oynayan hücresel ve moleküler süreçlerin ortaya çıkarılmasında temel araçlar olarak hizmet etmektedir. Standart modellere ek olarak, diyabetik, enfekte, iskemik ve immünsüprese yaralar için özel yaklaşımların yanı sıra rejeneratif tedavilerin değerlendirilmesinde kullanılan biyomateryal bazlı modeller de tartışılmaktadır. Bu derleme, Google Scholar, PubMed ve Web of Science gibi veritabanlarından elde edilen güncel yayınlara dayanmaktadır. Model seçiminde etik ilkeler, maliyet etkinliği ve translasyonel (klinikle uyumlu) geçerlilik gibi temel faktörler ele alınmaktadır. Ayrıca, yara iyileşmesinin değerlendirilmesinde global ölçekte ve Türkiye'de yaygın olarak kullanılan makroskobik gözlem, histolojik analiz, moleküler testler ve görüntüleme teknikleri gibi yerleşik yöntemler de özetlenmiştir.Son olarak, fizyolojik geçerliliği artırma ve klinik uygulamaya geçişi hızlandırma potansiyeline sahip yeni teknolojiler vurgulanmıştır.

Anahtar Kelimeler: Cilt yaralanması, deneysel hayvan modelleri, yara iyileşmesi.

EXPERIMENTAL ANIMAL MODELS IN WOUND HEALING RESEARCH: MECHANISMS, APPLICATIONS, AND LIMITATIONS

ABSTRACT. The skin is the largest organ of the human body and acts as a critical barrier, protecting underlying tissues from water loss and external threats such as pathogens. However, in performing this role, it is often exposed to a wide range of injuries - including mechanical trauma, thermal burns, chemical exposure, ischemia, and infections. Modelling these diverse injury types in animal studies enhances our understanding of wound healing mechanisms. This article presents a focused overview of commonly used experimental animal models in wound healing research, outlining their mechanisms, applications, and limitations. Rodents, pigs, and non-human primates are frequently employed to replicate different types of skin injury and are essential for uncovering the cellular and molecular processes involved in tissue repair. In addition to standard models, specialized approaches for diabetic, infected, ischemic, and immunosuppressed wounds as well as biomaterial-based models for evaluating regenerative therapies are discussed. The review is based on recent publications retrieved from databases such as Google Scholar, PubMed, and Web of Science. Key considerations in model selection such as ethical principles, cost-effectiveness, and translational relevance are examined. The review also summarizes established methods for evaluating wound healing such as macroscopic observation, histological analysis, molecular assays, and imaging techniques applied both globally and in Turkey. Finally, emerging technologies are highlighted for their potential to improve physiological relevance and accelerate clinical translation.

Keywords: Experimental animal models, skin injury, wound healing.

Makale atıf

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INTRODUCTION

As the body's first line of defense, the skin is constantly exposed to various environmental stressors, including physical injuries, chemical agents, and microbial threats (Lavers, 2017). In addition, systemic conditions like diabetes and poor blood circulation can weaken the skin's structure which can further weaken the skin and impair its ability to heal (Idrus et al., 2014). A wound is characterized by a break in the continuity of the skin's epithelial layer, potentially affecting the underlying tissue's structure and functional capacity. The severity of skin injuries is typically classified according to the depth of tissue involvement, with deeper and larger injuries generally correlating with more severe clinical outcomes, such as those seen in burn injuries (Subramaniam et al., 2021). Disruption of the epidermal and dermal structures can result in significant complications, including life-threatening conditions. Therefore, rapid and effective wound healing is essential for reducing morbidity and mortality rates (Idrus et al., 2014; Law et al., 2017).

