Wound healing is a complex process that involves the coordinated actions of many different tissues and cell lineages. It requires tight orchestration of cell migration, proliferation, matrix deposition and remodelling, alongside inflammation.[1]

Wound healing is a fascinating biological process, and understanding its intricacies is crucial for developing effective treatments. While human wounds offer the ultimate testing ground, ethical considerations and practical limitations necessitate the use of pre-clinical models. This article delves into the world of animal models employed to study wound healing.

Animal models, particularly rodents and pigs, have played a pivotal role in deciphering the fundamental principles of wound management. From the groundbreaking discovery of the benefits of moist wound healing in pigs to ongoing research, these models provide valuable insights. However, the perfect model remains elusive. Anatomical and physiological differences across species, including humans, introduce complexities. Age, sex, and even the location of the wound can significantly influence the results.

This review explores the strengths and limitations of various pre-clinical models, including mice, rats,
rabbits, pigs, and even zebrafish. We'll take a closer look at models specifically designed to mimic impaired wound healing, a significant hurdle in clinical practice. By understanding the advantages and disadvantages of each model, researchers can choose the most appropriate tool to investigate specific aspects of wound healing. The ultimate goal is to bridge the gap between pre-clinical research and clinical application. By ensuring reproducibility, quantitative data, and clinical relevance, these models pave the way for the development of novel and effective wound healing therapies.[2]

**Types of wound healing**

Acute wound healing exemplifies a well-orchestrated cascade of cellular and molecular events. The initial response involves immune cell infiltration (a) to combat invading pathogens and initiate the healing process. Simultaneously, keratinocytes at the wound edge become activated and upregulate genes associated with wound repair, enabling their collective migration towards the center to cover the exposed area (b). Underlying fibroblasts, both local and those recruited from the bloodstream, proliferate and migrate into the wound bed (c). These fibroblast populations play a critical role in establishing a provisional extracellular matrix (ECM) that provides structural support and transmits signals to orchestrate further repair. Notably, some fibroblasts differentiate into myofibroblasts, which contribute to wound contraction. Blood vessel formation (angiogenesis) is another key process in acute wound healing (d). New blood vessels perfuse the granulation tissue, delivering vital oxygen and nutrients to support the ongoing healing endeavor. Interestingly, research suggests a positive correlation between wound healing rates and nerve innervation (e). While nerves play a crucial role in coordinating the healing response, excessive innervation after wound closure can potentially contribute to neuropathic pain. In essence, acute wound healing represents a remarkable display of cellular cooperation driven by a complex interplay of molecular signals.[3]

Acute wounds, typically caused by trauma like burns or cuts, heal within 8-12 weeks. The healing process involves hemostasis, proliferation, maturation, and remodeling. Essential in acute wound management is removing debris, controlling infections, and achieving closure. Maintaining a moist wound environment and educating patients about proper care are key factors for quality healing.[4]

Split thickness skin grafts are the preferred treatment for acute burn wounds, but their use is limited by the amount of body surface they can cover.[5]

Chronic wounds resist healing due to high blood sugar, lingering inflammation, and missing growth factors. New therapies using stem cells or engineered skin grafts look promising, but require further testing.[6]

The process of wound healing involves several stages: haemostasis, inflammation, proliferation, and maturation. When wounds fail to progress through these stages in an organized manner, it leads to delayed healing and the development of chronic wounds. Common characteristics of non-healing wounds include excessive exudation, recurrent infections, tissue death, impaired skin regeneration, reduced blood vessel formation, and increased production of reactive oxygen species. Chronic wounds are typically classified into three main types: diabetic foot ulcers, vascular ulcers, and pressure ulcers, which are frequently observed in elderly individuals with conditions like diabetes, vascular issues, and obesity. Diabetes affects all phases of skin injury repair, leading to a heightened inflammatory response with elevated levels of inflammatory molecules such as TNF-α and decreased production of healing-promoting factors like IL-10 and TGF-β. This imbalance results in the polarization of macrophages towards a pro-inflammatory state, activation of CD8+ T-cells, and ultimately tissue necrosis.[7]

Macrophages play a pivotal role in the complex process of wound healing, orchestrating various stages to ensure proper tissue repair and regeneration. Upon injury, macrophages are among the first responders, infiltrating the wound site to initiate the inflammatory cascade. In chronic wounds, macrophages get stuck in a pro-inflammatory state (M1-like) and don't switch to the anti-inflammatory type (M2-like) needed for healing. This persistent inflammation is believed to be a major factor in why chronic wounds fail to heal properly.[8]
Wounds are initially categorized as acute, regardless of cause, with events like accidents, trauma, burns, or surgery leading to acute wounds. Chronic wounds, however, deviate from the normal healing trajectory, with healing times prolonged and resistance to treatment. These wounds often fail to achieve functional closure and may recur. The healing trajectory curve for chronic wounds is shifted away from normal, indicating delayed healing.\(^9\)

