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The Wound Reporting in Animal and Human Preclinical Studies (WRAHPS) Guidelines

Nkemcho Ojeh PhD[1,2] | Nicole M. Vecin MD, MPH[1] | Irena Pastar PhD[1] | Susan W. Volk VMD, PhD[3] | Traci Wilgus PhD[4] | Sarah Griffiths PhD[5] | Allison N. Ramey-Ward PhD[6] | Vickie R. Driver DPM, MS[7] | Luisa A. DiPietro DDS, PhD[8] | Lisa J. Gould MD, PhD[9] | Marjana Tomic-Canic PhD[1]

[1]Wound Healing and Regenerative Medicine Research Program, Dr Phillip Frost Department of Dermatology and Cutaneous Surgery, University of Miami Miller School of Medicine, Miami, Florida, USA

[2]Department of Preclinical and Health Sciences, Faculty of Medical Sciences, The University of the West Indies, Bridgetown, Barbados

[3]Department of Clinical Sciences and Advanced Medicine, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA

[4]Department of Pathology, The Ohio State University, Columbus, Ohio, USA

[5]Wound Care Collaborative Community (WCCC), Orlando, Florida, USA

[6]ProgenaCare Global, LLC, Marietta, Georgia, USA

[7]School of Medicine, Washington State University, Spokane, Washington, USA

[8]Center for Wound Healing and Tissue Regeneration, University of Illinois Chicago, Chicago, Illinois, USA

[9]South Shore Hospital Center for Wound Healing, Weymouth, Massachusetts, USA

Correspondence
Marjana Tomic-Canic, Wound Healing and Regenerative Medicine Research Program, Dr Phillip Frost Department of Dermatology and Cutaneous Surgery, University of Miami Miller School of Medicine, Miami FL, USA.
Email: mtcanic@med.miami.edu

Lisa J. Gould, South Shore Hospital Center for Wound Healing, Weymouth, MA, USA.
Email: lgould44@hotmail.com

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Abstract

Preclinical studies for wound healing disorders are an essential step in translating discoveries into therapies. Also, they are an integral component of initial safety screening and gaining mechanistic insights using an in vivo approach. Given the complexity of the wound healing process, existing guidelines for animal testing do not capture key information due to the inevitable variability in experimental design. Variations in study interpretation are increased by complexities associated with wound aetiology, wounding procedure, multiple treatment conditions, wound assessment, and analysis, as well as lack of acknowledgement of limitation of the model used. Yet, no standards exist to guide reporting crucial experimental information required to interpret results in translational studies of wound healing. Consistency in reporting allows transparency, comparative, and meta-analysis studies and avoids repetition and redundancy.

Abbreviations: 3D, three-dimensional; CFUs, colony forming units; CLSM, confocal laser scanning microscopy; CONSORT, Consolidated Standards of Reporting Trials; db/db, The genetically diabetic (db/db) mice, homozygous for a mutation in the leptin receptor ($Lepr^{db}$); DFU, diabetic foot ulcer; DM, diabetes mellitus; DRS, diffuse reflectance spectroscopy; FDA, US Food and Drug Administration; GIPRdn, dominant-negative glucose-dependent insulinotropic polypeptide receptor mutant; GLUT2, Glucose transporter 2; H&E, haematoxylin and eosin; ICH, The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; iPSC, induced pluripotent stem cells; MSS, Manchester Scar Scale; NIR, near-infrared; NONcNZO, a recombinant congenic strain that models human obesity-induced Type 2 diabetes and Metabolic Syndrome; ob/ob, mice homozygous for the obese spontaneous mutation, Lep^{ob}; OCT, optical coherence tomography; PDGF-BB, platelet-derived growth factor-BB; PS-OFDI, polarisation-sensitive optical frequency domain imaging; qPCR, quantitative polymerase chain reaction; STZ, streptozotocin; TEWL, transepidermal water loss; VSS, Vancouver Scar Scale; WCCC, Wound Care Collaborative Community; WHS, Wound Healing Society; WIHN, wound-induced hair neogenesis; WRAHPS, Wound Reporting in Animal and Human Preclinical Studies.

On behalf of the Wound Care Collaborative Community (WCCC) and the Wound Healing Society (WHS).

