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REVIEW ARTICLE

Experimental Skin-Wound Methods and Healing-Assessment in Animal Models: A Review

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A B S T R A C T

Studies on wound healing models in various disciplines of both human and veterinary medicines remain crucial in determining the potential accelerating effects of new drugs and various agents on healing and in providing insights into the mechanisms and pathophysiology of wound repair. Although the complex biological processes of wound healing are well understood, a thorough comprehension of the wound type, size, and impact on the healing process is essential to intervene in these processes effectively. The recovery of anatomical and functional characteristics of injured tissues relies on a comprehensive understanding of differences between wound models and animal species under investigation, which in turn necessitates the development of an appropriate study protocol. The primary objective of a researcher in wound healing is to develop novel methods, strategies, and medical products that intervene in natural wound healing, accelerate recovery, and prevent complications, including aesthetic considerations. To achieve this goal, numerous acute and chronic simulated wound models have been identified, encompassing a wide range of animal species. Nonetheless, wound healing continues to retain its significance and complexity, thereby amplifying the necessity for preclinical (animal model) studies in contemporary research. The reliable outcomes of an experiment depend on the researcher's meticulous planning regarding which stage of wound healing to intervene and how, the creation of an appropriate wound size and depth and the utilization of suitable wound measurement techniques for evaluating the healing process. This article presents and discusses current information on wound healing, influencing factors and criteria for evaluating the healing process.

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INTRODUCTION

The skin is the body's first line of defense and, in response to injury, activates a series of complex events to restore its own structure and thermogenic, endocrine and sensory functions. (Al-Masawa *et al.,* 2022). Wound healing encompasses a dynamic and complex natural process, shaped by the destruction or damage of skin or organ structure, in which various cells play a coordinated role (Bhattacharya *et al.*, 2020; Flynn *et al.,* 2023). In order to elucidate these complex processes, studies related to wound healing require the establishment of an appropriate wound model in the correct animal species (due to their different structural characteristics) (Lindblad, 2008*;* Dunn *et al.*, 2013). In addition to incisional and excisional partial or full-thickness acute wound models (Ansell *et al.,* 2014), various chronic wound types requiring prolonged healing (Davidson, 1998) continue to be the subjects of experimental wound studies. However, it is of great importance which aspect of the simulated wound model the researcher is concerned with (whether it is the early closure of the wound or the detailed identification of numerous interconnected parameters in the biological process of healing?). In this context, despite being the most preferred species due to the ease of maintenance and low cost, mice considered "loose-skinned animals" and their wound healing relies significantly on "wound contraction" through substantial contribution of the panniculus carnosus muscle rather than cellular processes (Abdeldjelil *et al.,* 2017). Therefore, to create an ideal model that reflects all the characteristics of wound healing in full-thickness excisional wounds, it is necessary to immobilize the wound initially to reduce or minimize the effect of wound contraction (Ren *et al*., 2012; Davidson *et al.*, 2013; Anuk *et al.*, 2016).

The body region where the wounds are created and, the number and dimension of the wounds should be taken into account, in wound healing studies. Familiarity with wound assessment criteria and software programs are also equally

essential (Baldassarro *et al*., 2022; Kuo *et al*., 2022; Fischer *et al*., 2023). This article focuses on reviewing research related to wound healing, factors influencing it, experimental wound models, animal species used, and wound assessment criteria.

Wound and wound healing

What is the wound?: Wound is the disruption of cellular, anatomical, and functional continuity of living tissue, which can occur due to physical, chemical, thermal, microbial, or immunological factors (Shrivastava *et al.*, 2018; Masson-Meyers *et al*., 2020; Aydın *et al*., 2022). For cases where the epithelial or mucosal layer is not completely destroyed, the term "closed wound" is issued, while for wounds that involve all layers of tissue integrity (such as incisions, penetrations, and lacerations), the term "open wound" is applied. Wounds are also classified as "acute wounds" and "chronic wounds" (Parlar Köprülü *et al*., 2022). Acute wounds (e.g. cuts or surgical incisions) can heal within a specific timeframe through the processes of wound healing. However, chronic wounds (such as local infections, circulation disorders, diabetes and immunodeficiency) often experience interrupted healing processes and fail to restore anatomical and functional integrity (Shrivastava *et al*., 2018; Baktır, 2019).