Chronic wounds are a major healthcare challenge, affecting millions and costing billions annually. Unlike acute wounds that heal in stages, chronic wounds get stuck in an inflammatory state, leading to pain, mobility issues, and increased risk of infection. As the population ages and chronic diseases become more common, the number of chronic wounds is expected to rise.\(^10\)

**Table 1: Key Differences between acute and chronic wound healing processes**

<table>
<thead>
<tr>
<th>Features</th>
<th>Acute wound healing</th>
<th>Chronic wound healing</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healing Time</td>
<td>Days to weeks</td>
<td>Weeks to months or even years</td>
<td>(^{11})</td>
</tr>
<tr>
<td>Progression</td>
<td>Follows a well-defined sequence</td>
<td>Stalled or stuck in one or more phases</td>
<td>(^{12})</td>
</tr>
<tr>
<td>Inflammation</td>
<td>Controlled and resolves within a short period</td>
<td>Persistent and excessive</td>
<td>(^{13})</td>
</tr>
<tr>
<td>Granulation Tissue</td>
<td>Forms normally and supports healing.</td>
<td>Formation is impaired or excessive.</td>
<td>(^{14})</td>
</tr>
<tr>
<td>Angiogenesis (New Blood Vessel Formation)</td>
<td>Occurs readily to deliver oxygen and nutrients</td>
<td>Impaired, leading to oxygen deprivation (hypoxia)</td>
<td>(^{15})</td>
</tr>
<tr>
<td>Cell Proliferation</td>
<td>Balanced between different cell types</td>
<td>Imbalance with excessive or insufficient cell activity</td>
<td>(^{16})</td>
</tr>
<tr>
<td>Remodeling</td>
<td>Collagen is deposited and organized to form a strong scar</td>
<td>Collagen deposition is disorganized, leading to weak or excessive scar formation</td>
<td>(^{17})</td>
</tr>
</tbody>
</table>

**Wound healing Models**

Rodents are attractive candidates for wound healing studies because of their availability, low cost, and ease of handling.\(^{18}\)

In the excision wound model the rate of wound closure (epithelialization) by creating a circular wound on the rats' backs is evaluated.\(^{19}\) The animals undergo anesthesia before and during the procedure. Excision wounds are induced following the method outlined by Morton and Malone. The dorsal fur of the rats is shaved, and the intended wound area is marked with methylene blue. A circular excision wound of 300 mm\(^2\) area and 2 mm depth is created using surgical instruments. The wounds are left open, and the animals are divided into control and test groups. Wound closure rate is assessed by tracing the wounds on days 1, 5, and 15 post-injury. All tracings are conducted by the same individual, and wound areas are measured using graph paper. The epithelialization period is determined as the day of eschar fall without any remaining raw wound.\(^{20}\) The tensile strength of the healed wounds and collected tissue samples for further analysis under a microscope (histological analysis).\(^{21}\)

In the incision wound model, rats undergo anaesthesia before and during the wound creation process. Their dorsal fur is shaved, and a longitudinal paravertebral incision of 6 cm is made through the skin and underlying muscle, following Ehrlich and Hunt's technique. Surgical sutures are then applied to close the incision at 1 cm intervals, and the wounds are left uncovered. Animals are randomly divided into control and test groups and treated accordingly. Sutures are removed on the eighth day post-wounding, and treatment continues.

In the secondary closure model of incised wounds, leaving the cuts open is preferred for studying scar phenomena in late stages (more than 65 days after incision) (Davidson, 1998). This model is also used
to assess the effectiveness of materials in reducing or preventing scar formation along with sutures in incisional and dermal flap models.\textsuperscript{[22-27]}

**Experimental wound models**

Various models have been devised for investigating human wound healing, with the goal of uncovering essential mechanisms and gaining deeper insights into the healing process. These encompass computational simulations (in silico), laboratory-based experiments (in vitro), tissue culture methods (ex vivo), and studies conducted within living organisms (in vivo).\textsuperscript{[28]}

These models, spanning from human skin-derived cells to in vivo animal models, vary in their ability to uncover the molecular, cellular, and structural mechanisms of scarring and test new therapies. They have significantly advanced skin scarring research.\textsuperscript{[29]}

An ideal wound healing model for adult human skin should replicate a wound in a system that mirrors and integrates all components, processes, and functions of adult human skin. It should also allow for testing therapies ex vivo and assess their impact on skin repair. However, existing models often fall short of this ideal. Monolayer and organotypic cultures offer insights into specific skin components but lack complexity and fail to mimic in vivo conditions.\textsuperscript{[30]}

**In silico studies**

In silico models lack the physical characteristics of native human skin. Although informative, animal models can differ significantly and raise ethical concerns. Human skin organ culture, while maintaining all skin components and interactions ex vivo, serves as a valuable tool for studying skin pathophysiology, testing treatments, and assessing xenobiotic effects.\textsuperscript{[31]}

**In vitro studies**

Wound healing assays with a focus on both 2D and 3D in vitro models.