Therefore, there is a critical and unmet need to standardise reporting for preclinical wound studies. To aid in reporting experimental conditions, The *W*ound *R*eporting in *A*nimal and *H*uman Preclinical *S*tudies (WRAHPS) Guidelines have now been created by the authors working with the Wound Care Collaborative Community (WCCC) GAPS group to provide a checklist and reporting template for the most frequently used preclinical models in support of development for human clinical trials for wound healing disorders. It is anticipated that the WRAHPS Guidelines will standardise comprehensive methods for reporting in scientific manuscripts and the wound healing field overall. This article is not intended to address regulatory requirements but is intended to provide general guidelines on important scientific considerations for such studies.

KEYWORDS
human wound models, preclinical testing, reporting guidelines, wound-healing animal models

1 | INTRODUCTION

Wound healing disorders pose significant challenges to patients and healthcare providers,[1,2] necessitating extensive preclinical studies to translate discoveries into effective products and therapies. Numerous preclinical studies comprising in vitro, in vivo, and ex vivo models have been established and modified over the years and have contributed to the advancement of knowledge and understanding of the molecular and cellular mechanisms that contribute to wound repair.[1,3] Despite this, the pathophysiology of wounds remains unclear, contributing to a paucity of effective treatments for patients. The development of effective therapies has further been hampered partly due to difficulty in translating results from preclinical models to clinical use, as no single model can convey a comprehensively realistic representation of the disease state and environment that contributes to impaired wound healing in humans. Moreover, the complexity of the wound healing process and mixture of biological variables introduces inherent variability into experimental design. Existing United States Food and Drug Administration (FDA) guidelines for animal testing and appropriate selection of preclinical models, such as General considerations for animal studies intended to evaluate medical devices,[4] ISO 10993-6:2016 Biological Evaluation of Medical Devices – Part 6: Tests for Local Effects after Implantation,[5] 2022 Biological Evaluation of Medical Devices – Part 2: Animal Welfare Requirements,[6] ICH S6 (R1) Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals – scientific guideline,[7] and the Guidance for Industry Chronic Cutaneous Ulcer and Burn Wounds – Developing Products for Treatment[8] provide important guidance for the investigator, but may fall short of clear recommendations for individual studies due to the specialised nature of wound healing experimental designs. Coupled with the paucity of guidelines on documentation and reporting of key information, and lack of standardisation in experimental design, reproducibility of animal wounds experimental design and results, as well as interpretation and comparative analyses of published data often becomes challenging and has created a barrier to develop new innovative therapies for patients who suffer with wounds.

Evidence to support such guidelines would be effective in increasing standardisation, and improving accuracy in interpretation is related to benefits gained from Consolidated Standards of Reporting Trials (CONSORT) guidelines. These guidelines for human clinical trials have been attributed to promoting consistency in reporting and enabling transparency, comparative, and meta-analysis studies, while avoiding redundancy and repetition in clinical research. Hence, there is a critical need for standardised reporting in preclinical testing for wound healing similar to CONSORT guidelines. To address this, we developed Wound Reporting in Animal and Human Preclinical Studies (WRAHPS) checklists and reporting templates which aim to provide standardised guidelines for reporting experimental conditions in the most frequently used preclinical animal and human ex vivo models in wound healing research.

In this paper, we present a comprehensive account of the WRAHPS checklist and reporting template for both animal wound models (comprising 40 items) (Checklist 1) and human ex vivo wound models (consisting of 24 items) (Checklist 2). In addition to Checklists 1 and 2 at the end of this article, a downloadable and fillable PDF checklists are provided in the supplement and can be used to accompany future submissions for any journal. The rationale for inclusion of these checklist items is explained below, along with illustrative examples from published preclinical studies showcasing the impact of these items on wound healing outcomes and emphasizing the importance of their inclusion for comprehensive reporting. In addition, an overview of the strengths and limitations of these preclinical wound models is presented. Factors and considerations that should be taken into account while employing these models are also discussed. This article also provides a summary of the most utilized animal and human ex vivo wound models in preclinical testing.

