Laboratory of WOUND MEDICINE

# Journal of Wound Medicine

**Volume 1 | Issue 2 | March–April 2026 | Pages 18-50**

## A Critical Review of Preclinical Models in Chronic Wound Healing Research: Challenges and Future Directions

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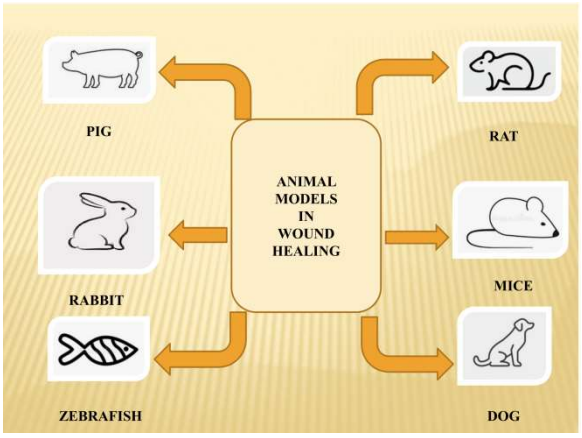
### Abstract

Chronic, non-healing wounds cause major morbidity, risk of amputation and death and high healthcare costs, but therapeutic development is hindered by repeated translational failures from preclinical to clinical translation. Based on the classic four-phase approach to wound healing (Hemostasis, inflammation, proliferation and regeneration, and maturation and remodeling), this paper critically synthesizes and evaluates the epistemic assumptions that underlie the selection of animal models for the study of chronic wound healing, with a particular emphasis on the question of clinical translatability and plausibility of human models. The analysis uses narrative comparative analyses of in vivo animal models (rodents, pigs, rabbits, guinea pigs, zebra fish), human wound models (skin grafts, skin, cutaneous, cuticle, excision model), and platform in vitro and ex vivo (in vitro, 3D skin and histocultures, computational simulation). Rodents, despite their practicality and genetic tractability (including chemically induced and db-db diabetic models), closure endpoints compared to human re-epithelialization; the rat tail model is noted for its reduction in contraction over prolonged periods. Pigs provide the closest overall architectural (anatomical) similarities in skin (thickness, sparse follicles, structures of the rete ridge), but are still limited by cost, accommodation, infection risk, differences in sweat glands, and the persistence of wounds of complete thickness. The rabbit ear model is unique in that it suppresses contraction by splinting the cartilage, allowing for a more human-like re-epithelialization and reproducible hypertrophic scarring and ischaemic site-specificity with respect to the agent and genetic factors. In disease models, especially in diabetes, short induction-to-wound intervals do not recapitulate long-term comorbidities (neuropathy, vascular disease) and in vitro 2D and 3D systems lack immunological, neural and vascular dynamics and are not well predictive in vivo. The bottom line is that no single model is enough; controlled human models, which are robust, well validated, and, despite ethical and practical limitations, remain the ultimate reference for moving from simple screening to more complex, disease-relevant systems, with the support of the emerging vascularized 3D constructs, microfluidics, and 3D bioprinting.

### Keywords

Chronic models ;Wound Healing ;Animal models; Rodents ;Rabbits; Pig ;Genetic Mouse ; Impaired wound healing ;Translation

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Received: February 10, 2026
Revised: March 5, 2026
Accepted: March 15, 2026
Published: March 16, 2026

doi:10.5281/zenodo.19037280

# 1 Introduction

## 1.1 The Foundational Theory of Wound Healing

The authors begin by summarizing the accepted physiological theory of wound healing, which serves as the foundation for their analysis but is not their own (Flynn et al. 2023). According to this theory, wound healing is a complicated process that consists of four separate but related phases (Davidson 1998; Dorsett-Martin 2004; Sharma 2013)

### *1.1.1 Hemostasis*

This phase starts right after an injury(Davidson 1998; Flynn et al. 2023). Vessel constriction, platelet activation to create a temporary plug, and the clotting cascade to create a stable fibrin plug are all involved. Growth factors like VEGF and PDGF are released to start the repair process (Davidson 1998; Fang and Mustoe 2008; Flynn et al. 2023; Grada, Mervis, and Falanga 2018; Sharma 2013).

### *1.1.2 Inflammation*

Cytokines and growth factors (Masson-Meyers et al. 2020; Sharma 2013). draw inflammatory cells like neutrophils and monocytes (Masson-Meyers et al. 2020; Sharma 2013). (which develop into macrophages) to the wound site to remove bacteria and dead cells (Masson-Meyers et al. 2020; Sharma 2013). The authors point out that this phase is frequently prolonged, resulting in tissue damage (Masson-Meyers et al. 2020; Sharma 2013) .

### *1.1.3 Proliferation/Regeneration*

Fibroblasts take over as the predominant cell type (Flynn et al. 2023).

### *1.1.4 Maturation and Remodeling*

This last stage can take up to two years (Flynn et al. 2023). Angiogenesis, or the creation of new blood vessels, takes place, and myofibroblasts shrink the edges of the wound (Flynn et al. 2023; Grada et al. 2018; Masson-Meyers et al. 2020). It increases the tensile strength of the new tissue by substituting stronger type 1 collagen for type 3 collagen (Flynn et al. 2023).

## 1.2 Prevailing claims

### *1.2.1 Rodent models are poor predictors of human wound closure*

The authors argue that rodents, although widely used for cost and convenience reasons, are fundamentally flawed in the study of wound healing (Flynn et al. 2023). This is because the wound is healed primarily by contraction, a mechanism controlled by the ***"panniculus carnosus "***muscle (Dorsett-Martin 2004). Humans, on the other hand, heal mainly by re-epithelialization (Dorsett-Martin 2004). They argue that this difference can obscure data and lead to findings that are not easily `translatable to humans`(Flynn et al. 2023).

### *1.2.2 Pigs are the superior animal model for anatomical and physiological similarity to humans*

Pigs are the best animal model currently available for studies trying to replicate the composition and healing mechanisms of human skin, according to the authors (Flynn et al. 2023; Sullivan et al. 2001). Their similar dermal and epidermal thickness, sparse distribution of hair follicles, and comparable cellular and growth factor responses all support this superiority (Flynn et al. 2023; Sullivan et al. 2001). Additionally, they draw attention to the fact that pigs' full-thickness wounds still heals with contraction to heal than human wounds, indicating that even pigs are not perfect(Flynn et al. 2023; Sullivan et al. 2001).

### *1.2.3 The rabbit ear model is uniquely advantageous for studying re-epithelialization and hypertrophic scarring*

One of the main arguments is that the rabbit ear has a unique property, whereby the underlying cartilage, which acts as a splint, prevents the wound from contracting in a natural way(Grada et al. 2018; Greenhalgh 2005; Sami, Heiba, and Abdellatif 2019). Consequently, the wound is forced to heal by re-epithelialization, which is a more realistic model for the healing process in human wounds (Flynn et al. 2023; Sisco and Mustoe 2003). They also argue that it is a reproducible model for the examination of hypertrophic scar formation, which is not possible in most other models of animal scar formation (Fang and Mustoe 2008; Flynn et al. 2023; Sisco and Mustoe 2003).

### *1.2.4 Most models of chronic disease are inadequate*

According to the authors, many attempts to model chronic conditions such as diabetes have failed to capture the true pathophysiology of these diseases

(Flynn et al. 2023). They criticize, in particular, diabetic rodent models where injuries (wound) is induced soon after the onset of disease (Flynn et al. 2023). They argue that the long-term systemic effects cannot be reproduced in this short time frame (e.g. neuropathy, vascular disease) which complicates chronic wounds in human patients, making the model inappropriate (Flynn et al. 2023).

#### *1.2.5 In vitro models are inherently limited and contribute to translational failure*

While acknowledging their utility for high-throughput screening, the authors claim that 2D in vitro models are a source of translational failure because they are overly simplistic (Flynn et al. 2023). They lack the physiological complexity of living tissue, including the extracellular matrix, immune responses, and crosstalk between different cell types(Flynn et al. 2023). Therefore, results from these models often fail to be replicated in more complex in vivo systems (Flynn et al. 2023).

#### *1.2.6 A 'progression of models' is a necessary strategy to improve success.*

The authors suggest that researchers should adopt a different approach in order to overcome the difficulties of translation (Flynn et al. 2023). To test this mechanism, they should start with simple models (in vitro) and gradually move to more complex models that are physiologically relevant (e.g. in vivo ) (Flynn et al. 2023). Models with a wide range of parameters (e.g. pigs) that mimic a structure similar to human skin (Flynn et al. 2023; Sullivan et al. 2001) The author asks whether such a strategy is necessary before moving to human trials, in order to increase the chances of success (Flynn et al. 2023).

### 1.3 Epistemic Assumptions

#### *1.3.1 The Ultimate Goal of Wound Healing Research is Clinical Translation to Humans*

Many researchers operate under the fundamental premise that the primary objective of the research using these wound healing models is the establishment of an effective treatment for chronic wounds in humans (Flynn et al. 2023). This is evident from their criticism of models that lack adequate translatability and their continued focus on animal-to-man translatability (Flynn et al. 2023). Researchers assume that research is translatable rather than questioning whether it should be, and they assess models based on this assumption (Flynn et al. 2023).

#### *1.3.2 A Model's Value is Proportional to its Physiological and Pathophysiological Similarity to Humans.*

Many authors' first assumption leads them to believe that the better model is one that is more similar to the skin biology of humans (Flynn et al. 2023). They criticize rodents for their disparate healing processes, while they praise pigs for their similarities in the anatomy and physiology (Flynn et al. 2023; Sullivan et al. 2001). This implies that, despite pragmatic considerations such as cost and ease of use, the most important metric to assess the value of a model is biological fidelity(Davidson 1998; Dorsett-Martin 2004; Flynn et al. 2023; Grada et al. 2018; Greenhalgh 2005; Sami et al. 2019; Sharma 2013)

#### *1.3.3 The Research Community is Currently Using Suboptimal Models.*

According to the authors, a significant portion of the current research on wound healing is based on models that were chosen more for practical purposes (like rodents) than for their scientific validity(Flynn et al. 2023). The general consensus is that translational failure is caused by this common problem (Flynn et al. 2023). The entire paper is structured as a counteractive, assuming that the field needs guidance on how to choose wound models more wisely (Flynn et al. 2023). The author demands that researchers justify the models they have selected, assuming that this is not being done sufficiently at present (Flynn et al. 2023).

#### *1.3.4 Chronic Wounds in Humans are Fundamentally Different from Acute Wounds in Healthy Animals.*

The premise of the authors' argument is that merely inflicting a wound on a healthy animal is insufficient to simulate a chronic wound in humans (Flynn et al. 2023). They believe that the co-morbidities—such as diabetes, poor circulation, and a dysregulated inflammatory environment—are essential to the pathophysiology of wounds that do not heal (Flynn et al. 2023). For this reason, they criticize disease models that prevent the full manifestation of these chronic conditions (Flynn et al. 2023).

#### *1.3.5 A Stepwise, Multi-Model Approach is Superior to Relying on a Single Model.*

According to the authors, a more sensible and effective research strategy is a “progression of models”, which moves from “simple to complex” (Flynn et al. 2023). This assumes that results can and should be validated across different levels of

biological complexity before proceeding to human trials (Flynn et al. 2023). It also assumes that this approach will reduce the rate of clinical failure, but this is not a proven fact; rather, it is stated as a logical proposition (Flynn et al. 2023).

In conclusion, the fundamental premise of the authors is that wound healing research is experiencing a crisis of translation, which is caused by a mismatch between the experimental models employed and the human conditions they are intended to represent (Flynn et al. 2023). Based on the premise that more physiologically relevant models will produce better clinical results, their entire review aims to identify this issue and provide a solid solution (Flynn et al. 2023).

## 2 Epistemological limitation of Existing Frameworks

### 2.1 The Problem of Flawed Translatability

The main problem is that current models are not sufficiently human-translatable (Flynn et al. 2023). The author does not merely state this as fact; he explains why this is a problem.

#### *2.1.1 Misleading Results from Common Models*

Paper poses a problem due to the heavy reliance on rodent models (Davidson 1998; Flynn et al. 2023; Grada et al. 2018; Greenhalgh 2005; Masson-Meyers et al. 2020; Sami et al. 2019). Although these models are logistically appropriate (low cost, easy to use), their basic healing mechanism ("panniculus carnosus "causes contraction of the wound) differs from that of human healing (re-epithelialization) (Flynn et al. 2023; Grada et al. 2018; Greenhalgh 2005; Masson-Meyers et al. 2020; Sami et al. 2019). This poses a critical problem therapies that appear to be effective in rodents may act on a biological process that is not relevant to humans, and translational failures are inevitable (Flynn et al. 2023). The authors state that this may "obscure the data collected on wound healing and render some data poorly translatable to humans" (Flynn et al. 2023).

#### *2.1.2 Failure to Recapitulate Chronic Conditions*

The authors point out a serious problem with animal disease modeling that leads to chronic wounds (Flynn et al. 2023). The authors argue that most models fail to accurately depict the complexity of chronic wounds in humans that are impacted by co-morbid conditions like diabetes, instead simulating acute wounds in healthy animals (Flynn et al. 2023). The long-term systemic damage seen in human diabetic patients, for example, is difficult to replicate in diabetic rodent models where the wound is made too soon after the disease is induced (Flynn et al. 2023). This suggests that the model is not testing the treatment in a relevant pathological context (Flynn et al. 2023).