**In 2D assays**, a confluent cell layer is wounded, and cell migration into the injured area is observed and analyzed. These assays are conducted under standard laboratory conditions and are cost-efficient. However, their limitation lies in their inability to capture the complexity of wound healing mechanics comprehensively.

**3D in vitro models** employ scaffolds seeded with cells to mimic complex wound healing processes, including cell-matrix and cell-cell interactions. These models offer a more realistic representation of the wound healing process compared to 2D assays. They provide insights into the interplay of different cell types and the effects of various factors on wound healing.\textsuperscript{[32]}

**Ex vivo studies**

These contain various cellular elements of human skin and have been used in dermatology studies. They offer a closer representation of human skin compared to in vitro models. However, there are challenges in maintaining these cultures and limited characterization of their properties in previous studies.\textsuperscript{[33]}

The biomechanical characteristics of healing skin wounds, including load relaxation, ultimate tensile strength, ultimate tensile stress, stiffness, and energy absorption capacity, are assessed outside of a living organism.\textsuperscript{[34]}

The study utilized an ex vivo wound model to investigate antibacterial treatment effects on wound healing. Skin samples from euthanized Göttingen minipigs were prepared, stored, and thawed. Standardized burn wounds were created, followed by infection induction and treatment administration. Analysis methods included bacterial count assays, histology, immunohistochemistry, SEM, and longitudinal bioluminescence imaging using IVIS. This approach enabled controlled experimentation and detailed examination of wound healing processes in a laboratory setting.\textsuperscript{[35]}

**In vivo studies**

In vivo models play a crucial role in wound healing research, allowing for the evaluation of healing processes in living organisms. These models replicate the complexity of wound healing in a physiological context, providing valuable insights into the efficacy of potential therapeutic intervention.

1. **Incision Wound Model**: Creates controlled incisions in the skin to study healing responses to treatments.
2. **Excision Wound Model**: Removes defined areas of skin to assess wound closure and tissue regeneration.
3. Burn Wound Model: Mimics thermal injuries to study healing processes following burns.

4. Dead Space Model: Creates cavities or spaces within the body to study tissue regeneration and the effects of interventions. These models are vital for understanding wound healing mechanisms and evaluating potential treatments.\[35\]

**Preclinical Wound Models for Novel Wound Therapies**

**In Vitro wound healing models.**

**2D Wound Scratch Assay**

The in vitro scratch wound healing assay, a cost-effective method often paired with image analysis tools, stands as one of the prevalent 2D techniques for assessing cellular migration and proliferation in scenarios like regeneration and disease. While open-source software like ImageJ exists for analyzing these assay images, they typically demand manual adjustment of parameters, which can be labor-intensive and restrict image processing speed.\[36\]

- Prepare protein solution (fibrinogen or collagen) in 12-well plate; use polystyrene plates as controls.
- Incubate for 1 hour; remove excess solution and wash with DPBS.
- Seed 80,000 cells/cm\(^2\); mark reference points for imaging.
- Incubate overnight for cell adhesion.
- Create scratch wounds using pipette tip; capture images with Leica DM IRB microscope.
- Repeat imaging hourly to monitor cell migration and wound closure.\[37\]

Boyden chamber assay, devised by Boyden in 1962, is a commonly used technique for studying cell migration dynamics.

- The assay employs a specialized chamber comprising a transwell insert within a cell culture plate well.
- A porous polycarbonate membrane in the transwell facilitates cell migration from the upper to the lower chamber.
- Cells are seeded onto the upper surface of the transwell, while the therapeutic agent under investigation is placed in the lower chamber.

- Migrating cells extend protrusions through the porous membrane toward the therapeutic agent, reaching the underside of the membrane.
- Microscopic imaging is conducted after a defined incubation period, typically 16 hours, to visualize and quantify the migrating cells.
- This assay provides valuable insights into cell migration behavior in response to therapeutic agents, aiding in their evaluation and development.\[38-39\]

The modified Boyden chamber assay is a refined method tailored to explore the intricate interactions between dermal fibroblasts, especially in wound healing. Unlike the conventional assay, this modified approach measures fibroblast migration within a controlled environment. By evaluating migration rates, researchers aim to uncover the influence of soluble chemotactic agents and discern any cell-to-cell interactions. Integrating this method with others allows for a more nuanced understanding of dermal fibroblast behavior during the wound-healing process.\[40\]

Organotypic cultures are advanced in vitro models mimicking native tissue architecture.

- Collagen is diluted and neutralized to form a gel matrix, with fibroblasts embedded to create a scaffold.
- Keratinocytes, epithelial cells found in skin, are seeded onto the collagen matrix.
- Transwell inserts are used for culturing, with media added above and below for nutrient supply.
- Absence of ROCK inhibitor allows for epithelial differentiation of keratinocytes.
- Cultures are incubated at 37°C, then lifted to air-liquid interface to promote further differentiation.
- After 14 days, cultures are cryopreserved for future use or analysis.\[41\]

**Organ-on-a-Chip Model**

- In the realm of tissue engineering, significant strides have been taken to cultivate human-derived cell types in suitable biomaterials to create precise tissue-specific models.
- Stem cell engineering breakthroughs, especially in induced pluripotent stem cell (iPSC) technology, have played a pivotal role in enabling the
development of patient-specific 'Organ-On-A-Chip' (OOC) models for preclinical studies tailored to individual patients.