2 | ANIMAL WOUND MODELS

Laboratory animals (e.g., rodents, rabbits, and pigs) are extensively utilised in preclinical studies for human wound healing disorders and to

support regulatory submissions for wound care products. These important models provide valuable mechanistic insights to wound healing processes and how experimental interventions affect said processes through in vivo experimentation. These models comprise different species, anatomic differences, and techniques, each with distinctive attributes that must be considered when examining particular aspects of the wound healing process and chronic wound pathology. However, no animal model has been shown to generate results that are completely translatable to human wound healing physiologically or to be able to recapitulate full pathological chronic wound conditions due to differences in skin anatomy, species and wound-specific physiology, and mechanisms.[9–12] Conceptually, these models are not bona fide 'chronic' wound models – rather, these are acute skin wound models with one or more imposed conditions introduced into acute wound setting that lead to impaired healing. Careful consideration should be taken when selecting a suitable model for preclinical testing as each model has its strengths and limitations.

3 | WRAHPS CHECKLIST AND REPORTING TEMPLATE FOR ANIMAL WOUND MODELS

The WRAHPS checklist and reporting template for animal wound models include the most frequent models and experimental details reported in scientific wound literature. This should serve to assure that specific key elements of experimental design are consistently being reported in a standardised manner, and to guide discussion of results in the context of the model used. We recognise that there will be unique characteristics of experimentation and the checklist cannot serve as one fit for all. Such unique experimental features should be reported in the narrative in addition to the information in the universal checklist.

4 | POTENTIAL UTILISATION

This checklist is intended to standardise reporting of nonclinical wound healing studies and is not intended to define regulatory expectations. We envision this checklist being used by authors as a guide for the experimental design of preclinical testing studies, and to ensure comprehensive standardised reporting of their studies. It is intended as an open-source living document whereby updates will occur based on its utilisation and feedback. To promote widespread adoption and accessibility, we suggest that journals consider incorporating this checklist into their submission guidelines or recommending its inclusion as Supplementary material S1.

We anticipate that WRAHPS guidelines will serve as a comprehensive primer on various types of wound models and a thorough reporting template, encouraging both researchers and readers to consider and be aware of the important scientific variables and considerations that apply to these models. It is intended for preclinical testing studies that will serve to support further development for applications to human use. By using this approach, WRAHPS aims to:

1. guide wound healing researchers in accurately documenting and reporting experimental conditions to regulatory agencies, ensuring compliance with animal testing recommendations;
2. standardise methods reporting in scientific journals to promote consistency and facilitate the dissemination of reliable and reproducible findings;
3. provides guidance and assistance in designing wound healing preclinical testing studies;
4. help guide scientific review regarding the assessment of experimental design;
5. complement existing animal research experiment reporting (such as ARRIVE) that lack specific details and requirements for wound healing studies.

4.1 | Section I: Animal wound model

Section 4.1 on 'Animal Wound Model' of WRAHPS checklist and reporting template (Checklist 1) captures key information regarding the types of animals used, including biological variables (e.g., species, strain/breed/genotype, age, sex, weight, genetically modified), the type of wound simulated (e.g., chronic, acute) and wound aetiology simulated. In addition, if diabetic models are employed, the method of induction, documentation of diabetic state, and other diabetes-related information should be disclosed. The section on Section 6 highlights the most common animals used in preclinical studies as well as their relevance, strengths, and limitations.

4.1.1 | Biological variables

Animal models of impaired wound healing may include magnetic pressure-induced, burns, surgical, redox manipulated, ischaemic, diabetic, and induced infection models, among others.[1,13] Data obtained from using these models may vary considerably depending on the species chosen and other biological variables, such as sex, age, hair cycling, microbiome diversity, metabolic underpinnings, wound type, and simulated wound aetiology.[3,13] Factors such as cost, required expertise, availability, animal welfare requirements, and ease of handling also impact selection of models with the highest fidelity of human wound healing.

The phases of wound healing across species may follow a similar pattern to humans; however, timeline, healing mechanisms, and skin and underlying tissue anatomy may be significantly different (and differ between animal models), so physiological and anatomical relevance to humans should be considered when choosing a preclinical model. Some of the differences between rodents and humans that may affect wound healing studies are listed in Table 1. Rodents' loose skin that is associated with the underlying panniculus carnosus muscle layer enables wounds to more efficiently contract compared with human skin, which is more firmly attached to a thicker hypodermis on top of the muscle. Consequently, rodent wounds heal primarily by contraction while human wounds heal by re-epithelialization and granulation

TABLE 1 Examples of rodent and human differences that affect wound healing studies.