### 2.2 The Problem of a 'One-Size-Fits-All' Mentality

The notion that a single model can serve as a gold standard is challenged by the authors (Flynn et al. 2023). They show that the lack of careful consideration when selecting a model is a major problem in the field (Flynn et al. 2023).

#### *2.2.1 No Single Superior Model*

There is no single model of wound healing which is superior and which can provide a translatable result for human research, the author says in a clear tone (Flynn et al. 2023). The problem, author argues, is that researchers may not match the unique benefits of the model to the research question (Flynn et al. 2023). For example, re-epithelialization is not appropriate to study in rodents, when a rabbit ear model would be more appropriate (Flynn et al. 2023; Sisco and Mustoe 2003).

### 2.3 The Consequences of the Problem

To show that this is a major issue, the authors highlight the consequences for the real world

#### *2.3.1 Clinical Failure and Healthcare Burden*

Chronic, non-healing wounds can lead to a poor quality of life and even death for patients, as well as excessive costs within the health system (Flynn et al. 2023). The author stresses the importance and urgency of their criticism by linking the flaws in the research models to the ongoing clinical problem (Flynn et al. 2023)

#### *2.3.2 Stagnation in Therapeutic Development*

The paper suggests that translational failures due to poor models are the main reason why, despite interventions, many wounds remain untreated and can lead to complications leading to amputation (Flynn et al. 2023).

### 2.4 Proposed Solution as Part of the Problematization

Last but not least, the authors' suggested solutions are a component of their problematization since they

draw attention to the gaps in the field of study (Flynn et al. 2023).

#### *2.4.1 Lack of Rigorous Justification*

The author asks that the manuscript should specify the reasons for the "choice of the animal model, the similarities and differences with the human condition", which suggests that this is not a standard practice (Flynn et al. 2023).

#### *2.4.2 Need for a New Research Strategy*

The authors propose a step-by-step "progression of models" from "simple to complex", challenging the current approach of relying on a single, often inadequate, model before moving on to human testing (Flynn et al. 2023).

In conclusion, the authors problematize the field by portraying the selection of experimental models as a crucial flaw that jeopardizes the entire research-to-clinic pipeline rather than as a straightforward methodological step (Flynn et al. 2023). Their work is a call to action for more deliberate and physiologically relevant research methods as well as a critique of the current state of research practices (Flynn et al. 2023)

### 2.5 The Primary Research Gap Lack of Clinically Translatable Models

The lack of a single, perfect model that can accurately and consistently replicate the intricate physiology of human wound healing, especially for chronic wounds, is the main gap that the authors have identified (Flynn et al. 2023). This primary gap leads to several specific areas where research is deficient or flawed (Flynn et al. 2023).

#### *2.5.1 Gap in Modeling Chronic Diseases*

The long-term consequences of chronic diseases that result in non-healing wounds are not well simulated (Flynn et al. 2023). The authors note that the majority of animal models, particularly for diabetes, only portray the acute stage of the illness (Flynn et al. 2023). Since wounds are frequently created soon after hyperglycemia begins, they are unable to account for the decades of systemic damage (such as neuropathy and vascular damage) that define chronic wounds in humans (Flynn et al. 2023).The authors state that there has been little success in attempts to "replicate the human co-morbidities that lead to chronic non-healing wounds" (Flynn et al. 2023)

#### *2.5.2 Gap in Understanding and Replicating Human Healing Mechanisms*

There are significant differences between the primary healing mechanism in humans and the most widely used animal models, rodents (Conn 2008; Davidson 1998; Dorsett-Martin 2004; Grada et al. 2018; Greenhalgh 2005; Masson-Meyers et al. 2020; Sami et al. 2019; Sharma 2013). In contrast to rodents, humans heal wounds through re-epithelialization (Flynn et al. 2023; Masson-Meyers et al. 2020). Treatments that promote contraction-based wound closure in mice may not be effective for human wounds that require re-epithelialization, which results in a significant gap in translatability (Flynn et al. 2023). Most animal models do not result in hypertrophic scars, which restrict our ability to study and develop treatments for this specific pathological outcome in people (Flynn et al. 2023).

#### *2.5.3 Gaps in In Vitro (Laboratory) Model Complexity*

There is a significant complexity gap in existing in vitro models, especially 2D cultures (Flynn et al. 2023). They are unable to replicate the environment in which the injury occurred, such as
Extracellular matrix (ECM) remodeling
The immune system, blood supply, and innervation all have an impact on the physiological significance of intricate signaling and "crosstalk" between different cell types (such as "fibroblasts", " keratinocytes", and "immune cells") (Flynn et al. 2023). However, vital elements like vascular networks are often absent from sophisticated 3D models and skin equivalents (Flynn et al. 2023). Their physiological relevance is compromised by their inability to accurately depict blood circulation and nutrient delivery due to the lack of vascular networks (Flynn et al. 2023).

#### *2.5.4 The Concluding Research Gap Bridging Basic Science and Clinical Practice*

Lastly, the authors identify a systemic gap in the research process itself. They argue that a lack of "close collaboration and communication between basic researchers and the clinicians who treat human wounds" prevents the development of better models (Flynn et al. 2023). This disconnects between the lab and the clinic continues to use models that are convenient but not clinically relevant, leaving a significant gap in the translation of research findings into effective human therapies (Flynn et al. 2023). From the document, the authors' novelty is not derived from new experimental data or a new biological theory. Instead, they offer a critical synthesis and a prescriptive framework for future wound healing research (Flynn et al. 2023). The

author identifies important research gaps and proposes a clear and structured approach to address them (Flynn et al. 2023).

### 2.6 Identified Research Gaps

The analysis of the authors reveals a number of critical gaps in current wound healing research

#### *2.6.1 The Clinical Relevance Gap*

A valid gap is the lack of models for reliably predicting human outcomes (Flynn et al. 2023). The authors state that no ideal model is currently available that would effectively reproduce "human physiological or pathological conditions" (Flynn et al. 2023).

#### *2.6.2 The Chronicity Gap*

Current models are unable to accurately depict chronic, non-healing wounds (Flynn et al. 2023). "The majority of animal models can only simulate acute wounds and the attempts to recapitulate the human co-morbidities that lead to chronic non-healing wounds have had limited success," the authors criticize (Flynn et al. 2023). They specifically note that diabetic models are unsuitable for studying complex, chronic wounds because they frequently fail to account for the long-term effects of the disease (Flynn et al. 2023).

#### *2.6.3 The Mechanistic Gap*

Rodents, the most popular animal models, heal in a different way than people (contraction vs. re-epithelialization), resulting in a discrepancy in the translation of wound closure outcomes (Flynn et al. 2023).

#### *2.6.4 The Complexity Gap in In Vitro Models*

Crucial components like a dynamic immune response, blood vessel networks, and intricate cell-to-cell crosstalk are absent from "*in vitro*" models, which range from 2D cultures to even 3D equivalents (Flynn et al. 2023).

## 3 Central Question

How can researchers choose the best and most efficient experimental models (ex vivo, in vitro, in vivo, etc.)? to guarantee that their research on the healing of chronic wounds is appropriately translatable to human physiology and pathophysiology?
This overarching question is not mentioned in a single sentence but is clearly the guiding principle of the whole analysis of the research paper. The authors do not ask what causes wounds, but rather how to study them properly. They examine this central issue by dividing it into several sub-questions

### 3.1 What are the major physiological and anatomical differences between the human and animal models of wound healing (rodents, pigs, rabbits) and what are the implications for the translatability of the research results?

To address this, the authors contrast methodically the structure of the skin and the processes of healing, in particular "wound contraction" and re-epithelialization. The author points out that the rodent is a poor model for the closure of human wounds due to its "panniculus carnosus", which is a key distinguishing feature.

### 3.2 Which models are most suitable to study specific aspects of wound healing or specific diseases?

By weighing the advantages and disadvantages of each model for the particular parameters that need to be evaluated, the authors' review attempts to address this question. For instance, they find that pigs are superior in terms of overall anatomical similarity, rabbits are superior in studies involving re-epithelialization, and rodents are more economical in the initial research studies.

### 3.3 How well do current experimental models replicate complex human chronic wound conditions such as diabetes or ischaemia?

This issue is at the heart of their criticism of current disease models. The author examines whether common procedures, such as short-term induction of diabetes in rodents, really mimic the long-term, systemic nature of human disease, which leads to non-healing wounds.

### 3.4 What are the limitations of non-animal models (in vitro, in silico) and how could new technologies be developed to overcome these?

The paper addresses this by challenging the simplicity of 2D cell culture, which lacks the complexity of living tissue. Author will then look at how advanced technologies such as 3D bioprinting and skin-on-skin chips could provide more dynamic and physiologically relevant platforms for future research.
The whole paper is essentially a comprehensive response to the central question of how to bridge the gap between preclinical research and successful

human therapies by making more informed and critical decisions about experimental models.

## 4 Objectives

### 4.1 To Improve Translatability to Humans

The main issue is the difficulty of translating the findings from animal models into successful treatments for humans. The paper critically examines why some models (e.g. rodents) often produce data that are not easily translatable to humans, because their wound healing mechanisms, such as "wound contraction", are significantly different from human re-epithelialization.

### 4.2 To Address a Significant Healthcare Problem

A significant burden on the healthcare system, chronic wounds can result in poor patient outcomes, including amputation and even death. The study emphasizes how important it is to find better treatments, which calls for more effective and relevant wound healing models.

### 4.3 To Provide a Comprehensive Overview of Available Models

The document covers a broad range of models and explains their specific applications

#### *4.3.1 In vivo animal models*

In vivo models (e.g. rodents, pigs, and rabbits) and how their distinct skin structure makes them appropriate for the study of different aspects of the wound healing. For instance, a rabbit ear model is ideal for studies on re-epithelialization and hypertrophic scarring, and pigs are excellent at mimicking human skin.

#### *4.3.2 In vivo human models*

The most clinically relevant data are obtained from excision wounds, suction blisters, and skin stripping; however, these methods have limitations such as voluntary participant recruitment and ethical concerns.

#### *4.3.3 In vitro, ex vivo, and in silico models*

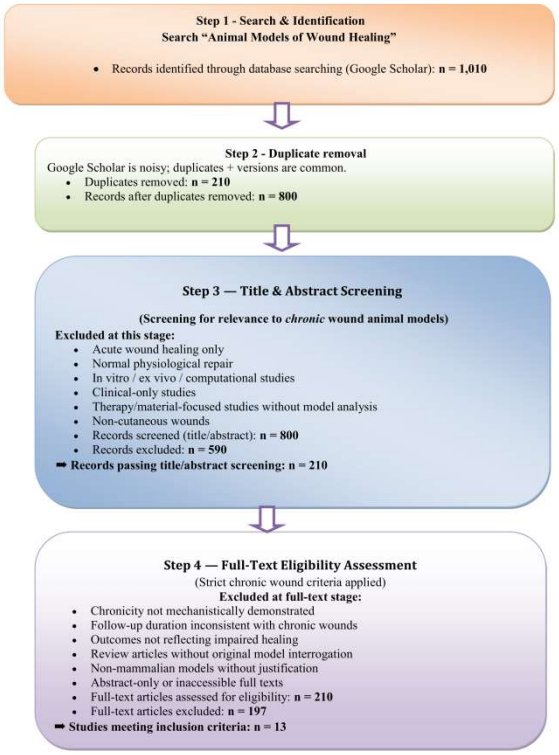
Computer simulations, 3D bioprinting, and cell cultures are helpful for high-throughput screening and studying particular cellular mechanisms, but they frequently lack physiological complexity.

### 4.4 To Advocate for Better Research Practices

The authors conclude that scientists should use a range of models, from simple to complex, to support and validate their findings, and that this should be done in a cost-effective and scientifically sound way. The author also emphasizes that scientific manuscripts should clearly justify the choice of models and explain their similarities and differences in relation to the human condition being examined.

## 5 Methodology

A Google Scholar search of literature turned up 1,010 hits. After removing duplicates and non-unique entries (n = 210), 800 articles were screened by titles and by abstract. Of these, 590 were excluded due to the focus on acute wound healing, non-surgical wounds, in-vitro studies or only in the clinical setting, or non-animal model therapeutic interventions. The full text of 210 articles was considered for eligibility, of which 197 were excluded due to insufficient validation of chronic wound status, insufficient translational utility, insufficient explanatory material on wound patterns, lack of mechanistic relevance or the accessibility of the full text. Thirteen studies met all inclusion criteria and were included in the quantitative summary of animal models for chronic wound management. (Figure 1 PRISMA FLOW DIAGRAM)



### 5.1 Reviewing and Analyzing Existing Models

They provide a detailed overview of a extensive range of models, including
***In vivo animal models*** (rodents, pigs, rabbits, etc.) (Flynn et al. 2023)
***In vivo human models*** (suction blisters, excision wounds, etc.) (Flynn et al. 2023)
***In vitro, ex vivo, and in silico models*** (cell cultures, 3D bioprinting, computational models, etc.) (Flynn et al. 2023)
The advantages, disadvantages and specific applications are systematically discussed for each model.

### 5.2 Emphasizing Translatability to Humans

The main focus of the analysis is the question of "translatability to humans" (Flynn et al. 2023). The authors point out that many promising results from preclinical studies have failed in human trials because the animal models used are not fully replicable for human physiology (Flynn et al. 2023). Data on rodents may be `poorly translatable to humans' because of the difference between healing mechanism in humans (re-epithelialization) and healing in rodents (contraction) (Flynn et al. 2023).