Wound healing is a highly organized and dynamic process involving multiple cell and tissue types. These complicated cascades are regulated through complex interactions among cell migration, proliferation, inflammation, matrix remodeling, and angiogenesis (Mayet et al., 2014). While minor skin injuries typically heal within days, with complete tissue regeneration, larger wounds from trauma or surgery often take weeks to heal and frequently result in fibrotic scarring. The process of healing starts with clot formation to restore the skin barrier, followed by coordinated migration and proliferation of keratinocytes, fibroblasts, and endothelial cells driven by chemical and mechanical signals, many originating from inflammatory cells. While angiogenesis is transient, scar formation leads to lasting architectural and functional impairment. Recent advances have also focused on regenerative strategies inspired by principles of developmental biology, focusing on cellular reprogramming strategies that aim to reactivate embryonic-like healing programs (Li et al., 2024; Peña and Martin, 2024). Additionally, growing insights into the roles of extracellular vesicles, biomechanical cues, and immune regulation have substantially improved our understanding of mechanisms that enhance wound healing (Las Heras et al., 2024). In this context animal models are key to understanding the molecular and cellular events in wound healing and for evaluating novel therapeutics, including stem cell therapies, bioengineered skin substitutes, and growth factor delivery systems (Choudhary et al., 2024; Hussen et al., 2024). Different models offer unique insights depending on their physiological similarity to human skin, ease of genetic manipulation, and ethical considerations. However, no single model perfectly replicates human wound healing, and careful selection based on study objectives remains critical (Choudhary et al., 2024).

This review aims to provide an updated and critical overview of the most commonly used animal models in wound healing research, emphasizing their applications, advantages, and limitations. Furthermore, it discusses the evaluation parameters used to assess healing outcomes and the ethical principles guiding animal research in this field, indicating the importance of translational strategies for advancing regenerative medicine.

Physiology of Wound Healing

The skin consists of two primary layers: the epidermis, which forms a protective barrier against the external environment, and the dermis, composed of connective tissue that provides mechanical strength. The epidermis is made up of stratified keratinized epithelium embedded within a network of hair follicles and glands. The dermis lies just below the epidermis, which is subdivided into the upper papillary dermis and the lower reticular dermis, distinguished by differences in collagen fiber density (Bielefeld et al., 2013). Disruption of this layered structure due to physical trauma, chemical exposure, surgical procedures, burns, infections, or diabetic complications results in a wound, characterized by both structural and functional damage to the skin (Summer et al., 2024).

During healing, skin cells work together—proliferating, migrating, and maturing—to rebuild the barrier and restore the skin's strength (Gurtner et al., 2008).

This dynamic repair process occurs in four overlapping fundamental phases: hemostasis, inflammation, proliferation, and tissue remodeling (Shaw and Martin, 2009). Acute wounds typically resolve within 8–12 weeks, while chronic wounds persist beyond this period, often due to repeated injury or underlying health conditions (Boateng et al., 2008). Compared to chronic wounds, acute wounds tend to heal faster and with fewer complications. Cutaneous wound repair aims to restore the integrity of damaged tissue and, in many aspects, mirrors embryonic skin development. Both processes involve coordinated behaviors of various cell types to reconstruct the multilayered structure of the skin (Bielefeld et al., 2013).

Phases of Wound Healing

Hemostasis

The wound healing process begins with hemostasis, characterized by coagulation and the formation of a fibrin clot (Kanji and Das, 2017). Upon injury, blood vessels constrict to minimize blood loss, initiating the hemostatic phase. Primary hemostasis involves the rapid adhesion and aggregation of platelets at the wound site, mediated by interactions with extracellular matrix proteins such as fibronectin and collagen. This is followed by secondary hemostasis, where activation of the coagulation cascade converts fibrinogen into fibrin, forming a stable fibrin mesh that entraps red blood cells and reinforces the platelet plug, effectively cover the wound and prevent further hemorrhage (Opneja et al., 2019).

Inflammation

The inflammatory phase, lasting several days, begins with the release of tumor necrosis factor-alpha (TNF-α) and platelet-derived growth factor (PDGF) from platelets and mast cells, initiating the inflammatory response (Eming et

al., 2007; Rodrigues et al., 2019). Local mediators such as histamine and activated complement proteins contribute to redness and swelling (Rodrigues et al., 2019). Neutrophils are the first immune cells to infiltrate the wound site, clearing cellular debris and pathogens. They are soon followed by monocytes, which differentiate into macrophages to further aid in debris removal and infection control (Eming et al., 2007; Wilgus et al., 2013). Platelets and white blood cells sustain the inflammatory process by releasing additional cytokines and growth factors, including PDGF and transforming growth factor-beta (TGF-β), which promote fibroblast migration, proliferation, and differentiation necessary for collagen production and tissue repair (Rodrigues et al., 2019; Wilgus et al., 2013). Effective wound healing relies on the controlled interplay between immune cells and signaling molecules, modulating the initial inflammatory response and guiding the subsequent phases of tissue repair and remodeling (Mamun et al., 2024).