- The 'Organ-On-A-Chip' in vitro wound healing model utilizes these advancements to create personalized platforms that mimic the wound healing process with high fidelity.

- By incorporating patient-specific iPSC-derived cells into the microfluidic devices, researchers can recreate the unique cellular responses and interactions observed in individual patients.

- This personalized approach allows for more accurate assessment of wound healing dynamics and the evaluation of potential treatments in a patient-specific context.

- Additionally, the use of iPSC technology in 'Organ-On-A-Chip' models offers the potential to study disease mechanisms and test personalized therapeutic strategies in a controlled laboratory setting.

- Overall, the integration of iPSC technology into 'Organ-On-A-Chip' platforms represents a promising avenue for advancing personalized medicine and improving preclinical models for wound healing research.[42]

Human Skin-On-A-Chip (SOC) Models: Attract attention for dermatological studies, wound healing, risk assessment, and transdermal drug administration research. 3D skin-on-a-chip models better mimic in vivo physiological conditions, overcoming limitations of 2D cell cultures.

Categories of In Vitro Skin Models

Epidermis, dermis, and subcutaneous tissue, each with distinct cell components and functions.

- Reconstructed Human Epidermis (RHE): Utilizes only keratinocytes, suitable for risk assessment but limited in drug efficacy testing.

- Dermo-Epidermal Human Skin Equivalents (HSE): Includes dermis compartment with collagen I and fibroblast cells, enabling investigation of cell-cell crosstalk and wound healing.

- Human Skin Equivalents with Additional Cell Types: Incorporate melanocytes, immune cells, stem cells, and neurons to study various skin functions and disorders.

- Vascularized Human Skin Equivalents: Allow extended tissue survival and assessment of transdermal penetration of drugs into the bloodstream.

Advancements in skin-on-a-chip technology promise to revolutionize drug discovery and personalized medicine in dermatology and wound healing research. The 'Organ-On-A-Chip' in vitro wound healing model revolutionizes drug discovery by mimicking human organ functions on a miniature scale. Its advantages include precise environmental control, personalized cell incorporation, and high-throughput screening capabilities. Challenges like scalability persist, but the technology promises to accelerate personalized wound healing treatments and enhance patient outcomes.[43-45]

**Ex vivo wound healing Model**

In wound healing research, while in vitro models have contributed significantly to our understanding of the underlying mechanisms and potential therapies, they often lack the complexity of the native wound microenvironment. To address this limitation, ex vivo models have emerged as valuable tools. These models preserve the native tissue architecture and cellular interactions, offering a more physiologically relevant platform for studying wound healing processes. For example, ex vivo organ culture models have been developed using tissue samples from wounded skin, allowing researchers to study wound healing in a controlled laboratory setting while maintaining the intricate cellular and extracellular components present in vivo. Such ex vivo models enable the evaluation of various wound healing parameters and the testing of therapeutic interventions, providing insights that can inform clinical practice. Ex vivo organ culture models have been developed to accurately maintain the natural microenvironment found in vivo, offering a valuable method for investigating wound healing processes.[46] Ex vivo human skin has been used for years as model to study skin physiology and drug penetration. For ex vivo studies, an optimal wound healing model using adult human skin should mirror a wound created and assessed within a system that faithfully replicates all components, processes, and functions of adult human skin. Additionally, it should facilitate functional testing of therapies outside of the body and enable
assessment of how these processes contribute to skin repair.[47-49]

**Murine Skin Explant**

Murine skin explant culture offers another approach for evaluating keratinocyte migration. By removing the fascia from mouse skin samples, the skin can adhere to tissue culture plates while submerged in culture media. Over a period of up to 7 days, keratinocyte migration out of the skin explants can be observed. This model serves as a valuable tool for investigating collective cell migration in transgenic or knockout mouse skin, as well as for assessing the impact of pharmacological treatments on keratinocyte migration in response to the effects of overexpressed or knocked out genes or specific drug interventions.[50]

- Anesthetize mice and shave the dorsal skin, then clean with antiseptic solution.
- Create wounds using standardized methods like punch biopsy or surgical blade.
- Apply treatments, such as therapeutic compounds or dressings, as per experimental design.
- Monitor wound healing progress regularly, assessing closure and inflammation.
- Sacrifice mice at predetermined time points to collect skin tissue samples.
- Analyze tissue sections to evaluate wound healing extent, including collagen deposition and angiogenesis.
- Compare experimental groups to assess treatment efficacy in promoting wound closure and tissue regeneration.[51-53]