Characteristic	Rodents	Humans
Skin Structure	Thinner epidermis with fewer keratinocyte layers; loose skin with underlying panniculus carnosus muscle; eccrine and apocrine sweat glands in skin lacking	Thicker epidermis with more keratinocyte layers; skin firmly attached to thicker hypodermis on top of muscle; eccrine and apocrine sweat glands present in skin
Muscle Location	Panniculus carnosus muscle located directly beneath the hypodermis in most body regions, except the tail	Muscle located below the fascia, which is beneath the hypodermis and is not equivalent to the panniculus carnosus muscle present in most rodent body regions
Wound Healing Pattern	Primarily by rapid contraction due to loose skin and panniculus carnosus muscle layer	By granulation tissue formation and re-epithelialization
Hair Follicles	Dense hair follicle population with faster healing in areas of higher density; shorter hair cycles that affect healing rates; quicker healing in anagen phase	Fewer hair follicles; hair cycles are longer, highly variable and region-dependent; hair follicle stem cells aid in re-epithelialization
Healing Timeline	Faster healing cycles completed within 7 days in mice and 12–14 days in rats	Slower healing rates which can take up to 2 years
Other Factors	Differences in immune, inflammation, and genetic background compared with humans	Differences in immune, inflammation, and genetic background compared with rodents

tissue formation.[3,9,10,14,15] There are also differences in skin thickness, and variations in the number, distribution, and behaviour of skin appendages, that is, hair follicles, apocrine sweat glands, eccrine sweat glands,[16] follicular patterns, and hair growth cycle. For example, mice have a thinner epidermis, fewer keratinocyte layers, and a dense population of hair follicles compared with humans,[17,18] and wounds in areas with higher hair density have been shown to heal quicker than those in less hairy or non-hairy areas.[19] During wound healing, hair follicle stem cells residing in the bulge region[20] migrate to the epidermis to aid re-epithelialization in humans and mice.[21,22] The hair growth cycle comprises anagen (active growth phase), catagen (partial degeneration phase), telogen (resting phase), and exogen (hair shedding).[23] Ansell et al.[19] reported faster wound healing rates during the anagen phase of the hair cycle compared with the telogen phase in mice. In addition to accelerated re-epithelialization, increased vascularization, and reduced inflammation were also observed in anagen skin wounds, indicating that the hair cycle stage greatly influences wound healing.[19]

The wound healing cycle is also different in humans and animals; as remodelling phase can take longer in humans (up to 2 years) compared with mice or rats which heal much faster,[9,24] although one can argue that we do not follow animal experiments as long. Sex of animal may also affect healing. For instance, male rodents have a thicker dermis, and their skin is 40% stronger than female rodents, who have a thicker epidermis and hypodermis.[25] Furthermore, a potential protective role for oestrogen is suggested by an increase in wound healing impairment observed in young male diabetic rats compared with female subjects.[26] Other factors to consider when translating wound healing from animal studies to humans are the immune, inflammatory, and genetic background and age of the animals.[27–29]

4.1.2 | Wound type and wound aetiology simulated

Acute wound models, including excisional, incisional, and thermal injury burn models, have been extensively characterised with well-defined protocols.[30] In contrast, the emulation of chronic wounds in animal models represents a more intricate challenge due to the inherent complexity of chronic wound pathophysiology and the fact that experimental (laboratory) animals do not naturally exhibit chronic wound states.[9,24,31,32] As such, transformation of acute wound models into those that model the chronic wound state must occur through the induction of diabetes, obesity, mechanical pressure, ischaemia, reperfusion injury, and infection. These models seek to mirror the underlying pathophysiology of human chronic diseases in animal systems, enabling the study of wound healing processes under specific conditions and the evaluation of therapeutic interventions for complex wounds. However, these experimental paradigms carry inherent limitations, stemming from the dissimilarity between impaired animal and chronic human wound responses. Distinctive species-specific variations in wound healing mechanism, tissue architecture, and immune responses contribute to the challenge of accurately representing human chronic wound states in animals. Consequently, no singular animal model has succeeded in fully reproducing all aspects of human chronic wounds; instead, each model captures specific facets of chronic ulcer characteristics. Hence, the careful selection of an appropriate wound model, along with thorough reporting of methodology and results, becomes crucial to ensure the production of translatable outcomes.