Proliferation

This phase is marked by the formation of granulation tissue, re-epithelialization, and neovascularization, processes that can extend over several weeks. Neovascularization occurs through both angiogenesis, involving the branching of new blood vessels from existing vasculature, and vasculogenesis, driven by endothelial progenitor cells (EPCs), restoring nutrient and oxygen delivery to the wound site (Boulton et al., 2005; Subramaniam et al., 2021). Endothelial cell migration, stimulated by factors such as VEGF, FGF, angiopoietins, and TGF-β, supports this vascular growth. Granulation tissue provides a scaffold for keratinocyte migration from the wound edges, initiating re-epithelialization and gradually minimizing wound size (Rhett et al., 2008)**.** The bidirectional interaction between keratinocytes and fibroblasts further promotes healing. As macrophages transition to the M2 phenotype, they release anti-inflammatory mediators that stimulate fibroblast proliferation, collagen deposition, and angiogenesis. Maturation begins as the initial fibrin scaffold is replaced by collagen fibers, strengthening the tissue (Subramaniam et al., 2021; Werner and Grose, 2003).

Remodeling

Following complete wound closure, tissue remodeling occurs beneath the epidermis, a process that may continue for a year or longer**.** Remodeling involves the degradation of excessive collagen, reorganization of the extracellular matrix, and wound contraction. During this phase, fibroblasts play a central role by modulating ECM composition and secreting matrix metalloproteinases (MMPs), which degrade damaged proteins and facilitate matrix reorganization. One key biochemical hallmark of this process is the shift in the ratio of collagen type III to type I. Initially, collagen type III, which provides flexibility and is rapidly synthesized during the early healing phase, dominates. However, as remodeling progresses, collagen type I gradually replaces type III, resulting in a denser and more rigid matrix that contributes to scar strength and structure. Although scar tissue restores much of the skin's structural integrity, it only regains approximately 80% of the original tensile strength (Gurtner et al., 2008; Velnar et al., 2009). In adults, ideal wound healing is typically characterized by the formation of a flat, nonerythematous linear scar (Stewart, 1995). To provide a clearer understanding of these phases—particularly the transition from hemostasis to remodeling—Table 1 summarizes the timeline, primary cellular players, and key biological processes involved in each stage of wound healing.

Overview of Animal Models

Factors Affecting Model Selection

For ethical reasons, animals are often the first choice for testing certain pathologies or drugs. Therefore, animal models play a critical role in understanding the complex biological processes involved in wound healing. However, model selection depends on many factors:

Table 1. Summary of Wound Healing Phases (Eming et al., 2007; Gurtner et al., 2008; Velnar et al., 2009; Kanji and Das, 2017; Opneja et al., 2019; Rodrigues et al., 2019; Subramaniam et al., 2021).

Phase	Time Frame	Key Cellular Players	Main Biological Events
Hemostasis	Immediate (minutes to hours)	Platelets, endothelial cells	Vasoconstriction, platelet aggregation, coagulation cascade activation, fibrin clot formation
Inflammation	First few days	Neutrophils, monocytes/ macrophages, mast cells	TNF-α and PDGF release, immune cell infiltration, clearance of pathogens and debris, initiation of cytokine signaling
Proliferation	Several days to a few weeks	Fibroblasts, keratinocytes, endothelial cells, M2 macrophages	Granulation tissue formation, neovascularization, re-epithelialization, deposition of type III collagen
Remodeling	Weeks to months (may exceed 1 year)	Myofibroblasts, fibroblasts	ECM remodeling, degradation of excess collagen, type III → type I collagen transition, wound contraction, scar maturation

Ethical Factors: Concerns about the ethical aspect of animal studies brought some universal standards and encourage the use of in vitro models where convenient (Russell and Burch, 1959; Chittasupho et al., 2021; Hofmann et al., 2023).