**Pressure ulcer ex vivo model**

- Anesthetize human skin donors and obtain skin samples from appropriate anatomical sites.
- Clean and prepare the skin samples by removing excess tissue and sterilizing the area.
- Apply standardized mechanical stress or pressure to the skin samples using a mechanical explant test system.
- Load the skin samples with static stress for different durations as per experimental design.
- Collect skin tissue specimens at various time points to assess morphological changes, protein expression levels, and RNA expression levels in response to mechanical load.
- Analyze the collected tissue specimens to evaluate the induction of inflammasome and other relevant molecular mechanisms.
- For porcine models, apply pressure to ex vivo wounds to evaluate the effectiveness of pressure-reducing devices.
- Monitor the skin samples under various environmental and experimental conditions to assess the durability and flexibility of the ex vivo wound model.
- Use appropriate controls and compare results to mimic acute and chronic wound conditions accurately.
- Employ ex vivo skin explants in conjunction with other in vitro and ex vivo models, such as in vitro cell culture and skin-on-a-chip models, to gain comprehensive insights into wound healing mechanisms and evaluate novel pharmacological treatments and devices.[54-57]

The porcine skin explant model offers several advantages for wound infection research and antimicrobial therapy evaluation. Its physiological resemblance to human skin allows for clinically relevant observations, while its accessibility from meat processing facilities makes it a cost-effective option for researchers. Moreover, the model enables the study of biofilm dynamics and the evaluation of diverse antimicrobial interventions, including iodine-containing dressings, silver-containing dressings, surfactant-based dressings, and honey ointments. However, limitations such as the absence of a commensal microbiome and a reduced host immune response should be considered when interpreting results from this model.[58]

Porcine skin explant models represent a valuable tool for investigating wound infections and evaluating antimicrobial therapies. Their ability to simulate chronic wound environments and assess various treatment modalities makes them indispensable in preclinical research. Future studies should aim to refine and optimize the model to better recapitulate in vivo conditions while addressing its inherent limitations. Overall, porcine skin explant models hold promise for advancing our understanding of wound infection pathogenesis and
facilitating the development of effective therapeutic strategies.\textsuperscript{[59-61]}

\textbf{In Vivo Models wound healing models.}

\textbf{Rodent Wound Healing Models (e.g., Incisional, Excisional)}

Mice and rats are the go-to animals for wound healing research due to several advantages. They are cost-effective, develop quickly, require less research material due to their small size, and heal faster leading to quicker experiments.\textsuperscript{[62]}

Additionally, the availability of inbred strains and genetically modified options allows for consistent results and targeted studies of specific healing pathways. Since these animals are widely used in biological research, a vast array of tools and tests are readily available for analysis. Furthermore, scientists can manipulate mice and rats to mimic various wound conditions, making them ideal for studying different wound healing processes. However, there are some limitations. Wound healing in these animals involves more contraction compared to humans, and there are fewer genetically modified rat strains available compared to mice. Additionally, rats are larger and more expensive to maintain than mice.

Hair growth cycles in mice and rats can influence wound healing. Wounds heal faster when hair follicles are actively growing (anagen phase) because these cells contribute to healing. To minimize this influence, experimenters carefully plan and monitor the age of the animal and its hair growth stage. Ideally, wounds are created during the resting hair growth phase (telogen) to minimize the contribution of hair follicles to healing.\textsuperscript{[63-65]}

In the realm of preclinical wound investigation, researchers commonly employ two primary in vivo wound models: excisional and incisional wounds.

Excisional wounds involve the complete removal of tissue, resulting in open wounds of variable sizes, typically ranging from 2 mm to 20 mm in diameter. These wounds can be generated using various tools such as biopsy punches, surgical scissors, or lasers, and may be covered with occlusive dressings or nonocclusive bandages. Conversely, Incisional wounds consist of making cuts into the tissue, often with consistent lengths of 10 to 15 mm, using surgical scalpels. While incisional wounds are usually allowed to heal without closure, some are sutured, potentially influencing the healing process. Familiarity with the intricacies of these wound models is essential for researchers to craft experiments that faithfully replicate clinical scenarios and effectively assess therapeutic strategies.\textsuperscript{[66-68]}

\textbf{Diabetic Wound Healing Models}

Diabetic wound healing poses a formidable challenge due to disruptions in the intricate cellular and molecular processes involved in wound repair. While healthy individuals follow a coordinated sequence involving various cell types like keratinocytes, platelets, macrophages, endothelial, and fibroblast cells, diabetic wounds exhibit a dysregulated response. Factors such as fibrin cuffs around small blood vessels hinder the formation of new blood vessels and granulation tissue, while the inflammatory response remains chronic with a distinct profile of neutrophils. Moreover, keratinocytes exhibit hyper-proliferative activity, while fibroblasts lose their capacity to proliferate and respond to growth factors. These alterations signify fundamental changes in the wound healing process, complicating the identification of a single intervention target.\textsuperscript{[69-70]}