Wound healing mechanism and affecting factors: After an injury, tissues can produce tissue factors from the cells and matrices in the injured region to accelerate wound healing. The wound healing process, which emerges as a natural response to the injury, encompasses numerous complex and coordinated dynamic processes. While these processes may proceed regularly in wounds with optimal conditions, various adverse factors can disrupt this progression. Wounds that experience interrupted healing can lead to high morbidity and mortality rates, causing severe pain, loss of function, sepsis, and gangrene formation, ultimately impairing the patient's quality of life. In this case, the cost of treatment will significantly increase. Several factors, such as hypoproteinemia, hyperglycemia, hypovolemia, edema, malnutrition, various cytotoxic agents, radiation, diabetes, uremia, systemic infections, and aging, alter the anatomy and biology of the skin, affecting the normal repair cycle impeding wound healing (Sami *et al.*, 2019; Wilkinson and Hardman, 2020; Lux, 2022). The healing of a skin wound involves a complex biological process comprising a coordinated sequence of cellular and molecular events (Wilhelm *et al.*, 2017; Wilkinson and Hardman, 2020). At each stage of this process, a series of microscopic events unfolds, encompassing mediators of wound healing such as growth factors, cytokines, and chemokines (Table 1). In wounds, multiple stages of healing can occur simultaneously, even if the preceding stage has been completed (Lux, 2022). The process encompasses four stages: 1) coagulation, 2) inflammation, 3) proliferation and 4) remodeling processes (Shrivastava *et al.*, 2018; Al-Masawa *et al*., 2022; Baldassarro *et al*., 2022; Fischer *et al*., 2023). However, the first and second stages are often combined for the purpose of categorizing wound healing into three phases (Jimi *et al.*, 2017; Baktır, 2019; Lux, 2022).

1- Hemostasis: In this initial phase constituting the first step of wound healing, numerous external and internal clotting factors become activated within the shortest period of time following injury (Verma *et al.*, 2019). Vasoconstriction, platelet degranulation, and aggregation, as well as fibrin accumulation, are characteristic processes of this phase (Wang and Xu, 2020). As a result, a clot (scab) composed of fibrin, fibronectin, vitronectin and thrombospondin forms, effectively sealing the wound and preventing bleeding. The scab also serves a range of secondary functions, including providing protection against bacterial invasion, forming a structural scaffold for immune cells, and contributing to early repair (Wilkinson and Hardman, 2020).

2- Inflammation: Inflammatory phase has been reported to occur from several minutes to 24 hours after injury (Verma *et al.*, 2019). The inflammation phase is characterized by increased capillary permeability and cellular migration to wound site (Wang and Xu, 2020; Parlar Köprülü *et al*., 2022). During this phase, the initial cells that appear at the wound site to clear debris and bacteria are neutrophils. Subsequently, monocytes that reach the wound area transform into macrophages, regulated by monocyte chemotactic protein-1 (MCP-1), transforming growth factor-beta (TGF- β) and other cytokines. Macrophages play a pivotal role in repair; apart from facilitating bacterial phagocytosis, they are involved in processes such as fibrin clot resolution, angiogenesis, and fibroplasia (Verma *et al.*, 2019; Wang and Xu, 2020). The inflammatory phase persists for about 72 hours in a clean wound bed, but in the presence of infection and other adverse factors, this duration extends (Lux, 2022).

3- Proliferation (Repair Phase): When the number of inflammatory cells in the wound diminishes, the process of proliferation takes place; however, the migration of monocytes to the wound and their activation into macrophages continues. Initiated typically around the 2nd to 3rd-day post-injury and lasting up to 2 to 4 weeks, this process involves three cell types: fibroblasts, endothelial cells, and keratinocytes (Verma *et al.*, 2019). Migrating to the wound area, fibroblasts produce collagen fibers that contribute to wound contradiction and tensile strength formation. Endothelial cells, on the other hand, proliferate from intact venules near the wound and participate in the angiogenesis process. An important event accompanying this stage is wound contraction. Differentiating from dermal fibroblasts approximately four days after injury, myofibroblasts play a crucial role in wound contraction. Aligned along the lines of wound contraction, myofibroblasts facilitate wound contraction, which occurs along the lines of skin tension (Grada *et al*., 2018; Lux, 2022). In the later stages of the proliferation phase, keratinocytes migrate toward the wound area and play a role in forming bridges with the wound site (Lux, 2022; Flynn *et al*., 2023). As keratinocytes proliferate, the connections between wound edges strengthen, marking the approach toward the end of the proliferation phase. As granulation tissue forms, simultaneously, the process of reepithelialization takes shape from the wound edges towards the center, signifying the completion of the proliferation phase (Parlar Köprülü *et al*., 2022).