Cost: Small animals are easy to breed and handle which make them less expensive and easier to manage, however large animal models (monkeys, pigs or sheep) can also be used with much higher costs (Banstola and Reynolds, 2022; Mukherjee et al., 2022).

Translational Value: Diversity of skin types in different animals needs to be considered for a researcher in modelling the human skin for its translational value (Mieczkowski et al., 2022).

Those subjects will be discussed in the following sections.

Small and Large Animal Models

Small Animals: Mouse and rat models are popular due to their easy handling and cost-effectiveness. However, they have differences such as thinner epidermis and faster healing potential compared to human skin (Sanapalli et al., 2021). In addition, they have advantages to have wound contraction which is much different compared to humans.

Large Animals: Animals such as pigs, dogs and rabbits are closer to human skin in terms of anatomical and physiological aspects. Pig models are often preferred due to their similar structure to human skin. In addition, their wound healing process is more similar to human compared to rodent models but there are still differences with humans which does not make a complete model for humans (Hofmann et al., 2023). Wound healing is also studied in monkeys and apes which began quite early (Desjardins et al., 1986). In scientific literature a wide range of research is found in this subject (Yu et al., 2023). A study which compared human and monkey wound healing showed similarity in healing process with markedly slower healing in humans compared to non-human primates (Matsumoto-Oda et al., 2025). Despite the differences observed with non-human primates, these animals are the closest model for studying human wound healing.

Advantages and Limitations

There are advantages and disadvantages to modeling human wound healing in animals (Table 2). The selection of the model for research becomes more possible by evaluating these different parameters.

Acute and Chronic Animal Wound Models

The animal model for wound healing varies according to the type of wound targeted. Wound models are basically divided into two as acute or chronic models. These models then vary according to the type of wound formation desired, such as mechanical, thermal, circulatory, and infectious. In each of these models, the evaluation parameters regarding wound formation and healing may differ.

Acute Wound Models

Incisional Wound Model

A sharp object injury is a type of injury that almost every person experience in daily life. Healing and the subsequent scar tissue changes depending on the size of the wound. The simplest method is to create a standard in-

Table 2. Advantages and disadvantages of different animal models (Garner, 1998; Apikian and Goodman, 2004; Hofmann et al., 2023; Matsumoto-Oda et al., 2025).

Model	Advantages	Disadvantages
Mice	Low cost Easy housing and handling High breeding rate Short time period of experimentation Presence of genetic models	Skin tissue dissimilarity Differences in wound healing Differences in immune systems Low translational validity
Rat	Low cost Easy housing and handling High breeding rate Short time period of experimentation	Skin tissue dissimilarity Differences in wound healing Low translational validity
Rabbit	Moderate cost Similarity in response to different impacts Appropriate wound dimensions	Not diverse as mice in transgenic models Low translational validity Less ease in housing
Pig	Similarity in anatomy and physiology Similarity in wound healing with humans	High cost Requirement for a long time period Less ease in housing
Non-human apes	Similarity in anatomy and physiology	High cost Difficulty in housing

cision for the formation of the wound. As stated in the advantages and disadvantages section, mice and rats are the most commonly used species, although they do not model human wound healing exactly (Pascal et al., 2024). As in many other wound methods, in this method, an incision of known depth and size is made with a scalpel under anesthesia. Different suture methods and threads are used to try the sutures. The effects of different applications on this healing process are compared with the control group.

Excisional Wound Model

Another test method for incision wounds is modelled by without using any sutures. It offers to assess the regenerative capacity of the skin. In addition to rodent models, pigs are preferred. The wound array created with a special device, especially on the dorsal side of the animal, is left without any sutures. However, it can be covered with the desired material for the protection and healing of the wound. This can be a plaster or a different material. Wound can be covered with sterile gauze, elastic bandage, and mesh bandage and finally a fabric. As can be seen, a large animal like a pig provides a great advantage in terms of placing so many layers easily (Kuo et al., 2022).