Replicating this complexity in animal models presents challenges, as animal wounds differ in causative factors and healing mechanisms from human wounds. Despite the limitations, small mammals like rats, rabbits, and mice are commonly employed in wound healing research due to their cost-effectiveness and ease of handling. However, their anatomical and physiological differences from humans limit their suitability for in vivo wound healing studies. Conversely, pig skin closely resembles human skin both anatomically and physiologically, and porcine wound healing processes closely parallel those observed in humans.\textsuperscript{[71]}

In clinical practice, managing diabetic wounds necessitates a comprehensive approach addressing factors such as tissue debridement, infection control, and advanced therapies. Yet, achieving successful outcomes remains challenging due to the multifaceted nature of diabetic wound healing and the difficulties associated with conducting effective clinical trials, particularly in recruitment and stringent exclusion criteria. As a result, despite their limitations, animal models remain an area of interest
for researchers seeking insights into diabetic wound healing.\cite{72-73}

Hyperglycemia significantly disrupts wound healing processes in diabetic foot ulcers (DFUs). It affects the synthesis, migration, and proliferation of keratinocytes and fibroblasts essential for re-epithelialization. Additionally, hyperglycemia reduces the activity of antioxidant enzymes, leading to free radical damage and the accumulation of skin aging markers like advanced glycation end products (AGEs). This condition also triggers the overproduction of reactive oxygen species (ROS), damaging peripheral nerves and increasing the risk of DFU development. Overall, uncontrolled high blood glucose levels render the skin more prone to injury and infection, impairing wound healing in DFUs.\cite{74}

Diabetes plays a significant role in delaying wound healing, leading to what is known as the chronic wound model. To simulate diabetic conditions in animals, a single injection of a hyperglycemic drug (such as streptozotocin or alloxan) is administered intraperitoneally after overnight fasting, prepared in a citrate buffer solution. Blood glucose levels are measured 24 hours post-injection using a glucometer by drawing blood from the orbital plexus. To create a standardized wound model, an 8 mm circular wound is made on the pre-shaved and sterile dorsal surface of the animal using a biopsy punch. Prior to wound creation, the skin is sterilized with 70% alcohol.\cite{75-76} In this diabetic wound model, test drugs are either applied topically or administered within an appropriate concentration range. Blood glucose levels are monitored both at the time of wound creation and at wound excision to assess the potential antidiabetic effects of the test drug.\cite{77-78}

### Rabbit Ear Wound Model

The Rabbit Ear Wound Model procedure involves anesthetizing rabbits with a ketamine-xylazine mixture before creating dermal wounds on their ear ventral sides. Each rabbit receives six 6-mm diameter full-thickness wounds per ear, totaling 12 wounds, which are then dressed with Tegaderm. Some wounds remain sterile for control purposes, while others are inoculated with specific bacterial strains on postoperative day 3. Topical antibiotics are applied on postoperative day 4 to target planktonic bacteria, followed by antimicrobial absorbent dressings to maintain a biofilm-dominant infection. Wounds are harvested at various time points for histologic analysis, scanning electron microscopy, quantitative reverse transcription-polymerase chain reaction, and viable bacterial counts. Sampling from both ears of multiple animals ensures representation and accounts for potential variability. Additionally, one extra animal is used for safety assessment but not included in analyses, with daily dressing checks conducted throughout the study. This model enables controlled investigation into wound healing, infection dynamics, and treatment responses using rabbits as an animal model.\cite{79-80}

A study compared wound healing in alloxan-induced diabetic rabbits to normal controls using stereological analysis. Alloxan was administered 7 days before surgery, with four wounds created on each ear. Analysis at 7 and 14 days post-surgery revealed increased inflammatory cells and fibroblasts in diabetic rabbits at 14 days. Additionally, diabetic rabbits showed reduced blood vessel density, indicating less efficient nutrient exchange. This study uniquely employed stereological methods to assess diabetes's impact on wound healing in a noncontractile model, offering valuable insights into diabetic wound physiology.\cite{81}

By inducing ischemia in one ear and comparing it to a non-ischemic control ear, researchers can closely investigate the effects of reduced blood supply on wound healing processes. The surgical procedure, which involves creating three small incisions on vascular pedicles and a circumferential subcutaneous tunnel, effectively interrupts blood flow to the ischemic ear while minimizing skin disruption. This results in a reliable and reproducible model with minimal surgical complications. Furthermore, the model allows for paired comparisons between ischemic and non-ischemic wounds within the same animal, reducing individual variations. Postoperative care involves monitoring skin temperature, dressing changes, and assessing wound healing times. This model holds promise for advancing our understanding of diabetic wound healing and evaluating potential therapeutic interventions.\cite{82-83}

### Ischemic Wound Models
Inducing ischemic wounds in rats involves creating full-thickness excisional wounds within a narrow bipedicile dorsal skin flap. This design ensures random blood supply, leading to ischemia in the wounds located at the midpoint of the flap. To prevent reperfusion and readherence, a silicone sheet is inserted beneath the skin flap, also inhibiting wound contraction. Removal of the panniculus carnosus muscle from the wound bed reduces wound contraction. Full-thickness excisional wounds are created within the skin flap, and control wounds without the flap and silicone sheet serve as internal controls. The skin flaps and silicone sheet are sutured to maintain ischemic conditions, and an occlusive dressing is applied for a controlled environment. This model enables researchers to investigate the effects of ischemia on wound healing and assess the efficacy of various agents in promoting healing under ischemic conditions, with parameters such as tissue oxygen tension, wound surface area, tissue lactate, tensile strength, and molecular analysis used for validation.