### 5.3 Arguing Against a 'One-Size-Fits-All' Approach

The central claim of the paper is that no model is ever better than another (Flynn et al. 2023). The authors argue that different models are useful for investigating specific stages or processes of wound healing (Flynn et al. 2023). For example, a rabbit ear model is particularly useful for the examination of hypertrophic scarring and re-epithelialization, while a pig model is excellent because of its anatomical similarity to human skin (Flynn et al. 2023; Sisco and Mustoe 2003; Sullivan et al. 2001).

### 5.4 Advocating for Improved Research Practices

In their conclusion, the authors recommend a more cautious, rigorous and in-depth selection of models. The author recommends that researchers should do the following.
In their publications clearly state the reasons for the choice of a particular model (Conn 2008; Dorsett-Martin 2004; Flynn et al. 2023; Grada et al. 2018; Greenhalgh 2005; Masson-Meyers et al. 2020; Sami et al. 2019).
Describe the similarities and differences between their model and the studied human condition (Davidson 1998; Dorsett-Martin 2004; Fang and Mustoe 2008; Grada et al. 2018; Greenhalgh 2005; Lindblad 2008; Masson-Meyers et al. 2020; Sami et al. 2019; Sisco and Mustoe 2003).
Consider using the "progression of models from simple to complex" to validate the findings before moving on to human testing (Flynn et al. 2023; Grada et al. 2018; Greenhalgh 2005; Masson-Meyers et al. 2020; Sami et al. 2019).
In short, the authors aim to provide a guide for the scientific community to critically evaluate tools of their trade (wound healing paradigms) (Flynn et al. 2023). By highlighting the strengths and weaknesses of each approach, they aim to improve the quality and clinical relevance of future (Flynn et al. 2023).

### 5.5 Analytical Framework to Evaluate Utility and Limitation

The authors' examination of models of wound healing is structured around a number of themes which have been synthesized (Flynn et al. 2023; Masson-Meyers et al. 2020). These topics provide an analytical framework for assessing the advantages and disadvantages of each model (Flynn et al. 2023).

### 5.6 Translational Relevance to Humans

This is the dominant theme and the main focus of the analysis (Flynn et al. 2023). The authors consistently assess each model on the basis of its ability to produce results that are meaningful and applicable to clinical situations in humans (Flynn et al. 2023). They explicitly state that they focus on the "translatability to humans" (Flynn et al. 2023) The models are either praised or criticized for their ability to mimic human wound healing (Flynn et al. 2023). For example, rodents are criticized for their inability to be translatable to humans because of their healing mechanisms, while pigs are praised for their anatomical and physiological similarities to humans (Flynn et al. 2023).

### 5.7 Physiological and Anatomical Fidelity

Directly related to translational relevance, this theme involves a detailed comparison of biological structures and processes in the models and humans (Flynn et al. 2023). Authors consistently analyze

#### *5.7.1 Skin Architecture*

Author does comparison of the density and type of skin appendages, presence of structures such as rete ridges, and thickness of the dermis and epidermis (e.g. sweat glands, hair follicles) (Flynn et al. 2023; Sullivan et al. 2001).

#### *5.7.2 Healing Mechanism*

The main point of the analysis is the difference between healing by contraction (characteristic in rodents) and healing by epithelialization (characteristic in humans) (Conn 2008; Davidson 1998; Dorsett-Martin 2004; Flynn et al. 2023; Grada et al. 2018; Greenhalgh 2005; Masson-Meyers et al. 2020; Sami et al. 2019). The main piece of evidence used in this analysis is the presence or absence of muscle of the "panniculus carnosus "(Dorsett-Martin 2004; Flynn et al. 2023).

### 5.8 The Challenge of Modeling Chronic Disease

The authors frame the theme around the critical difference between acute injuries in healthy animals and chronic wounds in humans with a co-morbid disease (Flynn et al. 2023). Analysis shows that most models fail to capture this complexity (Flynn et al. 2023). Author analyzes specific disease models, such as diabetes, and criticizes them for not replicating sufficiently the long-term systemic effects such as neuropathy and impaired circulation that are the key determinants of chronic human wounds (Flynn et al. 2023)

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### 5.9 "Fit for Purpose" Model Selection

Instead of searching for one perfect model, the authors suggest that researchers should analyze models and select models according to their suitability to address specific research questions (Flynn et al. 2023). This topic advocates a nuanced approach to the selection of models (Flynn et al. 2023)

#### *5.9.1 Pigs*

For general studies requiring a high degree of anatomical similarity, pigs are considered the best fit (Flynn et al. 2023; Sullivan et al. 2001).

#### *5.9.2 The rabbit ear model*

For studying hypertrophic scarring or re-epithelialization without the confounding factor of contraction, the rabbit ear model is found to be the most appropriate (Fang and Mustoe 2008; Flynn et al. 2023; Sisco and Mustoe 2003).

#### *5.9.3 In vitro models*

Despite their physiological limitations, in vitro models are considered suitable for high-throughput screening of compounds (Flynn et al. 2023).

### 5.10 The Progression from Simplicity to Complexity

This is reflected in the authors' analysis of the gaps in current research and how to overcome them (Flynn et al. 2023). The author examines how simple models (such as 2D cell cultures) fail to predict the success of complex life systems (Flynn et al. 2023). This leads to the conclusion that a more consistent and successful research strategy is to follow a series of models, ranging from simple to complex (Flynn et al. 2023). The analysis suggests that the more rational route for clinical translation is to test the mechanism in a simple system before moving to a complex animal model that is relevant to the disease (Flynn et al. 2023).

### 5.11 Technological Advancement and Overcoming Limitations

The potential of new technologies to address the shortcomings of older models is a point of interest in the analysis (Flynn et al. 2023). The authors analyzes the limitations of traditional in vitro models (e.g. blood flow deficit, lack of immune components) and then analyze how emerging technologies such as skin on chip and 3D bioprinting can overcome these specific constraints (Flynn et al. 2023).

## 6 Limitation

### 6.1 Limitations of *In Vivo* (Animal) Models

Animal models have been criticized for their fundamental biological and practical differences to man (Flynn et al. 2023)

#### *6.1.1 Different Healing Mechanisms*

Their primary mechanism of healing is most often referred to as restriction, particularly in rodents (Flynn et al. 2023). Humans heal mainly by re-epithelialization, but rodents' wounds heal mainly by contraction of the "panniculus carnosus "muscle to the wound (Davidson 1998; Dorsett-Martin 2004; Fang and Mustoe 2008; Flynn et al. 2023; Grada et al. 2018; Greenhalgh 2005; Masson-Meyers et al. 2020; Sami et al. 2019; Sharma 2013; Sullivan et al. 2001). Due to this difference, wound closure data is poorly translatable (Flynn et al. 2023)

#### *6.1.2 Inability to Model Chronic Conditions*

An important disadvantage is the inability of animal models to mimic the long-term systemic effects of chronic diseases resulting in non-healing wounds in humans (Flynn et al. 2023). For example, rodent

diabetic models are often short-term, which prevents neuropathy or advanced vascular complications, which are important components of human diabetic ulcers (Flynn et al. 2023). Attempts to "recapitulate human co-morbidities leading to chronic, non-healing wounds, have had limited success", the authors say (Flynn et al. 2023).

#### *6.1.3 Failure to Form Human-Like Scars*

The majority of animal models, such as pigs and rodents, do not develop hypertrophic scars, which restricts their application in studying this particular pathological consequence in humans (Flynn et al. 2023; Sullivan et al. 2001).

#### *6.1.4 Species-Specific Differences*

Even better models, like pigs, have limits (Flynn et al. 2023; Sullivan et al. 2001). Although their skin anatomy is similar to that of humans, they have mainly apocrine sweat glands instead of the large number of eccrine glands found in humans, which are a vital source of epidermal stem cells for the treatment of wounds (Flynn et al. 2023; Sullivan et al. 2001).

#### *6.1.5 Practical Limitations*

The use of more ideal models, such as pigs, is frequently restricted due to their "high cost", "maintenance requirements", and need for large, specialized housing facilities (Flynn et al. 2023; Grada et al. 2018; Greenhalgh 2005; Masson-Meyers et al. 2020; Sami et al. 2019; Sullivan et al. 2001). The lack of genetic traceability and species-specific reagents is a limitation for rabbits (Flynn et al. 2023; Sisco and Mustoe 2003).

## 7 Limitations of *In Vitro* (Laboratory) Models

In vitro models are useful for the study of specific cellular mechanisms, but have serious limitations in terms of physiological complexity(Flynn et al. 2023)

### 7.1 Oversimplification

Scratch test and other 2D models are criticised for not accurately portraying natural skin structure (Flynn et al. 2023). They are not capable of replicating complex pathways and signaling between different cell types or ECM remodeling, which often leads to in vivo failures (Flynn et al. 2023).

### 7.2 Lack of Systemic Components

In 2D, 3D, and even ex vivo models, the lack of essential biological systems is a fundamental limitation (Flynn et al. 2023). They are intrinsically devoid of migratory immune cells, innervation, and a dynamic blood supply—all of which are essential to the actual wound healing process (Flynn et al. 2023).

### 7.3 Technical Challenges

The popular scratch assay has reproducibility issues and frequently produces erratic scratches and cell accumulation that skew the results (Flynn et al. 2023). More complex 3D models are frequently difficult to produce and call for advanced technical abilities (Flynn et al. 2023).

## 8 Limitations of Human and *Ex Vivo* Models

### 8.1 Human Models

Although models using human volunteers are clinically relevant, difficulties in recruiting volunteers and ethical issues may limit participation in studies, which may lead to insufficient data for large-scale generalizations (Flynn et al. 2023).

### 8.2 *Ex Vivo* Models

Excised skin is used in ex vivo models, which have poor reproducibility and standardization (Flynn et al. 2023). The requirement for fresh tissue also limits their availability (Flynn et al. 2023).

### 8.3 Limitations of *In Silico* Models

#### *8.3.1 Lack of Physiological Reality*

The true physiological features of human skin are not present in computational models because they are theoretical in nature (Flynn et al. 2023). Usually, in vitro or in vivo experiments are required to validate their conclusions (Flynn et al. 2023).

#### *8.3.2 Collaboration Challenges*

The practical limitation is that accurate in-silico models require close cooperation between biologists and mathematicians, which may be a challenge (Flynn et al. 2023).

## 9 Translational Failure

### 9.1 Fundamental Differences in Healing Mechanisms

The most important cause of translational failure identified is the difference in how the standard

models of animals heal versus humans (Flynn et al. 2023).

#### *9.1.1 Rodent Models and Contraction*

Rodents, the most widely used species because of their low cost and ease of handling, heal mainly by contracting the wound (Davidson 1998; Dorsett-Martin 2004; Flynn et al. 2023; Grada et al. 2018; Greenhalgh 2005; Masson-Meyers et al. 2020; Sami et al. 2019; Sharma 2013). Their skin contains a layer of muscle called panniculus carnosus, which allows them to quickly pull the edges of the wound together (Davidson 1998; Dorsett-Martin 2004; Flynn et al. 2023; Grada et al. 2018; Greenhalgh 2005; Masson-Meyers et al. 2020; Sami et al. 2019; Sharma 2013).

#### *9.1.2 Human Healing and Re-epithelialization*

On the other hand, re-epithelialization, in which new skin cells (keratinocytes) migrate and multiply to cover the wound, is the main process by which humans heal (Flynn et al. 2023).

#### *9.1.3 The Translational Gap*

This difference means that treatment that accelerates wound closure in rodents may only promote contraction, which is an irrelevant mechanism for human healing (Flynn et al. 2023). The authors explicitly state that this difference may obscure data and make findings `untranslatable for humans`(Flynn et al. 2023).

### 9.2 Inability to Mimic Chronic Disease States

Models frequently fail to replicate the underlying diseases that cause chronic wounds in humans, which is another reason for translational failure (Flynn et al. 2023)

#### *9.2.1 Acute vs. Chronic Wounds*

The majority of animal models mimic acute (healthy) wounds, whereas co-morbidities such as diabetes, ischemia, and chronic inflammation complicate human chronic wounds (Flynn et al. 2023).

#### *9.2.2 Inadequate Disease Modeling*

The authors point out that wounds are often made 1-2 weeks after induction of diabetes and criticise diabetic rodent models (Flynn et al. 2023). This short time-span prevents the development of long-term effects of the disease (e.g. neuropathy and vascular damage), which makes the model insufficient to represent chronic diabetic wounds in humans (Flynn et al. 2023).

### 9.3 Lack of Physiological Complexity in Simpler Models

Although useful for high-throughput screening, in vitro and in silico models lack the complexity of a living organism, which leads to translational failure (Flynn et al. 2023).

#### *9.3.1 Missing Components*

These models lack components such as blood supply, migrating immune cells, and complex interactions (cross-talk) between different types of cells and the extracellular matrix (Flynn et al. 2023). As a result, promising results from such simple systems often fail to stand up when tested in complex in vivo environments (Flynn et al. 2023).

### 9.4 Authors' Recommendations to Avoid Translational Failure

To tackle this problem, the authors suggest a more rigorous research approach (Flynn et al. 2023)

#### *9.4.1 Choose the Right Model*

Researchers must carefully select a model that is physiologically consistent with the human condition being studied (Flynn et al. 2023). For example, the use of pigs for anatomical similarity or rabbit ears for re-epithelialization studies (Flynn et al. 2023; Sisco and Mustoe 2003; Sullivan et al. 2001).

### 9.5 Use a Progression of Models

Before beginning human trials, start with basic models to confirm a mechanism and work your way up to more intricate models that more closely resemble the disease state in humans (Flynn et al. 2023).