4.1.3 | Diabetic models

Diabetes mellitus (DM) is a major comorbidity of human chronic wounds.[1] DM can be induced in animal models by chemical, genetic manipulation, or diet. Type I DM arises from the autoimmune-induced inflammatory destruction of pancreatic beta-cells, leading to significantly impaired insulin production. Type II DM, highly prevalent globally typically affecting adults, is a chronic multifactorial condition that arises from the dysregulation of blood glucose utilisation as a fuel source due to insulin resistance.[9] Summarised characteristics and

TABLE 2 Characteristics and Limitations of Different Animal Models for studying Type I and II Diabetes Mellitus (DM).

Animal model	Induction method	Chemical/genetic name	Characteristics of model	Limitations of model
Rodents	Chemical	Streptozotocin Alloxan	Induction of diabetes caused by pancreatic islet β-cell necrosis results in pathophysiology and pathological characteristics similar to humans[173]	• Adverse effects; susceptibility and sensitivity to streptozotocin and alloxan varies among different rodent strains and gender, generalising findings[43] • Not suitable for studies >60 weeks; may not reflect aging-related aspects of diabetes[39] • db/db, ob/ob and zucker models are limited due to simplistic leptin abnormalities; whereas human Type 2 DM is polygenic[10] • Only NONcNZO10 male mice develop hyperglycaemia[174]
	Genetic (Type 1 DM)	Akita mouse	Develop insulinopenia and hyperglycaemia at 4 weeks post-birth due to pancreatic islet β-cell destruction; male mice exhibit more severe hyperglycaemia than females[36]	
	Genetic (Type 2 DM)	db/db mouse	Resistant to leptin; show progressive elevated insulin resistance, obesity and hyperglycaemia with advanced age[37] Natural disease progression with prolonged hyperglycaemic states[24]	
		ob/ob mouse	Exhibit obesity, hyperinsulinemia, hyperphagia, hyperglycaemia[173]	
		NONcNZO10 mouse	Polygenic background of diabetes with moderate obesity; exhibit insulin resistance and hyperglycaemia[35]	
		Zucker diabetic fatty rat (fa/fa rat)	Exhibit obesity, hyperphagia, and insulin resistance; fa/fa male rats develop diabetes as early as 10 weeks of age, reaching 100% incidence by 21 weeks of age[38]	
Rabbits	Chemical	Streptozotocin Alloxan	Induction of diabetes caused by pancreatic islet β-cell necrosis results in pathophysiology and pathological characteristics similar to humans[173] Survive up to a year post-induction with alloxan; exhibit long-term effects like fatty liver, kidney disease, impaired wound healing[40]	• STZ more toxic in rabbits, alloxan preferred over STZ; careful monitoring post-induction is important[35]
Pigs	Chemical	Streptozotocin Alloxan	Induction of diabetes caused by pancreatic islet β-cell necrosis results in pathophysiology and pathological characteristics similar to humans[173] Anatomically, physiologically, and metabolically similar to humans; tolerates chemically induced Type 1 DM treatment[41,42]	• Expensive animal model with higher cost for care and housing[173] • Streptozotocin less effective due to low GLUT2 levels; higher doses may induce hepatic and renal toxicity[45]
	Genetic (Type 2 DM)	GIPR(dn) transgenic pig expressing human dominant-negative GIP receptor mutant in pancreatic β-cells	Display decreased glucose tolerance due to delayed insulin secretion, and decreased insulin secretion and pancreatic β-cell mass with advancing age[139]	

limitations of different animal models for studying Type I and II DM are presented in Table 2. Several rodent models have been used to simulate both Type I and Type II diabetes. Type I DM is commonly induced in rodents and pigs with chemicals such as streptozotocin (STZ) or alloxan at high doses, causing destruction of insulin-producing pancreatic beta-cells, or by using genetically modified rodents.[24,31,33,34] Using STZ, mild to severe Type I DM can be produced with different parameters, including the route of administration, dosage, age, and strain.[35] Akita mice, which develop Type I DM through an autosomal dominant missense mutation of the insulin 2 gene develop features such as insulinopenia and hyperglycaemia at 4 weeks post-birth. Male mice exhibit more severe hyperglycaemia compared with females and may require more diabetes management, such as insulin administration, to survive,[36] compared with other types of models. The most common genetic animal models of Type II DM are the db/db mice, with a point mutation in the leptin receptor gene that exhibits progressively elevated insulin resistance, obesity, and hyperglycaemia as the rodent advances with age[37]; the obese ob/ob mice, which are leptin deficient and exhibit hyperphagia and insulin resistance; the NONcNZO mice, a recombinant strain with a polygenic background of diabetes with moderate obesity; and the Zucker fa/fa rats, which contain a missense homozygous mutation (fatty, fa) in the leptin receptor gene *LepR* and are resistant to leptin.[13,31,38]