Table 1: Natural processes and characteristics of wound healing

Wound Healing Stage	Time to Occur	Duration	Characteristic Process	References
Haemostasis	Immediately after injury	1-2 days	Vasoconstriction Platelet aggregation Coagulation Clot formation	Verma *et al.,* 2019 Wang and Xu, 2020 Wilkinson and Hardman, 2020
Inflammation	1st day	4-7 days	Increased capillary permeability Migration of neutrophils and macrophages Bacteria and dead tissue phagocytosis Homeostasis	Trøstrup *et al*., 2016 Verma *et al.,* 2019 Lux, 2022 Parlar Köprülü *et al.,* 2022
Proliferation(RepairPhase)	2nd-3rd days	2-4 weeks	Activation of fibroblasts, endothelial cells and keratinocytes Collagen synthesis Release of growth factors Granulation tissue formation Angiogenesis Wound contraction Re-epithelialization	Shrivastava *et al.,* 2018 Verma *et al.,* 2019 Lux, 2022 Parlar Köprülü *et al.,* 2022 Flynn *et al.,* 2023
Maturation (Remodeling)	Every stage of wound healing	3 weeks - 2 years	Apoptosis of cells whose role in healing ends Decrease in granulation tissue Vascular regression New matrix formation	Shrivastava *et al.,* 2018 Wang and Xu, 2020 Lux, 2022

4- Maturation and remodeling: Following proliferation stage (around the 3rd week post-injury), the final step wound healing, known as the remodeling phase, occurs. In this phase, the number of fibroblasts in the wound area diminishes, collagen production reaches a balance, epithelialization is completed, the wound color fades, wound tensile strength increases, and scar tissue volume decreases, ultimately leading to the formation of healed scar tissue. The maturation phase, the longest stage of wound healing, extends for weeks or months (Lux, 2022).

Animal species for wound healing models: In scientific studies, the choice of wound model is largely dependent on the hypothesis being tested. However, a good understanding of the chosen animal species' skin anatomy wound healing characteristic is necessary (Trøstrup *et al*., 2016; Grada *et al*., 2018; Saeed and Martins-Green, 2023).

Murine: Mice and rats are collectively referred to as "murine" (Saeed and Martins-Green, 2023). Although both are commonly used in wound healing studies due to their small size, low maintenance costs, and detailed knowledge of their anatomy and physiology, rats are preferred over mice for larger wounds due to their body size and thicker skin layers (Trøstrup *et al*., 2016; Saeed and Martins-Green, 2023).

While the skin in mice, like in humans and other animals, consists of three layers- the epidermis, dermis, and hypodermis- each layer exhibits unique anatomical and physiological differences (Wong *et al.,* 2011). For instance, rats have the ability to synthesize Vitamin C in their liver, which contributes to wound healing as an antioxidant and plays a role in collagen synthesis, in contrast, humans rely on external sources for Vitamin C (Abdeldjelil *et al.,* 2017).

The most significant anatomical difference of murine animals from others is the presence of the panniculus carnosus muscle, which is tightly connected to the skin and fascia and allows independent movement in the underlying muscle layer, enabling twitching and contraction (Zhou *et al.,* 2019). This can lead to premature closure of wounds through contraction before wound healing processes are sufficiently established (Wong *et al*., 2011; Zindle *et al.,* 2021 Saeed and Martins-Green, 2023). In humans, this muscle is a rudimentary structure without any function (Naldaiz-Gastesi *et al.,* 2018). Therefore, especially in the case of small-sized wounds, when a large-scale wound is not created or measures to inhibit contraction (such as splinting) are not taken, wound assessment parameters are disrupted due to early closure of the wound through contraction (Zomer and Trentin, 2018; Bhattacharya *et al*., 2020). In a study, it was revealed that while wound contraction plays a significant role in wound closure in rodent models, epithelialization dominates over contraction, and the effect of contraction continues after epithelialization (Chen *et al.,* 2015).

Rabbit: Factors such as short gestation period, low maintenance cost and larger body size make rabbits suitable for wound studies, and they are preferred for multi-wound models. Rabbit models are commonly employed in toxicological and pharmacological wound studies, as well as for topical application of various agents, due to their higher skin permeability compared to humans and other animals (Saeed and Martins-Green, 2023). Due to the influence of the panniculus carnosus muscle, skin contraction contributes significantly to wound healing in this species, similar to murine (Trøstrup *et al.,* 2016). However, the rabbit ear model, where wound contraction is minimized due to its tight connection to the underlying cartilage, is commonly used in wound studies (Trøstrup *et al.*, 2016). Additionally, the rabbit ear model is frequently preferred, particularly in ischemic wound studies, because of its well-developed vascularity (Zindle *et al*., 2021; Saeed and Martins-Green, 2023).