#### *9.5.1 Justify the Model*

The manuscript should clearly explain the reasons for the “choice of the model”, including its “similarities and differences” with human wound healing (Flynn et al. 2023).

In conclusion, the authors claim that translational failure is common in wound healing research because researchers frequently employ models that are practical but physiologically unrelated to human healing, producing false findings that are ineffective in clinical settings (Flynn et al. 2023).

## 10 Results/Synthesis

### 10.1 Methodological overview

The document provided is a review article and therefore does not provide details of any experimental methodology carried out by the authors. Instead, its core methodology is a comprehensive review and critical analysis of the different wound healing research models (Flynn et al. 2023). Authors examine, categorize and evaluate these models on the basis of their relevance and translational potential for humans (Flynn et al. 2023). The research models are examined and analyzed by the authors can be broadly classified as follows

### 10.2 *In Vivo* Models (Animal)

This methodology involves the creation of wounds in live animals in order to study the process of healing (Flynn et al. 2023). The authors examine models based on different species and disease states induced

**Species-Specific Models**

The paper analyzes the pros and cons of using different animals, such as

#### *10.2.1 Rodents (Rats and Mice)*

It is most widely used in research because of its low cost and ease of use (Conn 2008; Dorsett-Martin 2004; Flynn et al. 2023; Grada et al. 2018; Greenhalgh 2005; Masson-Meyers et al. 2020; Sami et al. 2019). The main methodological point is that they heal primarily by contraction, which is a constraint (Flynn et al. 2023) **(Reference: Table 1)**

#### *10.2.2 Pigs*

Pigs are considered to be the best model because their skin anatomy, thickness and healing process (re-epithelialization of partial thickness wounds) are very similar to that of humans (Flynn et al. 2023; Sullivan et al. 2001). **(Reference: Table 1)**

#### *10.2.3 Rabbits*

A model of the rabbit ear is specific and is used for studies of non-contractile healing (through splinting of the cartilage) and for modeling hypertrophic scars and ischaemia (Davidson 1998; Flynn et al. 2023; Sisco and Mustoe 2003) **(Reference: Table 1)**

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### 10.3 Impaired Healing Models

In order to simulate chronic human wounds, this method entails inducing a disease state in an animal (Flynn et al. 2023). The review includes .

#### *10.3.1 Diabetic Models*

Often induced by chemical induction (e.g. streptozotocin) or by the use of genotyped rodents (e.g. mice db/db) (Fang and Mustoe 2008; Flynn et al. 2023; Sharma 2013).

#### *10.3.2 Ischemic Models*

The “skin flap” model, which involves temporarily lifting and then suturing back a flap of skin in rodents—sometimes with a silicone sheet underneath to prevent revascularization—is a popular technique (Flynn et al. 2023).

#### *10.3.3 Infected Models*

This involves either inoculating the wound directly with bacteria or applying a biofilm to study the effect of infection on healing (Flynn et al. 2023; Grada et al. 2018; Masson-Meyers et al. 2020; Sami et al. 2019).

### 10.4 *In Vivo* Models (Human)

This method involves causing minor, controlled wounds on human volunteers (Flynn et al. 2023). The authors review a number of standard techniques (Flynn et al. 2023)

#### *10.4.1 Skin Stripping*

The main purpose of removing the stratum corneum with adhesive tape is to investigate the function and repair of the skin barrier (Flynn et al. 2023). **(Reference: Table 2)**

#### *10.4.2 Suction Blister Model*

This technique uses negative pressure to cleanly separate the epidermis from the dermis, enabling the collection of inflammatory cells and the study of re-epithelialization (Flynn et al. 2023). **(Reference: Table 2)**

#### *10.4.3 Abrasive Wound Model*

In this method, the epidermis is removed with a surgical brush, creating a wound similar to a real scrape, which is useful for testing the dressing (Flynn et al. 2023). **(Reference: Table 2)**

#### *10.4.4 Excision Wounds*

This technique uses a blade or punch biopsy tool to create split-thickness or full-thickness wounds in order to study the entire healing process, including angiogenesis and tissue re-growth (Flynn et al. 2023). **(Reference: Table 2)**

### 10.5 *In Vitro* Models (Laboratory-Based)

This technique makes use of engineered tissues or cells in a controlled laboratory setting (Flynn et al. 2023). These are divided into various categories by the authors

#### *10.5.1 2D Cell Monolayer Models*

The most common is the scratch test, in which a gap is made in the confluent monolayer of cells to allow migration and proliferation of the cells to be monitored (Flynn et al. 2023). **(Reference: Table 3)**

#### *10.5.2 Trans-well Systems (Boyden Chamber)*

This technique is used to analyze the migration of cells in response to chemical signals (chemotaxis) by means of a porous membrane that separates two chambers (Flynn et al. 2023). **(Reference: Table 3)**

#### *10.5.3 3D Wound Healing Assays*

More complex methods to better mimic human tissues
Histocultures: The process of cultivating intact skin fragments in a growth medium (Flynn et al. 2023).
Skin Equivalents Dermal and/or epidermal layers formed by bioengineered tissues made from skin cells and extracellular matrix (ECM) matrices like collagen (Flynn et al. 2023). **(Reference: Table 3)**

### 10.6Other and New Methodologies

The review also includes emerging and computational research fields (Flynn et al. 2023)

#### *10.6.1 Ex Vivo Models*

In this method, human or animal excised skin is grown alive in culture for testing compounds or for studying interactions between nanoparticles (Flynn et al. 2023).

#### *10.6.2 In Silico Models*

Computer or computational models that use mathematical equations to simulate biological processes such as angiogenesis and predict the outcome of various conditions (Flynn et al. 2023)

#### *10.6.3 New Technologies*

The authors highlight advanced methods such as microfluidic , skin-on-chip and 3D bioprinting, which offer greater control, automation and physiological relevance by incorporating features such as vascular networks (Flynn et al. 2023).

Finally, the methodology of the paper is a narrative literature review which organizes the vast body of wound healing research models into different categories and provides a critical assessment of each of them (Flynn et al. 2023).

### 10.7Empirical work

### Animal Models (*In Vivo*)

#### *10.7.1 Rodents (Rats/Mice)*

The most common models are used to study the general wound healing and also to study the induction of conditions such as diabetes (through streptozotocin), ischaemia (skin graft model) and obesity (high fat diets) (Flynn et al. 2023). A new discovery or novel approach mentioned is the rat tail model, which heals with less contraction for longer periods (21 days), making it more comparable to human healing and useful for testing treatments over long periods of time (Flynn et al. 2023).

#### *10.7.2 Pigs*

Because of its anatomical resemblance to human skin (diameter of the dermis, architecture of the hair follicle), it is regarded as a superior model. They are used as a model for diabetes and in the study of re-epithelialization (Flynn et al. 2023; Sullivan et al. 2001).

#### *10.7.3 Rabbits*

It highlights the rabbit ear model as the primary experimental instrument (Sisco and Mustoe 2003). Like humans, its cartilage structure prevents the wound from contracting and promotes healing through re-epithelialization (Sisco and Mustoe 2003). This makes it useful for researching hypertrophic scarring and ischaemia (Flynn et al. 2023; Sisco and Mustoe 2003). The ear's large vascularity also makes it easy to manipulate (Flynn et al. 2023).

### 10.8Human Models (*In Vivo*)

This document outlines several methods of creating and studying injuries in humans (Flynn et al. 2023).

#### *10.8.1 Skin Stripping*

Using adhesive tape to remove the stratum corneum is used to study the function of skin barrier and re-epithelialization (Flynn et al. 2023).

#### *10.8.2 Suction Blister Model*

Applying negative pressure to separate the epidermis from the dermis is used for the study of immune cells and wound closure without scarring (Flynn et al. 2023).

#### *10.8.3 Abrasive Wound Model*

Using a surgical brush to create a more realistic "scrape" type wound to test dressings (Flynn et al. 2023).

#### *10.8.4 Excision Wounds (Split- and Full-Thickness)*

It is invasive methods that remove skin down to the dermis or subcutaneous fat, allowing for detailed histological study of the entire healing process, including angiogenesis (Flynn et al. 2023; Grada et al. 2018; Masson-Meyers et al. 2020; Sami et al. 2019).

### 10.9 *In Vitro* and Emerging Technologies

#### *10.9.1 2D and 3D Models*

These include standard scratch assays on cell monolayers and more complex 3D skin equivalents built with scaffolds (Flynn et al. 2023).

### 10.10 New Discoveries (Advanced Technologies)

The authors point to several next-generation technologies designed to overcome the limitations of older models (Flynn et al. 2023)

#### *10.10.1 Vascularized 3D Skin Equivalents*

Integrating blood vessel networks into skin models used for angiogenesis study (Flynn et al. 2023).

#### *10.10.2 Microfluidics and Skin-on-a-Chip*

These devices mimic the dynamic environment of living tissue by incorporating fluid flow, which allows for better nutrient delivery and more physiologically relevant testing (Flynn et al. 2023) .

#### *10.10.3 3D Skin Bioprinting*

The use of techniques such as laser-assisted printing to precisely place different types of cells (fibroblasts, keratinocytes) and to create complex, multi-layered structures for the skin, which are highly reproducible (Flynn et al. 2023).

### 10.11 Authors' Conclusions

The authors synthesize information from previous empirical work published in peer-reviewed journals to draw a few key conclusions (Flynn et al. 2023).

#### *10.11.1 No Single Model is Superior*

The main conclusion is that there is no single ideal model of wound healing that can be applied in a general way (Flynn et al. 2023). Each model, whether animal, human or in vitro, has its own specific strengths and weaknesses (Flynn et al. 2023).

#### *10.11.2 Model Selection Must Match the Research Goal*

Researchers have to choose the model carefully, based on the particular aspect of wound healing they want to study (Flynn et al. 2023). For example, in the ischaemic studies, rabbits are best suited, while pigs are better at general skin regeneration (Flynn et al. 2023; Sisco and Mustoe 2003; Sullivan et al. 2001). The reasons for the choice of a particular model should be clearly stated in the research papers (Flynn et al. 2023).

#### *10.11.3 Animal Models Fail to Recapitulate Human Chronicity*

The main limitation of most animal models is that they are unable to simulate long-term, chronic diseases seen in humans, such as co-morbidities that progress with age and lead to non-healing wounds (Flynn et al. 2023).

#### *10.11.4 A Progression of Models is Needed*

The authors argue for a graduated approach to research. Studies should start with simple in vitro models to screen treatments and to test mechanisms, then move to more complex models mimicking the target human disease state, before moving to human studies (Flynn et al. 2023).

#### *10.11.5 Emerging Technologies are Promising but Not a Panacea*

Advanced technologies such as skin on chip and 3D bioprinting offer better ways of simulating human physiology and may ultimately reduce the need for animal models, but are still in the early stages of development (Flynn et al. 2023).

#### *10.11.6 The Ultimate Model is the Human*

The authors conclude that the human individual is the most clinically relevant model to study human wound healing, despite ethical problems, costs and variability (Flynn et al. 2023).

### 10.12 Rodent models are poor predictors of human wound closure.

#### *10.12.1 Anatomical Evidence*

The author provides direct anatomical evidence by identifying the panniculus carnosus, a thin layer of muscle in rodents, which is absent in most human skin (Conn 2008; Dorsett-Martin 2004; Fang and Mustoe 2008; Flynn et al. 2023; Grada et al. 2018; Greenhalgh 2005; Masson-Meyers et al. 2020; Sami et al. 2019; Sisco and Mustoe 2003). The authors state that this muscle allows the movement of the skin independently of deeper tissue and is the mechanism for rapid contraction of the wound (Flynn et al. 2023).

#### *10.12.2 Physiological Evidence*

They contrast this with human healing and state that in humans wounds heal by keratinocyte differentiation with less contraction of the wound (Flynn et al. 2023). Flynn (2023) cite a 2004 study by Galiano et al. in support (Flynn et al. 2023). The authors cite this basic physiological difference as the primary evidence that rodent wound healing is not similar to human wound healing (Flynn et al. 2023).

### 10.13 Pigs are a superior model due to anatomical and physiological similarity.

#### *10.13.1 Anatomical/Histological Evidence*

The authors provide a list of direct anatomical similarities as evidence
**Skin Thickness** Pig epidermis and dermis thickness are similar to humans(Flynn et al. 2023; Sullivan et al. 2001)'.
**Hair Follicles** Pig hair follicles are sparse and extend deep into the dermis/hypodermis, matching human architecture (Flynn et al. 2023; Sullivan et al. 2001).
**Rete Ridges** Authors cite evidence that pigs possess epidermal ridges containing alkaline phosphatase, which are structurally similar to the rete ridges in human skin (Flynn et al. 2023; Sullivan et al. 2001).

#### *10.13.2 Physiological Evidence*

The authors state that "collagen, elastin, immune cells and growth factors in pigs" respond in a similar way to human tissue, as shown by a study in Lanyu pigs, where partial thickness wounds regenerate the rete ridges while full thickness wounds cause scar tissue (Flynn et al. 2023; Sullivan et al. 2001).

### 10.14 The research community uses suboptimal models.

#### *10.14.1 Statistical/Observational Evidence*

The authors provide evidence by pointing out that rodents are the most commonly used species, attributing this widespread use to practical reasons such as availability, low cost, ease of handling and maintenance, rather than to scientific merit (Flynn et al. 2023). By contrasting this advantage with the strong evidence of rodent physiological differences from humans, they support their hypothesis that suboptimal models are selected for non-scientific reasons (Flynn et al. 2023).