Though these diabetic animal models are useful, there are some limitations. Human Type II DM does not simply involve leptin

abnormalities and is polygenic, so the db/db, ob/ob, and Zucker models can be less informative when it comes to predicting human outcomes. Additionally, there are inherent differences in fasting blood plasma levels in diabetic models compared with humans.[35] While rodents provide diverse utility in studying various aspects of diabetes-related pathophysiology, there are indications that mice and rats may not be ideal in conjunction with advanced age (>60 weeks).[39] The use of rabbits could offer an alternative, as they can survive for up to a year after Type I DM induction with alloxan, and after treatment with insulin, they can exhibit long-term effects of Type I DM, such as fatty liver disease, kidney disease, and impaired wound healing.[40] Pigs may offer additional utility over rabbits in that they are more metabolically similar to humans, share morphologically similar skin, pancreas, and tolerate chemically induced Type I DM treatment well.[41,42]

Certain factors need to be considered when using chemically induced models for experiments to simulate human chronic diseases. Chemical induction used to create Type I DM can have adverse effects on rodents and must be titrated appropriately to ensure viability of the animal. Hence, many studies create wounds as early as 1–2 weeks after hyperglycaemic states have been established, which may be too short to allow for the study of long-term effects of diabetes (e.g., reduced vasculature, neuropathy, and cardiovascular disease). db/db mice may be a more appropriate model for long-term effects, as they display natural disease progression with prolonged hyperglycaemic states,[24] although these time frames must be incorporated in model development. Some of the chemicals used for inducing diabetes are more toxic in some species, and no standardised consensus on the parameters regarding chemical induction currently exists, possibly due to sensitivity issues. For example, rodent strains and genders show high variability in sensitivity to STZ.[43] STZ is also more toxic in rabbits, so alloxan is preferentially used.[44] Pigs do not respond as well to STZ due to low GLUT2 levels.[45] However, this issue can be compensated for by increasing the STZ dosages, although doing so carries the risk of hepatic and renal toxicity.[45] Careful monitoring after the induction of Type I DM to reduce animal mortality rates is therefore important in animal management.[44]

4.1.4 | Impact of timing and sampling frequency on healing outcomes in diabetic wound studies

Published studies on animal diabetic wound models often overlook or do not report crucial information, such as the impact of the duration of diabetes on wound healing outcomes, the DM induction timeframe, confirmation of diabetes, time duration between diabetes induction and subsequent injury, monitoring and management of diabetes information, and any special treatments received prior to wound generation. These factors may significantly influence wound healing outcomes. One example is insufficient capture of healing deficits. Diabetic rats exhibited delayed re-epithelialization 2–3 weeks after STZ induction[26] but effects on angiogenesis and collagen deposition required longer timelines, up to 6 weeks post-induction.[26] Given that most studies typically utilise earlier endpoints of 3 weeks,[26] the impact on these important wound healing processes are often missed. Hence, methodology and reporting key variables in preclinical animal diabetic wound studies is essential to promote transparency, the ability to reproduce experiments, and allow for interpretation and comparative analysis of published data.

4.1.5 | Enhancing diabetic models to exhibit delayed wound healing

Diabetic rodent models have been shown to exhibit delayed healing, which is further exaggerated in aged individuals and in larger excisional wounds. Although healing is delayed relative to that seen in non-diabetic individuals, wound closure is achieved relatively rapidly, in contrast to human diabetic wound healing which occurs with prolonged delays in wound healing/closure. For example, 6 mm diameter wounds in STZ-induced Type I DM rats closed at 15 days compared with the 12 days observed with non-diabetic controls.[46] Similarly, in STZ-induced Type I DM pigs, 1.5 cm × 1.5 cm full-thickness wounds showed delayed wound re-epithelialization, with closure seen after 18 days compared with 12–14 days for non-diabetic pigs.[42] However, further refinement (including modelling other co-morbidities often accompanying human diabetic state) is needed to optimise utility of various diabetic models' impacts on impaired wound healing and associated clinical relevance. Huynh et al.'s[47] analyses suggest granularity is possible between diabetic and non-diabetic wound healing rates but additional studies are required.