Burn Wound Model

This model is chosen to model thermal injuries and is essential for studying burn pathophysiology and relevant therapies. Pigs and rats are commonly used. In order to obtain a burn model caused by thermal energy, a metal rod with a certain diameter, appropriate weight and structural properties is brought into contact with the shaved skin for a certain period of time (Arihan et al., 2021). The duration of this contact determines the degree of the burn. For example, touching an aluminum rod heated to 100 degrees to the skin for 10 seconds and touching it for 20 seconds can be evaluated as different degrees of burns and these two groups can be evaluated as groups with different degrees of burns. Another burn model is the use of a device that allows hot water to touch the skin for a certain period of time (Ciornei et al., 2024).

Mechanical Trauma Model

Mechanical injuries that do not result in skin cuts or perforations are also being modeled in animals. Those traumas due to blunt objects or abrasion with appropriate devices. Rats and non-rodent species such as rabbits are used commonly. In this model since more superficial and epidermal trauma is aimed standardized superficial abrasions are applied on the skin. Trauma is continued until uniform glistening and punctate bleeding are observed then the procedure is stopped. Lasers can be used for this purpose by choosing the correct laser type and adjusting the laser energy (Wilhelm et al., 2017). In addition, special contusion devices can be used to mimic blunt trauma.

Chronic Wound Models

Chronic wounds differ from acute wounds in terms of their formation and pathophysiological processes. For example, prolonged inflammation of loss of rapid healing is hindered in those wounds. In addition, recurrent infections may occur in chronic wounds. Therefore, different methods are applied in modeling these wounds than acute models.

Diabetic Wound Model

The presence of diabetes has a delaying effect on wound healing. For the impaired wound healing observed in diabetic patients, diabetes must first be triggered in animals. Diabetes induced rodents such as mice. In the induction of diabetes, IP injection of certain doses of agents such as streptozotocin (STZ) (Karakayalı et al., 2022) or Alloxan is preferred in order to model the type of diabetes, STZ injection can also be done after special diets. After the rodent becomes diabetic, a wound can be created with an incision or excision and the healing process can be monitored. Certain types of animals such as Zucker diabetic Sprague Dawley (ZDSD) rat or genetically modified animal models (db/db mouse or ob/ob mouse) can also be used (Rai et al., 2022).

Pressure Wound Model

The maintenance of circulation is very important for the tissues to continue their vitality through microcirculation. It can be expected that tissues under mechanical pressure will ulcerate and wounds will form if this situation lasts for a long time. This situation can be observed in cases such as Bedridden patients. Mice and rats can be used. The skin is compressed with a magnet or other method. Predetermined ischemia-reperfusion cycles are applied. After a certain number of repetitions of this process, there is a waiting period of several days for the wound to form. Presence of a repeated pressure in a chronic fashion enables a wound at the skin.

Venous or Ischemia-Induced Wound Model

These models mainly focus on insufficiency in blood flow via a venous or an arterial origin. Mice, pig and rabbits can be used. A variety of methods can be used to mimic venous or ischemia-induced wound models. Flap ischemia or ligation of the femoral artery can be administered (Hofmann et al., 2023). For the venous model venous outflow can be created by the obstruction via a vein ligation (Ghanbari et al., 2024).

Specialized Animal Models

Infected Wound Animal Models

Infected wound animal models are fundamental for investigating the mechanisms of infections and impaired healing. Models using mice, rats, and pigs allow the reproduction of chronic infection conditions with pathogens like *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) (Fila et al., 2016; Roy et al., 2020, Egro et al., 2022).

A key feature of these infections is biofilm formation, where microorganisms adhere to the wound surface and become embedded in a self-produced extracellu-

lar matrix. This biofilm structure shields pathogens from antibiotics and immune responses, leading to persistent inflammation and delayed wound healing (Bjarnsholt et al., 2008).

Typically, wounds are created by excisional or burn injuries, followed by bacterial inoculation to study biofilm formation, inflammatory responses, and delayed tissue regeneration (Fila et al., 2016; Wiegand et al., 2024). Recent studies have demonstrated how biofilm infections degrade collagen, alter immune responses, and significantly prolong wound healing time (Fila et al., 2016; Egro et al., 2022).