Porcine: Due to their anatomical and physiological similarities with human skin, the use of pigs has recently gained prominence as a model in wound healing studies. Anatomically, pig skin shares many features with human skin, including similar epidermal and dermal thickness, the presence of dermal papillae, elastic fibers in the connective tissue, protrusions in the dermal-epidermal junction, sparse hair coverage. Unlike murine and rabbit skin, another characteristic shared by pig skin with human skin is its attachment to the subcutaneous connective tissue. Due to their larger body size, they are also conducive to creating multiple wounds on the same individual (Zindle *et al.,* 2021; Flynn *et al.,* 2023; Saeed and Martins-Green, 2023).

Other mammals: Due to their resemblance to human skin structure and physiology, dogs and non-human primates

are also utilized as animal species in wound modelling. However, due to high care feeding costs, as well as logistic challenges, studies involving these animals are not widespread (Trøstrup *et al*., 2016). Although guinea pigs are small and cost-effective, wound healing in them also reflects a wound healing mechanism (contraction) similar to rodents, which is why they are not preferred extensively (Flynn *et al.,* 2023). However, due to their resemblance to human skin structure, they are chosen for some wound models (Parlar Köprülü *et al*., 2022).

Ethical approach to experimental wound studies: Currently, there exist nationally and internationally accepted ethical regulations that govern the use of animals in experimental studies. Obtaining approval from an "Animal Experiments Ethics Committee" for all planned experimental studies is a mandatory practice with legal consequences worldwide. Experimental protocols must include appropriate options for anesthesia, analgesia, and euthanasia. Furthermore, in order to ensure the application of ethical and humane procedures to animals while respecting animals'welfare, adherence to the principles of the 3Rs (replacement, reduction, refinement) should be observed (Dunn *et al.,* 2013; Masson-Meyers *et al.,* 2020; Kuo *et al.,* 2022).

Experimental wound models: Numerous experimental wound healing models have been developed with the aim of understanding tissue repair mechanisms, mitigating the adverse aspects of wound healing and devising novel treatment protocols. These models are generally categorized as *in silico*, *in vitro*, *ex vivo*, and *in vivo* models, each carrying its own advantages and disadvantages (Table 2). The first three models provide significant preliminary insights into the fundamental mechanisms of wound healing and contribute to the design of subsequent preclinical (animal model) investigations (Perez and Davis, 2008; Masson-Meyers *et al.,* 2020; Flynn *et al.,* 2023).

Animal preference, the variety of wounds to be created, their locations, diameters, and depth, as well as the number of wounds to be generated in each animal, are important parameters that affect the research outcomes. In addition, the planning of the number of groups, the number of subjects in each group, and the duration of the experiment should also be optimized according to standards (Dorsett-Martin, 2004).

***In virtuo/In silico* studies:** *"In silico"* is used to describe studies conducted in a virtual or cyber reality environment, often as computer simulations. These models are computational models derived from known and assumed kinetics of skin structure, providing opportunities to theoretically understand cellular responses and stages of wound healing. However, *in silico* models cannot progress beyond theoretical knowledge until they are biologically validated by *in* vitro or *in vivo* models (Grambow *et al.,* 2021; Flynn *et al.,* 2023).

***Ex vivo* studies:** In terms "*ex vivo*" is used to describe experiments or measurements of *in vivo* tissues that are conducted outside of the organism, yet in an environment as closely resembling natural conditions as possible. *Ex vivo* models provide valuable information for assessing wound healing, as they evaluate cellular proliferation rates and associated molecular information related to wound healing (Parnell and Volk, 2019; Sami *et al.,* 2019).

***In vitro* studies:** *In vitro* studies involve experiments conducted on biological materials (cells or tissue) outside of a living organism. Cell culture-based *in vitro* models encompass cell and tissue cultures along with 3D matrices, but they cannot replicate the physiological conditions of wound healing. *In vitro* models are employed for analyzing wound healing processes, particularly to minimize reliance on animal experiments; however, these models lack the ability to identify all factors involved in wound healing (Verma *et al.,* 2019; Grambow *et al.,* 2021).

***In vivo* studies:** *In vivo* models (animal models/preclinical models) are widely used to investigate the physiological and pathological processes related to wound healing, as well as all potential influencing factors and their effects. Nowadays, *in vivo* models have become an indispensable tool for researchers in almost every scientific discipline studying wound healing. However, it should not be expected that the result will be universally applicable; anatomical and physiological differences, along with species-specific variations in wound healing biology, should be taken into consideration (Grambow *et al.,* 2021).