#### *10.14.2 Evidence from Failed Translation*

The authors cite the limited success of in vitro model translation as evidence of their inadequacy (Flynn et al. 2023). The authors state that the "numerous results from the in vitro 2D models were not translated into successful in vivo studies" (Flynn et al. 2023).

### 10.15 Evidence for the Claim Most models of chronic disease are inadequate.

#### *10.15.1 Methodological Evidence*

The authors provide evidence by describing a common methodology for the development of diabetic rodent models (Flynn et al. 2023). They point out that in many experiments; the injury is induced "only 1-2 weeks" after the hyperglycaemic state has been established (Flynn et al. 2023). They cite this short time span as direct evidence that the models do not account for the long-term systemic effects of diabetes (e.g. neuropathy, vascular disease) that are a key component of human chronic wounds (Flynn et al. 2023).

#### *10.15.2 Evidence from Cited Studies*

They support this by citing studies showing that long-term diabetic models, such as one in rabbits that survived for up to a year, could demonstrate such long-term effects, including delayed healing of wounds and damage to organs (Flynn et al. 2023). This comparison serves as a demonstration that short-term models are indeed insufficient (Flynn et al. 2023) .
In essence, the authors base their argument not on any new experimental data, but on synthesizing anatomical facts, physiological principles, and methodological criticisms from existing scientific literature to provide evidence (Flynn et al. 2023).

### 10.16 Flaw Broad Generalization about Cellular Responses in Pigs

#### *10.16.1 The Claim*

The authors write that "collagen, elastin, immune cells and growth factors in pigs" respond in similar ways to human tissues (Flynn et al. 2023).

#### *10.16.2 Analysis of Flaw*

This is a very wide and powerful statement, covering a wide range of complex biological processes (Flynn et al. 2023). Although the document makes a strong case for anatomical similarities (skin thickness, hair follicles), it does not provide specific comparative data or cite studies that have shown that the entire set of immune cells and a complex cascade of growth factors in humans act in the same way during wound healing (Flynn et al. 2023). The cited citation [1] supports many of the claims made in relation to animal models, but may not contain the depth of evidence necessary to fully support such sweeping claims on complex molecular and cellular dynamics (Flynn et al. 2023). This claim is presented as a simple fact, but the evidence needed to fully substantiate this claim is extensive and not detailed in the document.

### 10.17 Superiority of Genetically Modified Rodent Models for Type II Diabetes

#### *10.17.1 The Claim*

When discussing diabetic models, the authors argue that "genetically modified rodents such as the db/db mouse represent a more natural progression of the disease" with long-term hyperglycaemic states and avoid some undesirable side effects caused by chemical induction (Fang and Mustoe 2008; Flynn et al. 2023; Masson-Meyers et al. 2020).

#### *10.17.2 Analysis of Flaw*

The authors prefer the genetic model to the chemical induction model, which they criticize for not allowing for long-term effects (Flynn et al. 2023). However, they do not provide specific evidence or citations to support the claim that db/db models are adequate for the study of long-term complications associated with chronic wounds (e.g., neuropathy, advanced vascular disease) (Flynn et al. 2023). They criticize one model as too short-term, but do not provide positive evidence that their preferred option fully addresses this fundamental issue in studies on wound healing (Flynn et al. 2023). The assumption that this is a significantly better model for chronic wound healing (and not just for diabetes progression) is not fully supported by the data in the paper (Flynn et al. 2023).

### 10.18 Lack of Hypertrophic Scarring in Pigs and Rodents

#### *10.18.1 The Claim*

In support of the rabbit ear model, the authors state that most animal models, including pigs and rodents, do not appear to develop hypertrophic scars, which is thought to be due to the fibromuscular layer that is found under the dermis of these species (Flynn et al. 2023; Sisco and Mustoe 2003).

#### *10.18.2 Analysis of Flaw*

Although it is generally accepted that these animals are not ideal for scarring research, the claim that it is caused by a fibromuscular layer is put forward as a speculative reason (it is believed that it is a fibromuscular layer) (Flynn et al. 2023). More importantly, the authors later in the document introduce the rat tail model, which they explicitly state is a new model, and which is similar to human wound healing and hypertrophic scars (Flynn et al. 2023). This contradiction renders the original broad statement flawed, because it ignores specific anatomical sites (e.g. the tail) where scarring behaviour may be different, thereby weakening the general statement (Flynn et al. 2023).

### 10.19 Efficacy of the Rat Ischemic Flap Model

#### *10.19.1 The Claim*

In describing the rodent ischaemic model, the authors note that the "addition of a silicone sheet under the skin flap" prevents revascularization and reduces contraction, allowing a more translatable wound healing physiology in humans (Flynn et al. 2023).

#### *10.19.2 Analysis of Flaw*

The claim that this particular manipulation would make the model more translatable is an assertion with no direct supporting evidence (Flynn et al. 2023). The authors explain what the silicone sheet does (prevents revascularization and contraction), but provide no citations or data to demonstrate that the resulting wound environment and the process of healing is physiologically homologous to that of an ischaemic human wound (Flynn et al. 2023). The logical leap from the concept of contraction to the concept of more translatable physiology is acceptable, but it is presented as a conclusion rather than as a hypothesis supported by the evidence in the text (Flynn et al. 2023).

Overall, the main arguments of the document are strong and well documented, but the specific cases

contain claims which are either too broad, based on assumptions which are not fully substantiated in the text, or contain contradictions within the text.

**10.20 There are fundamental, non-translatable differences in the healing mechanisms of common animal models compared to humans.**

Evidence
The most commonly used animal model, the rodent, has the muscle "panniculus carnosus "(Flynn et al. 2023; Masson-Meyers et al. 2020). This anatomical feature causes their wounds to heal mainly by contracting the wound site, pulling the skin together (Flynn et al. 2023; Masson-Meyers et al. 2020).
Evidence
Human skin, on the other hand, is attached to a thick hypodermis and wounds heal mainly by re-epithelialization, which is the regeneration of the cells in the surface of the wound (Flynn et al. 2023). This fundamental difference in primary wound healing mechanism may make rodent wound closure data difficult to translate into human data (Flynn et al. 2023).

**10.21 Certain animal models possess specific anatomical or physiological features that make them uniquely suitable for studying particular aspects of human wound healing.**

Evidence
The pig skin is presented as having significant anatomical and physiological similarities to human skin, including similar epidermis and dermis thickness, sparse hair follicles and similar cellular responses, which make it a better model for general studies (Flynn et al. 2023).
Evidence
The rabbit ear model shows that the dermis is attached to a cartilage layer, which acts as a natural splint to prevent the contraction of the wound (Flynn et al. 2023). This unique structure allows isolated studies of healing by re-epithelialization and granulation of tissues, processes that are very similar to human healing (Flynn et al. 2023; Masson-Meyers et al. 2020). This model is also useful for the study of hypertrophic scar formation, a condition that is not normally observed in other animals such as rodents and pigs (Flynn et al. 2023; Masson-Meyers et al. 2020; Sisco and Mustoe 2003; Sullivan et al. 2001).

**10.22 Laboratory models used to simulate chronic human diseases are often methodologically flawed and do not adequately represent the long-term pathophysiology.**

Evidence
The authors analyze diabetic rodent models and note that wounds often form only 1-2 weeks after hyperglycemic induction (Flynn et al. 2023).
Evidence
This short duration is not sufficient to produce the "long-term effects of diabetes" such as neuropathy and vasculature impairment that are the real cause of chronic, non-healing wounds in humans (Flynn et al. 2023). Therefore, these models may not be suitable for the study of complex, long-term diabetic wounds (Flynn et al. 2023)

**10.23 Simple *in vitro* models, while useful for initial screening, lack the biological complexity to be predictive of success in a living organism.**

Evidence
Standard 2D monolayer models do not represent the complete skin structure and do not mimic vital processes such as the remodeling of the extracellular matrix (ECM) and the complex interactions between the different cell types (e.g. immune cells, endothelial cells) (Flynn et al. 2023).
Evidence
The authors cite the fact that numerous "in vitro 2D models" did not translate into "successful in vivo studies" as a clear indication of their limited predictive power (Flynn et al. 2023).

**10.24 Emerging technologies offer a path to overcome the limitations of traditional models by creating more dynamic and physiologically relevant environments.**

Evidence
The authors describe how traditional 2D and 3D models lack a vascular network to provide nutrients, which makes it impossible to simulate the circulatory system. Evidence In response, technologies such as "skin-on-chips" and 3D bioprinting were introduced (Flynn et al. 2023). These technologies can integrate vasculature, immune cells, and dynamic fluid flow to create more robust models that better mimic the complexity of real tissue and allow for the study of processes such as angiogenesis (Flynn et al. 2023).

**10.25 Strength of Review**

***10.25.1 A Comprehensive Critical Review***

The most direct contribution is a thorough review that categorizes and analyzes a wide array of research models, including *in vivo*, *in vitro*, *ex vivo*, and *in silico* methods (Flynn et al. 2023). They critically

discuss the advantages and disadvantages of each, with a consistent focus on their translatability to human physiology. This serves as a valuable resource for researchers selecting a model (Flynn et al. 2023).

#### *10.25.2 Challenging the Status Quo A Shift in Perspective*

A significant contribution is their argument against the search for a single "perfect" model (Flynn et al. 2023). Instead, they contribute the perspective that different models are tools for specific questions (Flynn et al. 2023) . They argue that "there is not one model of wound healing that is superior. Rather, there are many different models that have specific uses for studying certain processes or stages of wound healing" (Flynn et al. 2023)This reframes the central problem in the field from finding one ideal model to intelligently selecting the most appropriate one (Flynn et al. 2023).

#### *10.25.3 Identifying and Articulating Key Research Gaps*

The critical analysis of the authors contributes to the field by clearly articulating the main shortcomings of the research (Flynn et al. 2023).
They point out that most models do not simulate chronic, non-healing wounds accurately, particularly those caused by long-term illnesses such as diabetes (Flynn et al. 2023).
A fundamental difference between rodent (contraction) and human (re-epithelialization) healing that makes the most common animal models impractical (Flynn et al. 2023).
Lack of complexity in in vitro models, which often lack key elements such as immune cells, blood supply and complex interactions between cells, which translate poorly into in vivo success (Flynn et al. 2023).

### 10.26 Proposing a Structured Framework for Future Research

Their most important contribution may be to prescribe solutions to the shortcomings identified (Flynn et al. 2023). They present a new paradigm for exploration (Flynn et al. 2023).

#### *10.26.1 A Progression of Models*

They recommend a structured approach moving "from simple to complex" (Flynn et al. 2023). Researchers should first use simple models to verify a specific molecular mechanism and then progress to more complex models that mimic the human disease state before attempting human trials (Flynn et al. 2023) .

#### *10.26.2 A Call for Rigor*

The author contributes to the call for higher standards in scientific communication, stating that manuscripts must justify the choice of models and explicitly describe their similarities and differences with the human condition (Flynn et al. 2023). The aim is to improve reproducibility and critical assessment in this area (Flynn et al. 2023).

### 10.27 Highlighting the Path Forward with New Technologies

Finally, they contribute by synthesizing information on cutting-edge technologies and positioning them as solutions to existing problems (Flynn et al. 2023). They advocate for the adoption of methods like **skin-on-a-chip**, **vascularized 3D equivalents**, and **3D bioprinting** to create more physiologically relevant and complex models that can help bridge the gap between "ab research and clinical reality" (Flynn et al. 2023).
In summary, the authors' contribution is a scholarly roadmap that diagnoses the problems in wound healing research, reframes the thinking around model selection, and provides a clear, actionable framework for improving the translatability and success of future therapeutic development.

## 11 Discussion

The main principles of this framework are

### 11.1 The Theory of Model-Specific Utility

They theorize that there is no single universal model that can accurately represent all the facets of the human wound-healing process (Flynn et al. 2023). Their central development idea is that the various models have specific, limited applications in the study of specific stages or healing processes (Flynn et al. 2023). The value of a model is not inherent but determined by its ability to replicate a specific process in the body or pathophysiology in a human being (Flynn et al. 2023).

### 11.2 The Theory of Progressive Complexity in Research

They propose a theoretical approach to research design, which goes from the simple to the complex (Flynn et al. 2023). Researchers should monitor the development of models, starting with simple in vitro models to test the effect of a treatment on a specific

molecular pathway (e.g. migration and proliferation of cells), and then moving to more complex animal or 3D models to mimic the target human state (Flynn et al. 2023). This structured approach is theorized as the most effective way to develop new therapies (Flynn et al. 2023).
(Refer: Table 3)

### 11.3 The Theory of Recapitulating Chronicity

Their main contention is that the model must accurately reflect the chronic nature of the human condition in order to be genuinely helpful in the study of chronic wounds (Flynn et al. 2023). They contend that models that solely replicate acute injuries or transient illnesses are theoretically unsuitable for studying long-term, chronic, non-healing wounds that are present in patients with concurrent conditions like chronic diabetes (Flynn et al. 2023).
The authors' contribution is not a new biological theory but a theoretical framework for methodological rigour in wound healing, stressing that the choice of experimental models should be carefully and rationally matched to the specificities of the complex wound healing process being studied, in order to ensure that results are clinically translatable s (Flynn et al. 2023)..