Immunosuppressed models

Immunosuppressed animal wound models are crucial for studying impaired healing mechanisms and evaluating therapies relevant to patients with compromised immune systems, such as those undergoing chemotherapy, organ transplantation, or chronic illness. These models replicate the delayed or non-healing wound conditions observed in immunocompromised individuals, allowing for translational research on infection risk, inflammation, and regeneration (Bootun, 2013; Appoo et al., 2024). Common approaches include chemically induced immunosuppression—such as the use of cyclophosphamide in mice to reduce neutrophil counts and mimic immune dysfunction (Karner et al., 2020) - Or corticosteroid administration (e.g., hydrocortisone) in rats which diminishes immune cell activity and delays wound closure (Zahran et al., 2024). Additionally, genetically modified models, such as IL-6 knockout mice, exhibit impaired reepithelialization and granulation tissue formation, providing insights into cytokine-dependent repair pathways (Gallucci et al., 2000). These models allow comprehensive examination into immune-mediated healing dynamics and facilitate the preclinical testing of novel therapeutic strategies aimed at restoring proper tissue repair.

Biomaterial-associated models

Biomaterial-associated wound models are essential tools in preclinical research for evaluating the safety and efficacy of novel wound dressings, scaffolds, and regenerative therapies. These models involve the application of natural or synthetic biomaterials—such as hydrogels, nanofibers, films, or decellularized matrices—to standardized wounds in animals, provided for evaluation of healing dynamics, inflammation, and tissue regeneration (Xiao et al., 2023; Arabpour et al., 2024; Talebi et al., 2025). To improve wound healing, extensive research on decellularized materials rich in bioactive cytokines and signaling molecules has guided the development of advanced wound dressings (Xiao et al., 2023). For example, decellularized extracellular matrix materials, such as ovine forestomach matrix (OFM), provide structural support and biochemical signals that facilitate cellular infiltration, angiogenesis, and remodeling during the healing process (Smith et al., 2022). In addition, hydrogel-based systems—especially those loaded with therapeutic agents like growth factors or antimicrobials—create a moist environment that supports cell proliferation, reduces infection risk, and improves wound healing (Lan et al., 2024). Electrospun nanofiber scaffolds, such as those incorporating nanodiamond-silk fibroin composites, have emerged as multifunctional platforms with antimicrobial properties, structural integrity, and biosensing capabilities for monitoring wound repair (Park et al., 2025). Overall, biomaterial-associated wound models bridge the gap between laboratory research and clinical application, offering controlled environments to study the interactions between materials, cells, and the immune system in the context of tissue repair.

Evaluation Parameters in Animal Wound Models

To achieve a comprehensive and reproducible assessment of wound healing dynamics, researchers commonly use a combination of four major evaluation domains: macroscopic assessment, histological evaluation, molecular analyses, and imaging techniques (Boateng et al., 2008; Au - Rhea and Au - Dunnwald, 2020).

Macroscopic assessment

Macroscopy offers a visual and quantifiable assessment of wound morphology, including parameters such as wound size, shape, and closure rate. It is commonly performed using calibrated digital photography in combination with planimetric software, permitting detailed tracking of wound contraction and re-epithelialization over time. This method is essential for identifying gross healing patterns and comparing treatment outcomes across experimental groups. For example, in a study involving equine limb wounds, planimetric analysis was used effectively to quantify epithelialization, contraction, and complete wound closure, demonstrating its value in assessing therapeutic efficacy (Mund et al., 2021; Gülbenat et al., 2022). As an indicator of wound tissue healing, resistance to mechanical tension is also measured by methods such as Tensile strength measurement (Kuo et al., 2022; Brandão et al., 2025).