To achieve better clinical representation of the healing of chronic wounds, diabetic animal wound models are often modified with the use of multiple factors that delay wound repair. For example, inoculation of *Staphylococcus aureus* biofilms into splinted excisional wounds of db/db mice delayed epithelization and wound closure.[48] In another study, wounds in aged db/db mice displayed healing with reduced stiffness and breaking load, along with decreased granulation tissue deposition that is independent of glycaemia.[49] Moreover, manipulating redox parameters in diabetic mice in combination with wound infection extends the 'chronicity' of the wound.[50–52] As no animal model can perfectly and comprehensively mimic the wound chronicity arising from diabetes in humans,[10] selecting the right animal model will be contingent upon how relevant it is to a particular aspect of the wound healing parameter being investigated.

4.1.6 | Ischaemic models

Ischaemic wound models have been established in rodents, rabbits, and pigs. The original flap model was developed in rodents by McFarlane et al.[53] Later, a modified model using a silicone sheet beneath the flap was shown to hinder revascularization from the underlying body wall, while still allowing lateral revascularization from the flap edges. This modification prevented healing by contraction, simulated the human disease state more closely, and allowed for greater translatability to human healing physiology.[54] It is important to consider the

impact of model modification, such as foreign body implantation, on the risk of infection or increased inflammation. The rabbit ear model, widely used to simulate ischaemic wounds and first described by Ahn and Mustoe,[55] circumvents this risk. Due to the large and easily accessible vasculature present in the large rabbit ear, it lends itself well to the ischaemic wound model. This wound model is generated by ligating the arterial blood supply, depriving the ear of blood flow,[9,55–57] and creating a full-thickness punch biopsy wound down to the cartilage layer.[55,58,59] Chien and Wilhelmi[56] refined this model by creating minimally invasive ischaemic wounds in rabbit ears which were effective and showed no signs of infection, bleeding, or skin rupture in the incisions. Another factor to consider is the length of time the ischaemic wounds stay open in order to directly imitate the chronicity of human wounds. New blood vessels develop after 10–14 days in the ischaemic rabbit ear model, while there is restoration of blood supply around 2–4 weeks in the skin flap model.[35]

Ischaemia–reperfusion (IR) models also exist and are created with the use of magnetic plates.[60,61] These animal models are useful in studying pressure ulcers, which mostly affect the elderly and immobile people.[62] However, data obtained from these studies have been inconsistent and direct comparisons difficult due to different parameters (type and strength of magnets used, duration, and number of IR cycles, etc.). Moreover, anatomic differences between rodent skin and that of aged human skin likely further negatively impacts model fidelity. Therefore, interpretation of data obtained from these studies may not be directly translatable to the human context.

4.2 | Section II: Wounding process

The current lack of standardisation of wounding process in animal preclinical testing renders comparisons across studies difficult.[63] Section 4.2 of the animal wound model checklist captures relevant information such as the use of anaesthesia, wound location, wound creation, depilation technique, type of tools used to generate wounds, splinted versus non-splinted, wound size, number of animals used, number of animals per experimental/treatment group, use of power analysis, and experimentally induced infection. These factors may impact wound healing outcomes and it is therefore essential that they are considered when developing animal protocols, and when reporting experimental information and results. We describe how these factors may affect wound healing outcomes below.

4.2.1 | Wounding and location

Wound healing may be affected by hair cycle stage[19] and depilation methods, such as plucking, shaving, clipping, and creams, which can influence hair follicle cycling. For instance, depilation through hair plucking is known to induce anagen hair cycling.[64–66] Moreover, wound location can significantly impact wound healing outcomes, depending on factors like skin thickness, hair density, hair cycle status, and skin anatomy.[10,19] For example, rodent tails lack the panniculus carnosus muscle and display reduced wound contraction.[67] Consequently, the tail wounds take longer to heal (around 21 days) compared with traditional dorsal wounds, which heal within days.[67] Rodent ear skin, which contains minimal subcutaneous tissue and a thin epidermal layer, exhibits rapid healing of ear punch wounds within 1 week, primarily through epithelialisation.[68,69] On the other hand, full-thickness excisional pedal wounds in the control group of non-diabetic rats exhibited slightly longer healing durations, with complete epithelialization observed between 13[70] and 16 days (15.9 ± 2.5 days),[71] depending on the initial wound size.