1- Incisional wound models: Skin or other tissues are incised with a scalpel, creating minimal damage to the surrounding tissues; however, other cutting devices such as electrocautery and surgical lasers, while causing less bleeding, can result in more collateral damage (Davidson, 1998). Due to the confined area of wound healing activity, these models are less suitable for the biochemical and historical evaluation of healing. However, this type of wound is ideal for the biomechanical analysis of wound tensile strength (Masson-Meyers *et al.,* 2020). Additionally, it is useful for studies involving different suture patterns characteristic of various suture materials. Incisional wound models typically involve creating a longitudinal incision, often centrally, spanning from the epidermis, through the dermis and subcutaneous tissue, down to the muscle layer on the back of mice and rats. In the case of primary closure or first intention suture models, stitches are removed on the 7^{th} to 8^{th} days after injury, and starting from the 10^{th} day, tensile strength is assessed using tensiometer (Sami *et al*., 2019; Verma *et al*., 2019). For the secondary closure model of incised wounds, also known as second intention, leaving the cuts open is preferred for investigating scar phenomena in late time periods (more than 65 days after incision) (Davidson, 1998). Additionally, this wound model is utilized to determine the effectiveness of materials that reduce/prevent scar formation along with sutures in incisional and dermal flap models (Çantay *et al.,* 2021; Aydın *et al.,* 2022).

2- Excisional wounds models: As the name suggests, this wound model involves the removal of a portion of the target tissue and provides an ideal environment for determining the histopathological aspects of wound healing (such as epithelialization processes), biochemical and mechanical properties, wound area measurements, and wound closure indices (Ansell *et al.,* 2014; Shrivastava *et al*., 2018). Additionally, they are suitable models for serial

Table 2: Commonly used experimental wound models and their characteristics

Wound Model		Purposes	Advantages	Disadvantages	References
In silico		Computational models	Provides fast and accurate results using various bioinformatics tools	The results are of low clinical significance unless confirmed by *in vitro* and *in vivo* experiments	Grambow *et al.,* 2021: Flynn *et al.,* 2023
Ex vivo		*Ex vivo* testing of *in vivo* tissues	They have advantages in analyzing cellular proliferation and molecular biological information	Requires study design in *in vivo* models	Sami *et al.,* 2019
In vitro		Performed in media outside the organism, such as cell culture and skin explants	Allows analysis of wound healing processes and affecting factors	Cannot provide physiological factors for wound healing	Verma *et al.,* 2019 Grambow *et al.,* 2021
In vivo	Incisional Wounds	Suture techniques Scar studies Flap models Biomechanical wound measurements	Ease of application	Not sufficiently suitable for biochemical and histological measurements	Davidson, 1998 Sami *et al.,* 2019 Masson-Meyers *et al.,* 2020
	Excisional Wounds	Use of topical agents Wound healing biology Mechanical measurements Serial biopsy Wound contraction Wound area assessment	Creates a suitable model for acute and chronic wound studies	Requires additional applications to prevent wound contraction Species characteristics of the animal affect healing	Davidson, 1998 Wong et al., 2011 Ansell *et al.,* 2014 Flynn *et al.,* 2023 Saeed and Martins-Green, 2023
	Burn Wounds	Burn pathophysiology Use of topical agents Flap and graft models	Creates a model for the first, second and third types of burns	Wound standardization is difficult Death risk	Cai *et al.,* 2014 Abdeldjelil *et al.,* 2017 Verma *et al.,* 2019 Masson-Meyers *et al.,* 2020
	Dead-Space Wounds	Granulation tissue Collagen synthesis Wound healing potential	Provides information for wound healing	Require special implants	Davidson, 1998 Verma *et al.,* 2019
	Diabetic Wounds	Biomechanical measurements of incisional wounds Excisional wound healing (topical agents) Wound implant studies Angiogenesis	Provides a model for studies to determine and prevent the extent of wound involvement from diabetes.	Deaths during induction of diabetes	Davidson, 1998 Wang *et al.,* 2010 Mendes *et al.,* 2012
	Pressure Ulcer Wounds	Pressure ulcer pathophysiology Studies on decubitus wound healing	Applicable to all experimental animal species	Does not adequately reflect the pathophysiology of pressure	Wang *et al.,* 2010 Zindle *et al.,* 2021 Saeed and Martins-Green, 2023
	Ischemia-Reperfusion Wounds	Decubitus ulcer Blood circulation studies Tissue perfusion studies	Creates an ideal model for tissue perfusion studies	Standardization is difficult Wound prognosis is poor	Grada *et al.,* 2018
	Septic Wounds	Wound sepsis processes Biofilm studies Efficacy of antimicrobial agents Efficacy of topical agents	There were standardized wound models	Systemic infection Death	Mendes *et al.,* 2012 Trøstrup *et al.*, 2016 Grada *et al.,* 2018 Zindle *et al.,* 2021

biopsy techniques involving specific stages of healing (Davidson, 1998). Excisional acute-chronic wound models also encompass various treatment studies, such as Thymus vulgaris (Bozlak *et al.,* 2022), Plantago lanceolata (Kurt *et al.,* 2018; Alan and Özen, 2021), and topical insulin applications (Özaydın *et al*., 2018; Gültekin *et al.,* 2020; Wang and Xu, 2020; Güngör Akbaş *et al*., 2022) and maggot therapy (Borkataki *et al.,* 2021; Uslu *et al*., 2021).