### 11.4 The most common animal models are fundamentally flawed for studying human wound closure.

The authors argue that the widespread use of rodents is a major problem (Flynn et al. 2023). Their reasoning is that rodents heal primarily by **"wound contraction"**, a process driven by the *"panniculus carnosus "*muscle (Dorsett-Martin 2004; Flynn et al. 2023; Masson-Meyers et al. 2020). Because humans heal mainly by **re-epithelialization**, the rodent model is not just a simpler version of human healing—it's a different biological process (Flynn et al. 2023). Therefore, they argue, data from these models on wound closure rates can be misleading and "poorly translatable to humans" (Flynn et al. 2023). They use this point to argue against choosing models based on convenience and cost alone (Flynn et al. 2023).
**(Reference: Table 1)**

### 11.5 Physiologically superior models exist for specific research questions.

To counter the reliance on flawed models, the authors argue that researchers should select models with specific strengths that match their experimental goals (Flynn et al. 2023).

#### *11.5.1 Pigs for Overall Similarity*

They argue that pigs are a better model for general studies because their skin structure, thickness and healing process (combination of re-epithelialization and contraction) are the closest to human ones (Flynn et al. 2023; Masson-Meyers et al. 2020; Soni 2025; Sullivan et al. 2001).
**(Reference: Table 1)**

#### *11.5.2 Rabbit Ears for Pure Re-epithelialization*

They argue that the rabbit ear model is uniquely suited to study the re-epithelialization in the context of isolation (Flynn et al. 2023; Masson-Meyers et al. 2020; Sisco and Mustoe 2003). The underlying cartilage acts as a natural splint, preventing the wound from contracting and allowing it to heal like a human skin (Flynn et al. 2023; Sisco and Mustoe 2003). This makes it a valuable tool for studying the key mechanism of human wound healing and for investigating the phenomenon of hypertrophic scars (Flynn et al. 2023). **(Reference: Table 1)**

### 11.6 Simulating chronic disease is critical but is currently done inadequately.

The authors argue that most models fail because they represent acute wounds in healthy animals, not the complex chronic wounds seen in human patients with co-morbidities (Flynn et al. 2023). They specifically argue that common diabetic models are insufficient because they do not allow enough time for the long-term systemic effects of diabetes (like poor circulation and neuropathy) to develop (Flynn et al. 2023). The argument is that a therapy tested in such a model is not being challenged by the same biological obstacles it would face in a chronically ill human, making the model a poor predictor of clinical success (Flynn et al. 2023).

### 11.7 A new, more rigorous research paradigm is necessary.

Finally, the authors argue for a change in research strategy to overcome these issues (Flynn et al. 2023). They propose that researchers should stop relying on single, inadequate models and instead adopt a "**progression of models, from simple to complex"** (Flynn et al. 2023)**.**Their argument is that a therapy should first be tested in a simple system (like an *in vitro* model) to confirm its effect on a specific cellular pathway (Flynn et al. 2023). If successful, it should then be moved to a more complex, physiologically relevant animal model that mimics the human disease state before ever proceeding to human trials (Flynn et al. 2023). They argue this

staged approach will improve the chances of successful translation from the lab to the clinic (Flynn et al. 2023).

### 11.8Analysis of the Central Question

The authors analyze this question by systematically dissecting it into several key components and evaluating the available models against these criteria.
**Analysis of Interspecies Physiological Differences**
The authors' primary analytical approach is to compare the fundamental biology of wound healing across species and contrast it with humans (Flynn et al. 2023).

#### *11.8.1 Rodents vs. Humans*

They analyze a significant variation in the mechanisms of healing (Flynn et al. 2023). Rodents heal primarily by "wound contraction", a process that is controlled by a special layer of muscle called the "panniculus carnosus "(Conn 2008; Davidson 1998; Dorsett-Martin 2004; Flynn et al. 2023; Grada et al. 2018; Greenhalgh 2005; Masson-Meyers et al. 2020; Sami et al. 2019; Sharma 2013). Humans, on the other hand, heal mainly by re-epithelialization (Flynn et al. 2023). This fundamental difference is presented as a major defect which makes rodent models unsuitable for the study of human wound closure (Flynn et al. 2023). **(Reference: Table 1)**

#### *11.8.2 Pigs vs. Humans*

The analysis highlights the pig as the best model, by detailing the anatomical and physiological similarities between pigs and humans (Flynn et al. 2023; Sisco and Mustoe 2003). This includes comparable skin thickness, similar skin follicle structure and similar responses to cells and growth factors (Flynn et al. 2023). **(Reference: Table 1)**

#### *11.8.3 Rabbits (Ear Model) vs. Humans*

They're analyzing the unique anatomy of the rabbit ear, where the underlying cartilage prevents the wound from contracting (Flynn et al. 2023; Masson-Meyers et al. 2020; Sisco and Mustoe 2003). This makes it an ideal model for specific analysis of re-epithelialization and hypertrophic scarring processes, processes which are difficult to study in non-animals (Flynn et al. 2023).

### 11.9 Analysis of Disease Model Fidelity

The authors analyze how well experimental models replicate the pathophysiology of human chronic diseases that lead to non-healing wounds (Flynn et al. 2023).

#### *11.9.1 Critique of Acute vs. Chronic Models*

Their analysis concluded that most animal models only simulate acute wounds in healthy subjects and do not capture the complexity of chronic wounds due to co-morbid conditions (Flynn et al. 2023).

#### *11.9.2 Case Study Diabetes Models*

They analyze standard rodent models of diabetes and find them inadequate (Flynn et al. 2023). Their analysis shows that the formation of wounds only 1 to 2 weeks after induction of diabetes does not allow time for long-term systemic effects (e.g. neuropathy and vascular disease) to develop (Flynn et al. 2023). They concluded that these models were therefore not suitable for investigating the real problems of healing a chronic diabetic wound (Flynn et al. 2023).

### 11.10 Analysis of *In Vitro* and Advanced Technology Models

The authors analyze the usefulness and limitations of non-animal models (Flynn et al. 2023).

#### *11.10.1 In Vitro Limitations*

Their analysis shows that traditional 2D in vitro models, although useful for initial screening, do not have the physiological complexity of human skin, including the immune components and extracellular matrix (Flynn et al. 2023). This leads them to conclude that the results of these models often do not translate into success in vivo (Flynn et al. 2023). **(Reference: Table 3)**

#### *11.10.2 Advanced Technologies*

They are analyzing new technologies such as 3D bioprinting and skin on chip as potential solutions (Flynn et al. 2023). Analysis suggests that these models may better mimic the dynamic environment of human tissue by including vascular networks and immune cells, and thus provide more robust and physiologically relevant data (Flynn et al. 2023).

### 11.11 Synthesis and Proposed Solution

Finally, the authors synthesize their analysis to propose a new research paradigm (Flynn et al. 2023). They conclude that no single model is perfect and argue for a **"progression of models, from simple to complex"** (Flynn et al. 2023). This strategy involves using simple models to test a specific mechanism, followed by validation in progressively more

complex, disease-relevant models before moving to human trials (Flynn et al. 2023). This, they conclude, is the most rational approach to increase the likelihood of successful clinical translation (Flynn et al. 2023).

There is no single animal species or experimental model that is superior or can perfectly replicate all aspects of human wound healing (Flynn et al. 2023).Each animal species offers unique physiological characteristics that make it advantageous for studying specific and independent elements of the wound healing process (Flynn et al. 2023).Researchers must carefully select not only the animal species but also the specific experimental model to best align with the human physiological or pathophysiological condition they intend to study (Flynn et al. 2023).The majority of animal models are only capable of simulating acute wounds and have had limited success in "recapitulating the complex co-morbidities" that lead to chronic non-healing wounds in humans (Flynn et al. 2023).To develop new treatments, researchers should consider using a "progression of models, moving from simple models" to those of increasing complexity that mimic the target human condition (Flynn et al. 2023).

Recent advances in computer modeling and 3D models (like skin-on-a-chip and bioprinting) may provide better insights into the complex interactions of wound healing and could potentially bypass the need for preclinical animal models in some cases (Flynn et al. 2023).The most "clinically relevant wound healing model"ultimately remains the human (Flynn et al. 2023).

### 11.12 Most animal models have fundamental physiological differences from humans, leading to non-translatable data.

Evidence

The paper provides strong evidence that the most widely used model, rodents, heal primarily through "wound contraction", a mechanism mediated by the "panniculus carnosus "muscle (Flynn et al. 2023). This is in stark contrast to the human treatment, which is based on re-epithelialization (Flynn et al. 2023). This mismatch in the basic physiological process leads to the conclusion that wound closure data from these models are inherently inaccurate, which makes the translation unsuitable for human use (Flynn et al. 2023). **(Reference: Table 1)**

### 11.13 Existing disease models fail to replicate the true complexity of chronic human conditions.

Evidence

The authors analyze rodent models of diabetes and conclude that these are inadequate (Flynn et al. 2023). They provide evidence that wounds are often induced only 1 to 2 weeks after hyperglycaemia has started (Flynn et al. 2023). This supports the conclusion that these models are not adequate, as the time-frame is too short to develop the long-term systemic complications (e.g. neuropathy and vascular disease) that are the real cause of diabetic ulcers that do not heal (Flynn et al. 2023). This lack of chronicity modeling supports the argument that therapies developed in these models may not be effective in clinical scenarios in the real world (Flynn et al. 2023).

### 11.14 Simple *in vitro* models lack the complexity to predict success in a whole organism.

Evidence

The document states that while 2D cell cultures are useful for basic screening, they do not contain extracellular matrix (ECM), immune components or complex pathways between different types of cells (Flynn et al. 2023). The authors cite as a direct argument for this conclusion the fact that "numerous results from in vitro 2D models" were not translatable into 'successful in vivo studies" (Flynn et al. 2023). This lack of predictability justifies the need for more complex and more downstream validation steps (Flynn et al. 2023). **(Reference: Table 3)**

### 11.15 The availability of specialized models for specific questions highlights the flaw in a one-size-fits-all approach.

Evidence

The authors describe a model of the rabbit ear, which, because of its underlying cartilage, prevents "wound contraction" and allows for specific studies of re-epithelialization and hypertrophic scarring (Flynn et al. 2023). The existence and the need for such a specialized model justify the conclusion that a single generic model (such as a standard rodent injury) is not sufficient for all research questions (Flynn et al. 2023).

The final argument It is logical to conclude from the combination of these arguments that the current animal models are misleading (11.12), the disease models are too simplistic (11.13) and the basic laboratory models are not predictive (11.14). The existence of highly specialized models (11.15) shows the need for a more adapted approach (Flynn et al. 2023). The most rational and reasonable conclusion is therefore that researchers must adopt a new strategy of `pushing models from simple to complex,` with the aim of systematically validating therapies at

all levels of biological complexity before trying them on humans (Flynn et al. 2023). This approach is justified as the most logical way of bridging the translation gap from laboratory to clinic (Flynn et al. 2023).

**11.16 Rodents heal primarily by wound contraction, a mechanism fundamentally different from human re-epithelialization.**

***11.16.1 Illogical Conclusion***

Because rodent models are so different from humans, any past or future wound healing research done in rodents is completely irrelevant and should be discarded (Flynn et al. 2023).

***11.16.2 Why it's Illogical***

The real point of the authors is that rodent models are not well translatable to wound healing studies, but may still be adequate for other purposes, such as dermal and epidermal drug research (Flynn et al. 2023). The illogical conclusion takes a specific restriction and over-generalizes it to an absolute, thereby discarding all the possibilities of a value (Flynn et al. 2023).

**11.17 Pigs have skin that is anatomically and physiologically very similar to human skin.**

***11.17.1 Illogical Conclusion***

All wound healing research should be done using pigs only, and any results from other animal models are scientifically inconclusive (Flynn et al. 2023).

***11.17.2 Why it's Illogical***

That conclusion ignores the explicit warnings of the authors (Flynn et al. 2023). They note that pigs are expensive, require large housing facilities and have a different distribution of the sweat glands, which may affect epidermal stem cell studies (Flynn et al. 2023). This illogical conclusion cherry picks positive evidence and ignores the stated constraints and practical problems, creating a de facto mandate (Flynn et al. 2023).

**11.18 Creating diabetic rodent models only 1-2 weeks before wounding does not allow long-term complications to develop.**

***11.18.1 Illogical Conclusion***

The only valid way to study diabetic wounds is to wait for years for complications to naturally develop in the rodent, which is impractical and impossible to perform in time (Flynn et al. 2023).

***11.18.2 Why it's Illogical***

That conclusion creates a false dilemma (Flynn et al. 2023). The authors criticize the short time frame as inadequate, but do not claim that a prohibitively long time-frame is the only option(Flynn et al. 2023). In fact, they highlight the need for models that better recapitulate chronicity, which may include different induction methods or species (e.g. the rabbit model in the case of chronic diabetes) (Flynn et al. 2023). An illogical conclusion makes the problem unsolvable, which is not the intention of the authors (Flynn et al. 2023).

**11.19 Numerous results from simple *in vitro* 2D models have not translated into successful *in vivo* studies.**

***11.19.1 Illogical Conclusion***

In vitro models are a complete failure and a waste of resources. All research should start with comprehensive animal models directly (Flynn et al. 2023).

***11.19.2 Why it's Illogical***

This is in direct contrast to the research strategy proposed by the authors (Flynn et al. 2023). They argue that in vitro models are a key first step in screening compounds for high throughput and understanding basic mechanisms, before moving to more complex, costly and ethically difficult in vivo tests (Flynn et al. 2023). The illogical conclusion misunderstands the word not always predictive as meaning never useful (Flynn et al. 2023).