In a twenty domestic white pigs were utilized, following approval from the Ohio State University Institutional Laboratory Animal Care and Use Committee. Pigs weighing between 70 to 80 pounds were anesthetized with Telazol and isoflurane, after which their dorsal regions were shaved and surgically prepared with alternating Betadine and alcohol scrubs. Four full-thickness bipedicile skin flaps, each measuring 15 by 5 centimeters, were created on each pig through parallel incisions using electrocautery. A sterilized 0.01-inch-thick Sil-Tec medical-grade sheeting was placed underneath each flap to prevent readherence and reperfusion of the flap from underlying tissue. The flap incisions and silicone sheets were sutured into position using continuous sutures. Ischemia of the flap tissue was confirmed through laser Doppler imaging of blood flow. Full-thickness excisional wounds were then made in the middle of each flap using an 8 mm disposable biopsy punch, with six more wounds developed similarly on nonischemic skin. The wounds were dressed with VAC Drape and monitored regularly, with dressing changes every three days and drainage of accumulated wound fluid as needed. Images of the wounds were captured digitally for wound closure data collection, and tissue analyses, including RNA, protein, and histological analyses, were conducted on harvested wound tissue at designated time points. Upon completion of experiments, pigs were either euthanized or transferred to another approved protocol.

Rodents are currently the most prevalent choice for studies like these due to their cost-effectiveness, as they require relatively minimal resources for food, water, and housing. However, their anatomical differences from humans pose a limitation. Larger animals offer a more human-like anatomy and physiology but demand greater expenses and housing space. The bipedicile skin flap, along with its modifications, serves as a popular model for studying ischemic wounds.

The murine incisional wound model allows for the examination of cell interactions and influences, as well as the biomechanics of wound healing and scarring. While the use of wound splinting can minimize contraction, this model is not optimal for assessing epithelialization due to differences in skin anatomy and physiology between rodents and humans. Similarly, the murine excisional wound model facilitates the study of cell interactions and treatment effects on wound contraction but is also limited in its ability to assess epithelialization. The murine full-thickness splinting model, however, can more closely model human epithelialization processes by attenuating the effects of contraction and providing greater reproducibility. Conversely, the parabiosis model is effective for studying circulation and cell infiltration in wound healing but is not ideal for assessing epithelialization. The dead space wound model isolates connective tissue formation and provides information on interstitial fluid accumulation but is not useful for epithelialization assessment due to anatomical variations between rodents and humans. Finally, the dorsal skinfold chamber model enables microscopic imaging of vasculature in vivo, allowing for real-time pathophysiological imaging but is also affected by differences in skin anatomy and physiology between rodents and humans.

Denervation Wound Models

Denervated tissue refers to tissue that has been deprived of its nerve supply. In the context of wound healing research, denervated tissue is often utilized to study the role of neural input in the healing process. Denervation models involve
surgically severing nerve endings in specific areas of the body, such as the T9 to L1 vertebrae in mice or porcine models. This deprivation of neural input mimics neuropathy, a condition commonly associated with chronic wounds like diabetic foot ulcers. Denervated tissue is used to investigate the effects of nerve signaling molecules like Substance P (SP) and calcitonin-gene-related peptide (CGRP) on wound healing. Additionally, denervated tissue can be used to study the cellular and molecular mechanisms underlying delayed wound healing observed in patients with peripheral neuropathies. By creating wounds within denervated tissue, the impact of neural input on various stages of the healing process can be assessed, providing valuable insights into potential therapeutic interventions for improving wound healing outcomes in neuropathic conditions.\textsuperscript{[90-91]}

Denervation models, utilized to study wound healing in the context of neuropathy, involve surgically exposing specific vertebrae in mice and porcine models and transecting nerve endings. After recovery, wounds are created by excision, and the success of nerve resection is verified by testing recovery, wounds are created by excision, and the porcine models and transecting nerve endings. After surgically exposing specific vertebrae in mice and healing in the context of neuropathy, involve denervation models, utilized to study wound conditions.