Excisional models are classified into three groups based on the depth of the wound.

Superficial Wounds: These models are typically created by stripping the shaved skin of rodents using adhesive tape. Thus, partial removal of the epidermis, especially the stratum corneum and stratum granulosum is achieved. Since the base layer of the epidermis and the basal membrane remain intact, there is no significant blood loss. Inflammation, epidermal hyperplasia, and decrease in the integrity of the skin barrier can be examined in these wounds. These superficial wound models have also been utilized to observe the effect of various therapeutic and cosmetic agents through topical applications in enhancing the skin barrier (Saeed and Martins-Green, 2023).

Partial-thickness (Split- Thickness) Wounds: Partial-thickness wounds extend into the dermal layer of the skin and are often created using a scalpel, dermatome or keratome, particularly in the thoracic and paravertebral regions (Flynn *et al.,* 2023). A significant portion of the dermis (reticular) and most of the epidermal appendages (sebaceous and sweat glands, hair follicles) remain intact. They are well-suited for studies, especially investigating re-epithelialization (Wilhelm *et al.,* 2017). Creating the model can be challenging due to the thinness of the skin in rodents; therefore, pigs is an ideal animal of choice (Davidson, 1998). These wounds are used for investigating acute wound healing, assessing the action mechanisms of topical agents, and preparing skin grafts from relevant regions of donor animals (Wilhelm *et al.,* 2017; Udegbunam *et al.,* 2021).

Full-Thickness Wounds: This model requires the complete removal of the epidermis and dermis down to the depth of fascial planes or subcutaneous fat (Wilhelm *et al.,* 2017; Özaydın *et al.,* 2018). It continues to be a significant research tool for examining modulations in repair processes such as cell populations, vascularity, and matrix

alterations, as well as for wound area measurement. Biopsy from healing tissue allows analysis of the chemistry, histological organization of connective tissue, angiogenesis and biochemical content of collagen or proteoglycans (Wong *et al.,* 2011). Various devices (e.g., punch biopsy, scalpel, dermatome, electrocautery, and lasers) can be used to create a standardized lesion in this model (Davidson, 1998; Wong *et al*., 2011; Gul Satar *et al.,* 2014; Ermutlu *et al.,* 2018). Wound measurement and the number of wounds in each subject should align with the objectives of the study (Ansell *et al.,* 2014).

3- Rabbit ear model: The absence of wound contraction and the ability to create multiple copies of wounds in a single animal offer more advantages compared to other excisional acute and chronic wound models, making rabbit ear model the preferred choice. Additionally, due to its rich vascularity, the model is suitable for ischemic models as well. It allows detailed examination of wound healing processes, including re-epithelialization and granulation. It possesses several advantages over other excisional wound models: there is no wound contradiction, lesions can often be managed with restraint instead of anesthesia, and multiple copies of wounds can be created in a single animal (Davidson, 1998; Davidson, 2013; Shrivastava *et al.,* 2018).

4- Burn wound models: Animal experimental models are important tools in the evaluation of burn treatments (Cai *et al.,* 2014). Chemical, thermal or radiation burns occurring in the skin or other tissues lead to significantly diverse healing responses due to their impact on cells and tissues lead to significantly diverse healing responses due to their impact on cell and tissue viability. Thermal burns, in particular, create a large open necrotic area containing dead cells and denatured burnt connective tissue. Beyond the visibly destroyed area, there exists a zone of coagulation necrosis caused by denaturation of plasma and cellular proteins, as well as the occlusion of blood vessels and lymphatics (Davidson, 1998; Abdeldjelil *et al.,* 2017). Burn wound models are used for the investigation of burn-related pathophysiological processes, including re-epithelization, granulation on tissue formation, angiogenesis, contraction, and scar formation, as well as for measuring wound tissue. Additionally, they are employed for evaluating the effectiveness of various topical agents and burn wound dressings (Sezer *et al.,* 2007). Moreover, they serve as models for flap and graft applications (Abdeldjelil *et al.,* 2017; Blaise *et al.,* 2020).

Burns wounds can also be partial or full thickness, yet creating a wound model (Espicial in small animals) may presents significant challenges (Masson-Meyers *et al.,* 2020) and due to their intense response to burn-induced trauma, they can lead to deaths of these animals during the wound model creation or subsequent stages (Baktır, 2019). Other factors include the proportional differences in body surface areas between various animal species and humans, and whether a wound of a specific size created in an animal produces the same systemic changes as those observed in extensive burns in humans remains uncertain (Abdeldjelil *et al*., 2017; Verma *et al.,* 2019).