**11.20 The rabbit ear model prevents contraction and is useful for studying hypertrophic scarring.**

***11.20.1 Illogical Conclusion***

Because the best model for hypertrophic scars is the rabbit ear, treatments that reduce scarring in the rabbit ear will work just as well for any scar anywhere in the human body (Flynn et al. 2023).

***11.20.2 Why it's Illogical***

That conclusion leaps in faith by ignoring the specificity of the tissues (Flynn et al. 2023). The authors state that the unique cartilage structure of the rabbit ear is what makes it useful. They also note that

tissue outside the ear is healed by contraction in rabbits (Flynn et al. 2023). This illogical conclusion assumes that the success of a treatment in one highly specialized anatomical site is universally applicable, ignoring the context in which the model works (Flynn et al. 2023).

In each case, the illogical conclusion stems from ignoring nuance, over generalizing a specific point, creating a false dichotomy, or failing to consider the authors' overall argument for a multi-faceted, context-dependent approach to wound healing research (Flynn et al. 2023).

### 11.21 All Animal Models Have Flaws

The paper systematically dismantles every model of animal (Flynn et al. 2023). Rodents heal in different ways (contraction versus re-epithelialization) Pigs, although superior, are expensive and have a different distribution of sweat glands (Flynn et al. 2023). Rabbits are only useful in specific scenarios, such as wounds to the ears and ischemia (Flynn et al. 2023). This overwhelming evidence suggests that all non-human models are imperfect surrogates (Flynn et al. 2023).

### 11.22 Artificial Models are Incomplete

The review shows that in vitro models lack physiological complexity (immune system, blood supply), and even advanced 3D models and chip-based skin technology are still attempts to mimic reality rather than fully replicate it (Flynn et al. 2023).

#### *11.22.1 Human-Specific Factors are Critical*

The authors repeatedly refer to factors unique to the complex human body, such as the progress of age-related co-morbidities, which cannot be successfully replicated in animal models (Flynn et al. 2023). They also discuss human-specific wound models (e.g. sutured blisters and abrasive wounds), and implicitly point out that to study human healing, one must end up studying human wounds (Flynn et al. 2023).

### 11.23 The Inferential Leap

The logical step from warrants to conclusions (Flynn et al. 2023). The warrants state that all alternative models, from rodents to pigs to 3D bioprinting, are imperfect or incomplete substitutes for the human system (Flynn et al. 2023). Therefore, by elimination, the only truly complete and accurate model to study the treatment of human wounds is the human individual (Flynn et al. 2023).

The authors do not present a specific experiment or data that would prove that man is the best model (Flynn et al. 2023). Instead, they lead the reader to that conclusion by proving the inadequacy of all the alternatives (Flynn et al. 2023). The conclusion is a capstone statement which logically follows from the overall criticism, but is not a scientifically tested hypothesis in the preface of the document (Flynn et al. 2023). It serves as a final justification for their call for improved preclinical models to better bridge the gap to the ultimate, inevitable human patient (Flynn et al. 2023).

## 12 Criticism

### 12.11. Poor Translatability Due to Fundamental Biological Differences

The main criticism is that rodents, the most widely used animals, have a very different primary healing mechanism than humans(Davidson 1998; Dorsett-Martin 2004; Flynn et al. 2023; Grada et al. 2018; Greenhalgh 2005; Masson-Meyers et al. 2020; Sami et al. 2019; Sharma 2013). Humans heal primarily through re-epithelialization, whereas rodents heal primarily through “wound contraction”, which is aided by a muscle layer called the “panniculus carnosus “(Flynn et al. 2023). This crucial distinction can obscure data and make results from rodent studies poorly transferable to human wound closure (Flynn et al. 2023). A study comparing genomic responses to trauma in humans and mice found that murine models are not translatable, though the authors note this conclusion is controversial (Flynn et al. 2023). **(Reference: Table 1)**

### 12.2 Flawed Simulation of Chronic Human Diseases

The authors criticize how chronic diseases like diabetes are modeled (Flynn et al. 2023). In many rodent experiments, wounds are created only 1–2 weeks after inducing a hyperglycemic state (Flynn et al. 2023). This short timeframe is inadequate to replicate the long-term systemic effects of diabetes (e.g., neuropathy, vasculature complications) that cause chronic, non-healing wounds in humans (Flynn et al. 2023).

Similarly, a diabetic pig model was criticized because the animals were only kept alive for 18 days, which was not long enough to determine the long-term effects of diabetes (Flynn et al. 2023).

The authors state that the majority of animal models can only simulate acute wounds, and attempts to truly replicate the co-morbidities that lead to chronic non-

healing wounds in humans have had limited success (Flynn et al. 2023) .

## 12.3Criticism of *In Vitro* (Laboratory) Models

### *12.3.1 2D Models are Too Simplistic*

Conventional 2D cell monolayer models (like the scratch assay) are criticized for not representing the actual structure of human skin. They fail to mimic crucial processes like extracellular matrix (ECM) remodeling and the complex "crosstalk" and signaling between different cell types (e.g., immune and endothelial cells) (Flynn et al. 2023).
The authors state directly that a major problem is that "numerous results from in vitro 2D models have not translated into successful in vivo studies" because of this lack of complexity (Flynn et al. 2023) .
Specific techniques like the scratch test have practical downsides, including creating irregular scratches and being difficult to reproduce consistently (Flynn et al. 2023) . **(Reference: Table 3)**

### *12.3.2 Even Advanced 3D Models are Incomplete*

While 3D skin models are an improvement, they are often strenuous to create and still lack critical systems found in living organisms, such as a blood supply, an active immune system, innervations, and hair follicles (Flynn et al. 2023).
Traditional 3D organotypic models cannot simulate *in vivo* blood circulation because they lack a vascular network to provide nutrients to the cells (Flynn et al. 2023). **(Reference: Table 3)**

## 12.4Overall Conclusion of the Critique

The authors' overarching criticism is that there is no single, perfect model that accurately recapitulates human wound healing (Flynn et al. 2023) . They argue that researchers often use these models without fully considering their limitations, leading to research that may not be clinically relevant (Flynn et al. 2023). They call for researchers to provide a clear rationale for their choice of model and to acknowledge its similarities and differences compared to the human condition being studied (Flynn et al. 2023).

### *12.4.1 Value of Translatability to Humans*

The authors' primary value is the **clinical translatability** of research to humans (Flynn et al. 2023). This is the main criterion they use to judge the usefulness of any mode (Flynn et al. 2023)l. They explicitly state this focus in the abstract and introduction.
They repeatedly criticize models for their lack of translatability (Flynn et al. 2023). For example, they state that the difference in healing mechanisms between rodents and humans can make rodent data "poorly translatable to humans" (Flynn et al. 2023).

### *12.4.2 Opinion on the State of Current Models*

**No Single Model is Superior**: The authors firmly believe that there is no single "ideal" or "superior" odel for wound healing (Flynn et al. 2023). They explicitly state that no model perfectly recapitulates human physiology, and their analysis suggests that different models are useful for different, specific purposes (Flynn et al. 2023).
**Pigs are the superior model**: Although they believe that no model is perfect; they consider pigs to be the superior model for studying wound healing because of their anatomical and physiological similarities to humans (Flynn et al. 2023). However, they quickly point to practical constraints such as costs and equipment requirements (Flynn et al. 2023). **(Reference: Table 1)**
**Animal Models for Chronic Disease are Inadequate**: The authors are critical of how current animal models simulate chronic human diseases like diabetes (Flynn et al. 2023). They opine that creating wounds shortly after inducing diabetes is "not the most appropriate" method, as it fails to capture the crucial long-term effects of the disease (Flynn et al. 2023).

### *12.4.3 Opinion on Methodological Rigor and Future Direction*

**Collaboration is Essential**: They hold the opinion that the development of better, more clinically relevant models requires "close collaboration and communication between basic researchers and the clinicians who treat human wounds" (Flynn et al. 2023)
**A Tiered Approach is Best Practice**: The authors strongly advise that researchers "should consider a progression of models, from simple to complex" (Flynn et al. 2023) They believe this structured approach is the most effective way to develop and validate new treatments (Flynn et al. 2023).
**Justification is a Mandate**: They firmly believe that researchers should identify the model they have chosen and explain in publications how it differs and is similar to the human condition (Flynn et al. 2023). Transparency and scientific rigor are valued (Flynn et al. 2023).

### *12.4.4 Ultimate Opinion on the "Best" Model*

The authors conclude with the strong opinion that, despite all the technological and animal models available, “the most clinically relevant wound healing model remains the human” (Flynn et al. 2023)They characterize the human model as “elusive and highly variable”, acknowledging the practical and ethical challenges but reaffirming that, for ultimate relevance, research must eventually be validated in people (Flynn et al. 2023).
On the basis of the document provided, the authors do not propose any new unique theory for wound healing. Instead, they present and analyze the existing theoretical framework for wound healing and use it to create a conceptual model for how to carry out research in this area (Flynn et al. 2023). Their central argument is the choice of models based on clinical translatability s (Flynn et al. 2023).

## 13 Addressing gaps and Novel insights

### 13.1 Novelty in Perspective No "Perfect" Model, Only "Appropriate" Models

The authors' central novel argument is to shift the field's perspective away from searching for a single “superior” model (Flynn et al. 2023). They assert that “there is not one model of wound healing that is superior and can give translatable results to human research (Flynn et al. 2023). “Rather, there are many different models that have specific uses for studying certain processes or stages of wound healing” (Flynn et al. 2023) . This reframes the problem from finding one solution to intelligently selecting the right tool for a specific job (Flynn et al. 2023).

### 13.2 Proposing a Solution The Progression of Models

The authors suggest a new, organized research pathway to bridge the gap between basic lab models and intricate human conditions (Flynn et al. 2023). They suggest that researchers should consider a progression of models, from simple to complex (Flynn et al. 2023). This entails first verifying the impact of a treatment on a particular molecular pathway using basic in vitro models. moving on to more intricate models that reflect the particular human condition that is being targeted (Flynn et al. 2023). Before proceeding to human trials (Flynn et al. 2023). This methodical approach is suggested as a more thorough and effective means of creating and verifying novel treatments, thereby bridging the translational success gap (Flynn et al. 2023).

### 13.3 Championing New Technologies to Fill Complexity Gaps

To get around the drawbacks of conventional models, the authors strongly support the adoption of new technologies (Flynn et al. 2023).
The lack of vascular networks and dynamic flow in static cultures is addressed by Vascularized 3D Skin Equivalents and Skin-on-a-Chip models (Flynn et al. 2023). Additionally, 3D Bioprinting is praised for its capacity to produce more robust, reproducible, and complex skin constructs, even embedding vasculature (Flynn et al. 2023).
Finally, In Silico (Computational) Models are suggested to help screen compounds and comprehend complex interactions in theory before costly lab work begins (Flynn et al. 2023).

### 13.4. Mandating Methodological Rigor

To address the issue of poor model selection, the authors prescribe a novel standard for scientific communication (Flynn et al. 2023). They state that manuscripts “should identify the rationale for the choice of the animal model, the similarities and differences from the human condition that is being modeled, and sufficient detail for other researchers to replicate the model” (Flynn et al. 2023) . This call for mandatory justification aims to fill the gap in research quality and reproducibility (Flynn et al. 2023).
In summary, the novelty of this paper is not in a discovery, but in its **prescriptive analysis** (Flynn et al. 2023). The authors diagnose the key gaps in wound healing research and offer a comprehensive, multi-pronged framework—combining a new philosophical approach to model selection, a structured research pathway, the adoption of advanced technologies, and stricter publication standards—to fill them (Flynn et al. 2023).
Since the given document is a review article, it does not describe any of the authors' experimental procedures (Flynn et al. 2023). Rather, a thorough review and critical analysis of the numerous research approaches employed in the field of wound healing constitute its fundamental methodology (Flynn et al. 2023). Based on their applicability and potential for human translation, the authors survey, classify, and assess these techniques (Flynn et al. 2023).

### 13.5Strength of review

#### *13.5.1 Critical Synthesis and Evaluation*

The main advantage demonstrated is the ability to synthesize and critically evaluate large amounts of disparate information from the literature (Flynn et al. 2023). Authors do not just list models; they analyze their pros and cons, and focus on their human

translatability (Flynn et al. 2023). This provides a consolidated, high level understanding which would be difficult to obtain by reading the individual research papers on their own (Flynn et al. 2023).

#### *13.5.2 Identifying Gaps and Inconsistencies*

A review provides the advantage of a bird's-eye view of a research field, making it easier to identify systemic problems and gaps (Flynn et al. 2023). The authors use this advantage to point out that there is no ideal model for human wound healing and that most animal models fail to replicate the chronic nature of human wounds (Flynn et al. 2023). This helps direct future research efforts toward the most pressing challenges (Flynn et al. 2023).

#### *13.5.3 Establishing Best Practices and Proposing New Frameworks*

The advantage of the review is its potential to be prescriptive. After analyzing the state of the art, the authors propose a new and more consistent paradigm for research (Flynn et al. 2023). They advocate the gradual progression of models from simple to complex and call for more stringent standards of manuscript reporting (Flynn et al. 2023). These guidelines can improve the quality and consistency of research in all areas (Flynn et al. 2023).

#### *13.5.4 Educating and Guiding Researchers*

The review serves as a fundamental educational tool (Flynn et al. 2023). For researchers entering the field or planning an experiment, this paper offers a significant advantage in providing a comprehensive overview of available models, ranging from animal and human models to in vitro and cryogenic methods (Flynn et al. 2023). It helps them to make an informed choice of the most appropriate model for their particular research topic, and it saves them time and resources (Flynn et al. 2023).