Histological Evaluation

Histology provides insights into the cellular and tissue-level changes during wound healing (Sami and Abdellatif, 2021; Vespa et al., 2022). The development of standardized histological scales like HEALS-A has facilitated consistent assessment of parameters such as epithelialization, angiogenesis, and scar tissue formation in murine models (Muñoz-Torres et al., 2025). In addition necrotic mass, enhancement or decrease in granulation tissue and microbiological evaluation such as number and diversity of bacteria are conducted (Arihan et al., 2021). In a study investigating the effect of *Mentha spicata* essential oil on wound healing in male Wistar-albino rats, healing was evaluated histologically over a 21-day period. Tissue samples were stained with Hematoxylin and Eosin (H&E) to assess general histopathological changes (Atille et al., 2023). In addition, levels of inflammatory cytokines are also assessed. In histopathological examination, 5 µm

thick sections can be stained with hematoxylin–eosin or Masson staining. Subsequent immunohistochemical staining procedures can also be performed (Yuan et al., 2023). In another study, Gülbenat et al. (2022) evaluated the effects of silk sutures, polypropylene, and skin staples on wound healing both macroscopically and histologically. Skin staples demonstrated superior outcomes in terms of wound closure and histopathological parameters in incisional wounds (Gülbenat et al., 2022).

Molecular analyses

Molecular analyses play a critical role in understanding the underlying biological processes involved in wound repair dynamics by assessing the expression of cytokines, growth factors, and other molecular markers. Techniques such as quantitative PCR (qPCR) and enzyme-linked immunosorbent assay (ELISA) are widely used to evaluate how therapeutic interventions influence these molecular pathways. For instance, in a study investigating the effects of a lupeol-based cream on wound healing in rats, researchers used RT-qPCR and ELISA to measure gene and cytokine expression, indicating accelerated tissue repair following the treatment (Pereira Beserra et al., 2020). Similarly, another study demonstrated that *Ganoderma lucidum* spore oil modulated inflammatory and healing-related pathways by measuring the expression of TLR4 and other markers through qPCR and ELISA, revealing how it supports wound repair (Jiao et al., 2020). Likewise, Karakayalı et al. (2022) investigated the effects of probiotics on molecular markers of wound healing in diabetic rats. Probiotic treatment significantly enhanced the expression of eNOS, Caspase-3, IL-10, VEGF, and Collagen I, suggesting a beneficial role in modulating wound healing pathways (Karakayalı et al., 2022).

Imaging techniques

Advanced imaging tools, including optical coherence tomography (OCT) and bioluminescence imaging (BLI), offer non-invasive methods to monitor wound healing processes in real-time. These techniques help visualize structural and functional changes in the tissue, clarifying healing dynamics (Close et al., 2011; Schuh et al., 2024; Tavecchio et al., 2025).

OCT in particular, delivers high-resolution, cross-sectional images of biological tissues and has been successfully employed to analyze wound repair by quantifying morphological alterations (Cobb et al., 2006; Schuh et al., 2024).

BLI on the other hand is a highly sensitive, imaging technique that enables real-time monitoring of biological processes in live animal models. It has been widely applied in wound healing studies to track gene expression, cell proliferation, and infection dynamics (Close et al., 2011). For example, Benjamin et al. employed BLI to monitor *Staphylococcus aureus* infection in murine incision wounds using bioluminescent bacterial strains, facilitating ongoing evaluation of antimicrobial treatment efficacy without sacrificing the animals (Tavecchio et al., 2025). Those techniques which are non-invasive, helps diagnostic procedures and also provides both investigation of molecular fibrosis mechanisms and patient management strategies (Moroni et al., 2023).

Ethical and Regulatory Aspects

Since wound and wound healing involve complex biological processes, it is very difficult to model certain conditions experimentally without using animals. However, this use is regulated by certain ethical rules for humanitarian reasons.

Principles in Wound Healing

As a result of the ethical processes that began with the Nuremberg Code and have been increasingly detailed, studies conducted with experimental animals are now determined according to the 3R (Russell and Burch, 1959) and currently the 4R principles. In the context of wound healing these rules become important where repeated tissue damage and pain are involved due to slow healing of wounds.

Replacement: *In vitro* human skin models or 3D bioprinted skin which mimics human skin can be tested in suitable conditions (Hofmann et al., 2023).

Reduction: Although reducing the number of animals used also reduces statistical power, ethically it is preferable to use the fewest number of animals possible.

Refinement: For humane treatment of animals, pain management and procedural refinement should be considered since wound models involve painful operations such as incisions, excisions, or burns. Appropriate anesthetics before the operations, analgesics following the procedures, and humane endpoints should be concerned to prevent suffering of the animal.