Two of the most commonly used burn models in rats are scalded burn and contact burn models created using a hot metal instrument (Abdeldjelil *et al*., 2017; Verma *et al*., 2019; Güngör Akbaş *et al*., 2022). To mimic clinical scenarios, appropriate techniques have been identified for models that reflect the first-degree (where epidermis and stratum corneum are damaged), second-degree (partial-thickness dermal wound) and third-degree (full-thickness) levels of burns (Davidson, 1998; Abdeldjelil *et al.,* 2017; Masson-Meyers *et al.,* 2020). In addition to these methods, various chemical agents, electrical devices, heated stainless steel, wires apparatuses like hot wax, or alternative methods are also used for creating wounds (Cai *et al*., 2014; Qu and Nourbakhsh, 2017; Shrivastava *et al.,* 2018; Parlar Köprülü *et al*., 2022). The selected method for experimental cutaneous burns should be simple, safe, and reproducible, and capable of creating burns of similar width and depth in each animal. The size, location, and depth to burn can be successfully established when the temperature of the applied object, the duration of exposure, and the pressure exerted by the burning instrument are well-planned (Abdeldjelil *et al.,* 2017).

5- Dead space wound models: The dead space model is used to assess tissue wound healing potential and collagen strength. Although they vary slightly in design, all such models function by creating an artificial tissue area into which plasma flows. The dead space wound is created by making a small transverse incision of the dorsal paravertebral lumbar skin area. The implant materials are quite diverse: viscous/cellulose sponges, polyvinyl alcohol sponges, stainless steel or nylon mesh reservoirs, porous Teflon tubes, perforated silicone tubes containing sponge material, or just an air bubble. Depending on the implant material, scar-like maturation can occur, often characterized by a connective tissue capsule composed of several collagen fibers surrounding the implant. After ten days of treatment, the materials implanted in the area and the granulation tissue formed over it are carefully excised, and the tensile strength of the granulation tissue is measured (Davidson, 1998; Shrivastava *et al*., 2018; Verma *et al*., 2019).

6- Diabetic wound model: In diabetes mellitus, microangiopathy induced by arteriosclerosis and neuropathy leading to sensory loss in the skin results in impaired peripheral circulation, weakens the ability of the organism to fight infections and additionally, numerous metabolic changes associated with hyperglycemia takes place, disrupting wound healing (Özaydın *et al*., 2018). While studies mostly involve local blood circulation in rodent models, rabbits have been indicated as more suitable animals for diabetic models due to their specific skin structure, body size, docile temperament, longer lifespan, and ease of experimental procedures. The use of transgenic rodent models for diabetes has gained popularity (Wang *et al.,* 2010). Rodent models with diabetes-induced conditions are commonly employed to study various parameters of wound healing, including the biomechanical characteristic of incisional wounds, the closure of excisional wounds, the formation of granulation tissue in porous implants, and the development of new blood vessels in various angiogenic analyses (Wang *et al.,* 2010; Masson-Meyers *et al.,* 2020). Rodent models are extensively utilized to investigate the immunological mechanisms and metabolic effects of diabetes (Graham *et al.,* 2011).

Toxic chemicals like streptozotocin (Graham *et al.*, 2011; Park *et al*., 2014) and alloxan (Wang *et al.*, 2010) are commonly used to induce diabetes in animals. A single intramuscular injection of streptozotocin at a dose of 55 mg/kg has been reported to be sufficient to induce diabetes (Davidson, 1998). However, the same dose or higher can also be administered intraperitoneally (IP) (Özaydın *et al*., 2018). Due to streptozotocin's toxicity in rabbits, a longer-lasting diabetes model has been created using alloxan at doses of 75 mg/kg IV (Davidson, 1998) or 100 mg/kg IV (Özaydın *et al.,* 2018). Following induction with either chemical substance, a blood glucose level ≥250-300 µg/dL is considered an indicator of diabetes.

7- Pressure ulcer wound model: It is a commonly used method for modeling pressure ulcers or decubitus ulcers in rodents. First, a cut is made on the animal's back skin and a steel plate or magnet is implanted under the skin (Wong *et al.,* 2011). It is also possible to fix the steel plate to peritoneal wall (Zindle *et al.,* 2021). Subsequently, another magnet is held externally over the implanted plate or magnet in a sandwich-like configuration. After determining the appropriate duration for the experiment's purpose, the area subjected to pressure is evaluated. Due to its inability to fully replicate the expected wound model, it encompasses several disadvantages (Baktır, 2019; Zindle *et al.,* 2021; Saeed and Martins-Green, 2023).