<table>
<thead>
<tr>
<th>Category</th>
<th>Model Type</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>In Vitro</td>
<td>2D Wound Scratch Assay</td>
<td>Easy to implement, low-cost, good for high-throughput studies, allows for assessment of cell migration</td>
<td>Lacks complexity of real wounds (no cell-cell interaction, immune response, etc.)</td>
<td>[95-97]</td>
</tr>
<tr>
<td>In Vitro</td>
<td>Transwell Migration Assay (Boyden Chamber Assay)</td>
<td>Useful for studying cell motility (chemotaxis and chemokinesis), assesses single-cell movement.</td>
<td>Limited physiological relevance, doesn’t capture the full picture of wound healing.</td>
<td>[98-100]</td>
</tr>
<tr>
<td>In Vitro</td>
<td>3D Organotypic Cultures Mimicking Wound Healing Disorders</td>
<td>Allows better cell behaviour study, allows for incorporation of primary human cells for disease modelling, good control over experimental conditions</td>
<td>Lacks some complex cellular interactions, media choice is crucial for proper cell behaviour.</td>
<td>[101-103]</td>
</tr>
<tr>
<td>In Vitro</td>
<td>Organ-on-a-Chip Model</td>
<td>Organ-on-a-chip models accurately mimic human organ</td>
<td>Require specialized expertise in microfluidics.</td>
<td>[104-106]</td>
</tr>
</tbody>
</table>

| Ex Vivo | Human Ex Vivo Wound Model | Uses human skin, maintains some resident immune cells, good for studying epithelialization, allows for testing topical treatments. | Lacks blood supply and immune cell infiltration, may have variability due to skin source, basement membrane integrity may not fully reflect in vivo conditions | [107-108] |
| Ex Vivo | Marine Skin Explant Model | Useful for studying collective cell migration, allows for testing effects on genetically modified skin. | Limited to studying keratinocyte migration, doesn't represent the full complexity of wound healing in humans | [109-110] |
| Ex Vivo | Pressure Ulcer Ex Vivo Model | Can mimic chronic wounds, allows for studying pressure-induced changes, can be used with human or porcine skin. | Limited to studying a specific type of wound, may not fully capture all aspects of pressure ulcers. | [111-112] |
| Ex Vivo | Porcine Ex Vivo Wound Infection Model | Easy to obtain skin, good representation of human skin physiology, allows for studying wound infections and antimicrobial therapies. | Lacks full human immune response, lacks commensal microbiome due to sterilization. | [113] |
| In Vivo | Rodent Wound Healing Models e.g., Incisional, Excisional | Relatively inexpensive, small size allows for using less reagent, rapid healing allows for shorter experiments, genetic homogeneity facilitates reproducibility, allows for studying wound healing under different conditions (diabetes, infection, etc.). | Differences in skin anatomy and physiology compared to humans, hair follicle cycle can influence epithelialization, not ideal for studying epithelialization itself | [114-116] |
| In Vivo | Diabetic Wound Healing Models | Allows for studying wound healing in the context of diabetes, can be used to introduce bacterial biofilms for mimicking | Difficulty in accurately replicating the multifaceted nature of human diabetic wound healing in animal models. | [117-118] |

In Vitro 3D Wound Scratch Assay

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Advantages of preclinical wound models

- Results are obtained quickly.
- Variability in wound characteristics, age, and nutrition is relatively low.
- There are no compliance issues with animals.
- Wounds tend to be more homogeneous in animals compared to humans.
- The splint model closely resembles human wound healing, facilitating re-epithelialization and new tissue growth.
- It offers flexibility, being applicable for studying wound healing in both normal and diseased conditions such as diabetes.
- Each mouse serves as its own control in this model, cutting down on the required number of animals.
- The surgical procedure is simple, making it accessible even to researchers with limited surgical expertise.
- Preclinical models are essential for testing safety, toxicity, and efficacy of new wound-healing therapies.
- Incorporating patient-derived cells and tissue engineering advances can enhance preclinical models.
- Technologies like iPSCs and microengineering offer opportunities for more accurate preclinical testing.
- Better models can reduce time and costs associated with drug development. \[123\]

**Limitations**

- Differences in healing mechanisms between animal models and humans.
- Anatomical and physiological disparities between animal models and human skin.
- Limited clinical relevance of findings from animal studies to human treatments.
- Ethical considerations surrounding the use of animals in research.
- Challenges in accurately modeling complex chronic wounds in preclinical settings.
- Difficulty in predicting human responses due to genetic variability and other factors.
- Technical limitations such as variability in experimental procedures and standardization challenges. \[124\]

**CONCLUSION**

In conclusion, wound healing research relies heavily on animal models due to ethical and practical limitations in human studies. Rodents, pigs, and other animals have provided invaluable insights into wound management, from basic principles to complex chronic wounds. These models, while not perfect, offer significant advantages, including quick results, low variability, and relevance to both normal and diseased conditions. The diverse array of in vitro, ex vivo, and in vivo models offers researchers a range of options for studying various aspects of wound healing. However, differences in healing mechanisms, anatomy, and physiology between animals and humans remain significant limitations. Future improvements in modeling, such as incorporating patient-derived cells and advanced technologies, hold promise for enhancing the relevance and accuracy of preclinical wound healing studies. The translational relevance of findings from animal studies to human treatments remains a challenge, often due to differences in healing mechanisms and the complexity of chronic wounds. Ethical considerations and the need for better translation to human treatments underscore the importance of continually refining and validating these models for more effective wound care strategies.
Conflicts of Interest: The authors declare that there are no conflicts of interest.

Acknowledgement: NA.

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