Responsibility: This recently added 4th R highlights the researcher's ethical responsibilities in awareness, continuing education of the research staff, and monitoring animal welfare (Kang et al., 2022).

Model Selection and Ethical Considerations

Appropriate wound model (burn, excisional, ischemic, infected wound) and animal species (rodents, pig, rabbit) must be scientifically sound and ethical.

Regulatory Frameworks for Animal Wound Models

Ethical committee approval, researcher training and guidelines for animal welfares are standard for many countries.

Recent Advances and Future Directions

Recent advancements in wound healing research have introduced innovative model systems and digital technologies aimed at improving the physiological relevance and clinical applicability of experimental approaches. Innovative *in vitro* models, such as skin organoids and mi-

crofluidic platforms, have shown promise in replicating the detailed structure and dynamic microenvironment of human skin.

Organoids derived from stem cells have the ability to self-organize into structures that closely replicate both the epidermal and dermal layers of human skin, including appendages such as hair follicles and sweat glands. These complex 3D models offer a physiologically relevant platform for investigating mechanisms of wound repair and skin regeneration (Wang et al., 2025). A recent study introduced the development of epidermal organoids (EpiOs) from induced pluripotent stem cells (iPSCs), demonstrating their potential to replicate key features of epidermal biology. These EpiOs provide a promising tool for studying epidermal regeneration and wound healing research (Kwak et al., 2024).

Similarly, microfluidic platforms, often referred to as "skin-on-a-chip" systems, have gained importance as reliable tools in wound healing research, offering controlled *in vitro* environments that closely mimic the dynamic conditions of skin repair. These systems enable precise regulation of chemical gradients, fluid shear, and multicellular interactions—factors critical to studying keratinocyte migration, re-epithelialization, and immune responses (Kwak et al., 2024; Teertam et al., 2025).

Recent innovations include bioelectronic microfluidic platforms designed to deliver direct current stimulation to human keratinocyte cultures under both normoglycemic and diabetic conditions, revealing critical information about electroceutical strategies for wound treatment. By mimicking molecular, cellular, and tissue-level features of the *in vivo* wound environment, these small-scale systems improve the accuracy and consistency of *in vitro* wound models (Shaner et al., 2023). In parallel, the integration of artificial intelligence (AI) and digital wound monitoring tools has greatly improved wound assessment and care. AI-based image analysis can detect wound problems, track healing, and help guide treatment decisions using predictive algorithms. These tools facilitate earlier interventions and have been shown to improve diagnostic accuracy and treatment efficiency, particularly in the management of chronic wounds (Maleki Varnosfaderani and Forouzanfar, 2024; Reifs Jiménez et al., 2025). These approaches are especially valuable in managing chronic wounds and lowering healthcare burden via personalized, data-driven care. Despite significant advances in preclinical wound healing research, translating experimental findings into effective clinical therapies remains a major challenge. This is largely due to physiological differences between animal models and human skin, particularly in immune responses and tissue repair mechanisms, which limit the predictive value of commonly used models such as murine excisional wound systems (Galiano et al., 2004). Furthermore, the complexity of chronic wound environments in humans, which involve prolonged inflammation, infection, and impaired vascularization, is difficult to replicate accurately in preclinical models (Aljamal et al., 2024).

To address these limitations, recent efforts focus on integrating patient-derived cells, iPSC-based models, and micro-engineered platforms to improve relevance and predictive accuracy (Rawat et al., 2024). Advancing therapeutic development in this field also requires the refinement of experimental models, standardization of research protocols, and strengthened interdisciplinary collaboration among scientists, clinicians, and biomedical engineers.

In conclusion, while animal models provide invaluable data about cellular and molecular mechanisms of wound healing and also for preclinical testing of novel therapies, their use requires a careful balance between scientific advancement and ethical responsibility. No model can perfectly replicate human skin physiology, emphasizing the importance of model selection based on research goals. Integration of the precise ethical supervision, and the continuous development of refined or alternative models—such as those using patient-derived cells, iPSC-based systems, and bioengineered platforms—are essential to conducting ethical and clinically relevant research. Ultimately, progress in wound healing research relies on innovative therapies and collaborative improvements in experimental models that translate lab discoveries into clinical applications.

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