8- Ischemia-reperfusion model: Diminished blood supply and fluid drainage are critical factors in the etiology and treatment of wounds. Chronic ischemia in the extremities is a key factor in ulcer development, and the prognosis remains a pool until blood flow and tissue perfusion are rectified. Acute cutaneous ischemia leads to pressure ulcers, although creating a standardized model in most laboratory animals is quite challenging. The model is often generated through vessel ligation. A simple model has been reported involving the placement of a material like a plastic syringe subcutaneously, constriction of the skin, area over the syringe with a bandage after suturing the skin, and then releasing the pressure after the desired duration (Trujillo *et al.*, 2015; Grada *et al*., 2018).

9- Septic wound model: Infectious burden is one of the most critical aspects of chronic wound care. As a general consensus, contamination exceeding $>10^5$ microorganisms/g of tissue (>1 million colony-forming units per mm3 tissue) indicates that the wounds will not heal (Lux, 2022). Specific sepsis models are employed to discern the processes of sepsis in wound healing and the effectiveness of antimicrobial agents. For this purpose, numerous reproducible quantitative sepsis and biofilm models have been developed using various aerobic strains (Trøstrup *et al.*, 2016; Grada *et al.*, 2018; Zindle *et al.*, 2021). Bacterial suspensions can be administered topically or through local injection (Mendes *et al*., 2012). In incisional and excisional models, 106 to 109 organisms have been employed for inducing wound sepsis (Davidson, 1998).

10- Other wound models: Parallel to recent technological advancements, scar-based wound healing studies, negative pressure research, stem cell investigation and pharmacological other wound models have been defined for a multitude of purposes (other methods have not been covered in this article).

Wound splinting: Rodents have found widespread use in full-thickness excisional wound studies, but in these models, the substantial closure of the wound through the panniculus carnosus has directed researchers toward efforts to prevent contraction. However, wound healing in human skin occurs through re-epithelialization rather than contraction. Currently, the necessity for "wound splinting" models to neutralize contraction has been acknowledged.

Splinting techniques that allowed wound healing through re-epithelialization and granulation tissue formation have enabled the creation of an excision murine wound model that is closer to human wound healing, thus allowing for a more relevant evaluation of molecular signaling and cellular metabolism occurring during wound healing (Davidson, 1998; Wong *et al.,* 2011; Ren *et al.,* 2012; Bhattacharya *et al.,* 2020).

To mitigate contraction, splinting has often been employed in rodent models by placing a ring tightly adhered to the skin surrounding the wound (Lindblad, 2008; Jimi *et al.,* 2017; Grada *et al.,* 2018; Flynn *et al.,* 2023). Additionally, various splits made from materials such as steel rings or other suitable substances are simply fashioned and secured to the wound area with stitches or adhesive (Davidson, 2013; Dunn *et al.,* 2013; Anuk *et al.,* 2016; Fischer *et al.,* 2023).

Wound assessments methods: Once an appropriate model and method is identified for a study, the creation of suitable and reproducible invasive and noninvasive protocols allowing the monitoring of wound changes over time is essential (Davidson, 1998; Ansell *et al*., 2014; Masson-Meyers *et al*., 2020). Current information regarding different methods used to evaluate wound healing, including incision, excision, burn wound, and dead space models, should be reviewed (Shrivastava *et al.,* 2018; Udegbunam *et al.,* 2021; Kuo *et al.,* 2022). For this purpose, researchers should formulate as strategy that addresses the following questions.

Which features of the wound will be assessed? (contraction rate, healing speed, scar-adhesion formation, epithelialization rate, vascularity, biophysical properties, etc.)

Which phases and/or days of wound healing are suitable for measurement, and why? Inflammation, proliferation, remodeling, etc.

Which measurement and assessment methods will be employed? Mechanical methods, biopsies, molecular techniques, histology, wound analysis through imaging, photographs, statistical analysis, computer-based measurement and assessment programs (software), etc.

Conclusions: While various experimental models and methods for wound healing have been developed, today's advanced clinical, laboratory, and computational technologies have significantly enhanced and transformed researchers' understanding of wound repair. These advancements are elevating the knowledge about wounds to increasingly intricate and intriguing dimensions. With a thorough understanding of the unique advantages,

disadvantages, and limitations of each model, scientists can devise research strategies with innovative potential. First and foremost, it should be remembered that the characteristics of creating wound model are specific to the animal used in the experiment. Additionally, factors such as the species of the experimental animal, the choice of the wound site, wound size, and protocols affecting the process such as splinting will contribute to modeling that more accurately reflects the biological processes in wound healing. The accurate utilization of measurement and assessment methods will facilitate the analysis of information about wound healing mechanisms and maximize interventions targeting factors affecting healing.

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