#### *13.5.5 Highlighting Future Directions and New Technologies*

A review offers the advantage of looking forward (Flynn et al. 2023). The authors consolidate information on emerging technologies like skin-on-a-chip, 3D bioprinting, and vascularized skin equivalents, positioning them as potential solutions to the limitations of older models (Flynn et al. 2023). This helps the research community stay abreast of cutting-edge methods and encourages innovation (Flynn et al. 2023).

In essence, the document itself is the best evidence for the advantages of a review paper it critically assesses the current state of knowledge, identifies major problems, provides a roadmap for future research, and acts as an authoritative guide for scientists in the field (Flynn et al. 2023).

## 14 Conclusions

### 14.1 Animal models are not universally translatable, and their selection must be specific to the research question.

**Warrant (Justification/Reasoning)**: The authors warrant this conclusion by providing a detailed comparative analysis of different species, demonstrating that each has unique characteristics making it suitable for some studies but not others (Flynn et al. 2023).

**Evidence for Rodents**: They show that rodents heal primarily by **"wound contraction"** due to the *"panniculus carnosus "*muscle, which is fundamentally different from human **re-epithelialization** (Flynn et al. 2023). This anatomical and physiological discrepancy warrants the conclusion that rodents are poor models for studying human wound closure but may still be useful for initial, cost-effective experiments where contraction is not a confounding factor (Flynn et al. 2023).

**Evidence for Pigs**: They warrant the conclusion that pigs are a superior model for many studies by presenting evidence of their high **anatomical and physiological similarity** to humans, including comparable skin thickness, hair follicle structure, and cellular responses (Flynn et al. 2023; Sullivan et al. 2001).

**Evidence for Rabbits**: They justify the use of the rabbit ear model for specific research by showing that its underlying cartilage **prevents contraction**, thus isolating re-epithelialization for study in a way other models cannot (Flynn et al. 2023; Sisco and Mustoe 2003). This makes it uniquely suited for studying processes like hypertrophic scarring (Flynn et al. 2023).

### 14.2 Most existing models of chronic disease are inadequate because they fail to replicate the complexity of human pathophysiology.

**Warrant (Justification/Reasoning)**: The authors warrant this conclusion by critiquing the methodology of common disease models, arguing they only simulate acute conditions rather than true chronic states (Flynn et al. 2023).

**Evidence from Diabetes Models**: They provide evidence that many diabetic rodent models are flawed because wounds are created only **1-2 weeks after hyperglycemia is induced** (Flynn et al. 2023). This

short timeframe, they argue, fails to allow for the development of long-term systemic complications like neuropathy and vascular disease, which are the real drivers of non-healing wounds in human patients (Flynn et al. 2023). This methodological flaw warrants the conclusion that such models is inappropriate for studying chronic diabetic wounds (Flynn et al. 2023).

**Evidence from Ischemia Models**: They describe surgically created flap models designed to mimic ischemia (Flynn et al. 2023). While these models are useful, the authors imply their limitation by contrasting them with the complexity of chronic ischemic states in humans, which develop over long periods due to underlying diseases (Flynn et al. 2023).

### 14.3 A progressive, multi-model approach is the most rational strategy for developing new wound healing therapies.

**Warrant (Justification/Reasoning):** This final, overarching conclusion is justified by a synthesis of the failures and limitations of the reliance on any single model (Flynn et al. 2023). The high failure rate in therapies that move from preclinical to clinical phase suggests that the current paradigm is flawed (Flynn et al. 2023)

**Evidence from *In Vitro* Models:** They show that simple models such as 2D cell cultures are useful for high throughput screening, but lack the physiological complexity of living tissue (e.g. immune system, blood supply) (Flynn et al. 2023). Their inability to predict in vivo success justifies further, more complex testing (Flynn et al. 2023)**. (Reference: Table 3)**

**Proposed Solution as a Warrant:** The solution proposed by the authors is a warrant in itself. They argue that the most effective way to avoid the risk of moving to human trials is to proceed logically, starting with simple models to test a specific molecular or cellular effect, and then moving to more complex models that mimic the human disease state (Flynn et al. 2023). They conclude that this structured approach will provide better insight and increase the chances of successful clinical translation (Flynn et al. 2023).

In summary, the authors conclude that the one-size-fits-all approach to wound healing research has failed (Flynn et al. 2023). They justify this by systematically identifying specific, evidence-based defects in current models and propose a more nuanced and multi-step verification strategy as the most logical way forward (Flynn et al. 2023).

## 15 Future direction

### 15.1 Advice on Model Selection and Experimental Design

#### *15.1.1 Choose the Right Model for the Job*

The main recommendation of the authors is that researchers should abandon a one-size-fits-all strategy (Flynn et al. 2023). They emphasize that the model selection must be carefully thought out to be in line with the particular research question (Flynn et al. 2023). For instance, instead of using a less appropriate model by default, use rabbit models for ischemia research or pig models for tissue regeneration (Flynn et al. 2023).

#### *15.1.2 Justify the Model Choice in Publications*

Researchers should explicitly state in their manuscript the reasons for their choice of animal model (Flynn et al. 2023). This justification should include a discussion of the "similarities and differences" of the model to the human condition studied, so that others can better assess the translatability of the findings of the study (Flynn et al. 2023).

#### *15.1.3 Improve the Simulation of Chronic Diseases*

Future models need to be more accurate in simulating the long-term nature of human wounds (Flynn et al. 2023). The authors suggest that long-term conditions should be better represented in models of diseases like diabetes (Flynn et al. 2023). Rather than merely replicating acute effects, this involves making sure animals are older and exhibit biomarkers that correlate with the human disease state (Flynn et al. 2023).

### 15.2 Advice on the Research Process

#### *15.2.1 Adopt a Progressive, Multi-Model Approach*

The authors advise using a tiered approach to research (Flynn et al. 2023). To confirm that a treatment affects the targeted pathway (e.g., in vitro cell cultures), experiments should start with simple models. (e.g. migration and proliferation of cells) (Flynn et al. 2023). After confirmation, research should move on to more sophisticated models that closely resemble the intended human condition "before moving on to human trials" (Flynn et al. 2023). This methodical process aids in the validation of results at every stage (Flynn et al. 2023).

#### *15.2.2 Foster Collaboration*

Clinicians who treat human wounds must closely collaborate and communicate with basic science researchers in order to develop better models (Flynn et al. 2023).

### 15.3 Advice on Adopting New Technologies

#### *15.3.1 Explore Advanced In Vitro Models*

To get around the drawbacks of conventional models, the authors advocate using emerging technologies (Flynn et al. 2023). These include
Vascularized 3D Skin Equivalents (Flynn et al. 2023), which can be used to better understand angiogenesis (Flynn et al. 2023).
Microfluidics (Flynn et al. 2023) and Skin-on-a-Chip s (Flynn et al. 2023), which can be used to create dynamic systems that more closely resemble the physiological environment of human tissue (Flynn et al. 2023).
3D Skin Bioprinting, which can be used to create complex and highly reproducible skin constructs (Flynn et al. 2023).

### 15.4 Leverage New Technologies for High-Throughput Screening

The authors point out that high-throughput screening of possible therapeutic molecules can be achieved through computer modeling and sophisticated 3D models (Flynn et al. 2023). This could eliminate the need for some preclinical animal models by enabling the direct testing of promising candidates in humans (Flynn et al. 2023).

**Data availability:** Not applicable
**Ethics statement**: No animal or human trials have been conducted. Paper is research review
**Competing Interests**: The author declares that he has no known competing financial interests or personal relationships which could appear to affect the work reported in this paper.
**Funding**: No funds was received for this project
**Credit statement:** The sole author was responsible for the conception, methodology, software, validation, formal analysis, research, resources, data mining, writing - original proposal, writing - review and editing, visualization, supervision, project management, and obtaining funding. The author also completed all stages of proofreading, plagiarism check and final validation.
**Acknowledgement:**

Cite:

## APA (7th edition)

Dr. Navneet O. Soni. (2026). *A critical review of preclinical models in chronic wound healing research: Challenges and future directions*. **Journal of Wound Medicine**, 1(2), 18–50. https://doi.org/10.5281/zenodo.19037280

ASA:
Dr. Navneet O. Soni. 2026. "A Critical Review of Preclinical Models in Chronic Wound Healing Research: Challenges and Future Directions." *Journal of Wound Medicine* 1(2):18–50. https://doi.org/10.5281/zenodo.19037280.

NLM:
Soni NO. A critical review of preclinical models in chronic wound healing research: Challenges and future directions. *Journal of Wound Medicine*. 2026 Mar–Apr;1(2):18-50. doi:10.5281/zenodo.19037280.

## Vancouver

Soni NO. A critical review of preclinical models in chronic wound healing research: Challenges and future directions. *Journal of Wound Medicine*. 2026;1(2):18-50. doi:10.5281/zenodo.19037280

**Table 1 In Vivo Models: Animal**

| Animal Model | Unique Features & Advantages | Limitations |
|---|---|---|
| Rodents (Mice & Rats) | Features:<br>Most common model; have a "panniculus carnosus "muscle.*<br>Rat tails offer a model with less contraction.*<br>Advantages:<br>Low cost, easy handling and extensive knowledge base.*<br>Can be genetically modified or chemically induced to model diseases like diabetes. * | Limitations:<br>Heal primarily by "wound contraction", unlike human re-epithelialization, making data on wound closure poorly translatable. *<br>Induced diabetes models often don't represent the long-term effects of the disease in humans. * |
| Pigs | Features:<br>Considered a superior model due to skin anatomy and physiology closely resembling humans. *<br>Skin contains rete ridge-like structures. *<br>Advantages:<br>Dermal thickness, hair follicle architecture, and immune responses are similar to humans.*<br>Partial-thickness wounds heal by re-epithelialization. * | Limitations:<br>High cost, large housing requirements, and greater risk of infection. *<br>Different sweat gland composition (mostly apocrine, few eccrine), which impacts the source of epidermal stem cells. * |
| Rabbits | Features:<br>The ear model has cartilage that prevents "wound contraction" (splinting effect). *<br>Large ear vasculature suitable for creating ischemic models*.<br>Advantages:<br>Excellent for studying re-epithelialization, hypertrophic scar formation, and reversible ischemia. * | Limitations:<br>Limited genetic traceability and availability of species-specific reagents.*<br>Tissue on the body (aside from the ear) heals by contraction.* |
| Guinea Pigs | Features:<br>Like humans, they cannot synthesize their own Vitamin C. *<br>Advantages:<br>Inexpensive and useful for studying collagen synthesis in a vitamin-deficient state. * | Limitations:<br>Heal by "wound contraction", limiting translatability. * |
| Zebrafish | Features:<br>Wound healing phases occur sequentially, not overlapping.*<br>Advantages:<br>Allows for the isolated study of each specific phase of healing (hemostasis, inflammation, etc.). *<br>Heal by re-epithelialization.* | Limitations:<br>Heal extremely rapidly. *<br>Less established as a model*. |

*(Dorsett-Martin 2004; Flynn et al. 2023; Grada, Mervis, and Falanga 2018; Masson-Meyers et al. 2020; Sami, Heiba, and Abdellatif 2019_ Soni2026)

**Table 2 In Vivo Models: Human**

| Human Model | Unique Features & Advantages | Limitations |
|---|---|---|
| Skin Stripping | Features:<br>Uses adhesive tape to remove only the stratum corneum. [2]<br>Advantages:<br>Minimally invasive; excellent for studying skin barrier function and recovery[1] | Limitations:<br>Creates a very minimal wound, limited to the most superficial layer. [1] |
| Suction Blister | Features:<br>Creates a clean separation between the epidermis and dermis using negative pressure. . [1]<br>Advantages:<br>Heals without scarring; allows for extraction of immune cells and fluid for analysis. . [1] | Limitations:<br>The induced wound may not represent a typical traumatic injury. [1] |
| Abrasive Wound | Features:<br>Removes the epidermis with a surgical brush, mimicking a scrape. [1]<br>Advantages:<br>More representative of a common real-life injury; useful for testing wound dressings. [1] | Limitations:<br>More invasive than stripping or blisters. [1] |
| Excision Wounds | Features:<br>Involves surgically removing either a partial (split-thickness) or entire (full-thickness) section of skin down to the subcutaneous fat. [1]<br>Advantages:<br>Most useful model for studying the entire wound healing process, including angiogenesis, tissue regeneration, and scar formation. [1] | Limitations:<br>The most invasive method. [1] |

[2] (Flynn et al. 2023)

**Table 3 In Vitro Models**

| In Vitro Model | Unique Features & Advantages | Limitations |
|---|---|---|
| 2D Cell Monolayer | Features:<br>A wound (e.g., a scratch) is created in a single layer of cells. [1]<br>Advantages:<br>Simple, inexpensive, and useful for analyzing cell migration, proliferation, and viability. [1] | Limitations:<br>Does not represent the 3D skin structure or complex cell-cell interactions. [1]<br>Results often do not translate to in vivo studies because they lack immune and vascular components. [1] |
| 3D Assays (Skin Equivalents) | Features:<br>Constructed from cells and ECM to mimic skin's dermal and epidermal layers. [1]<br>Advantages:<br>More closely mimics the physiological and cellular environment of skin compared to 2D models. [1] | Limitations:<br>Often lack crucial systems like blood supply, hair follicles, and immune cells. [1]<br>Technically strenuous to create. [1] |

[1] (Flynn et al. 2023)

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