Another point for consideration is which animal model is best suited for the type of wounds (incisional, excisional, pressure injury, burn) and the specific wound parameters and treatment being investigated. For example, a partial-thickness excisional model is commonly used to study re-epithelialization and its effects on processes like ageing[72] or the evaluation of topical therapeutics.[73] On the other hand, an incisional wound healing model using primary intention can be valuable for biomechanical analyses of wound strength. However, it may be less useful for histological assessment of healing or investigating epithelialization or wound tissue biochemistry due to the restricted volume of wound healing activity.[74] For a comprehensive study of wound physiology, including inflammation, granulation tissue formation, re-epithelialization, angiogenesis, and remodelling, excisional wounds are particularly relevant.[24] Wounds can be created in various ways depending on the specific human condition or wound type being investigated. Excisional wounds are a common model, but large methodological variations exist, such as the size of the excision (ranging from 2 to 20 mm in diameter), the number of wounds per animal, the tools used to perform the excision (surgical scissors, punch biopsy, lasers etc.), the use of splints, and in rodents, whether the panniculus carnosus is removed with the excised skin or is left intact in the wound bed. All of these factors can affect wound healing outcomes.[63] Conversely, incisional wounds tend to be more consistent across studies, with wounds ranging from 10 to 15 mm in length and being full-thickness wounds created by scalpel injury. However, sutures used to close wound margins have been demonstrated to affect the tensile forces across wounded skin.[63]

Splinting encompasses a range of techniques, including the use of native splinted models such as the rabbit ear model,[55] the mouse cranial model,[68] and tail model,[75] as well as the use of splinting devices including silicone rings, steel rings, polydimethylsiloxane devices, distraction devices (which distend the wound), and dorsal skin fold chambers. Most of these devices require fixation through gluing or suturing in place or come equipped with tooth edges that secure the device on shaved skin or without depilation. When using splints, it is important to consider factors such as the number of sutures, width of inner and outer diameter, depth, spacing, and thickness of splints as these factors may influence healing outcomes.[76–80] Studies have reported variable results obtained with splinting models depending on the type of wound healing model used. For example, recombinant human platelet-derived growth factor-BB, which is FDA approved as a diabetic foot ulcer treatment displayed variable efficacy in preclinical wound models ranging from improved closure of wound of 1.5 cm

excisional diabetic mouse wounds,[81] to a substantial increase in granulation tissue formation without major improvement in the wound closure time,[82] to having no effect on the re-epithelialization process in a murine splinted diabetic wound model.[83]

4.2.2 | Wound size and numbers

The size of the wound and the number of wounds that can be created depend on the size of the animal used in the model.[24] Mice have smaller wounds, and accordingly, their wounds will heal faster (7 days) compared with 12–14 days for slightly larger wounds in rats. Rats or rabbits, due to their larger size, allow for larger or a higher number of wounds.[24] For example, the rabbit ear excisional wound model allows an increase in size and number of test sites per animal, as each rabbit ear is large enough to hold up to six wounds, providing sufficient data for within-animal replicates.[24] This decreases animal usage and enhances the statistical robustness of the experimental results. Findings from a murine incisional and excisional wound model study indicated that individual wounds within the same mice were considered independent biological replicates.[63] Notwithstanding, the influence of systemic factors should be taken into account, especially when multiple wounds are used per animal. Moreover, as the use of individual wounds versus individual animals as replicates can impact the comparability of results across studies,[63] randomising the sites when multiple wounds and/or multiple animals are used to ensure robustness of the experimental design is recommended.

When using animal wound models for preclinical testing, animal welfare considerations should follow the Replace, Reduce, and Refine guidelines (3Rs)[84] set forth by the FDA, as described in General considerations for animal studies intended to evaluate medical devices[4] and ISO 10993-2:2022 Biological Evaluation of Medical Devices – Part 2: Animal Welfare Requirements.[6] These guidelines provide recommendations to reduce the number of animals used, refine test methods to minimise pain and distress in test animals, and suggest obtaining scientific evidence, such as in vitro data, prior to preclinical models. They also provide suggestions for replacing animal models using additional recommended strategies when possible. Hence, careful experimental design of animal wound models should help refine existing protocols, optimise models, and is critical for maximising the probability of achieving statistical significance and reducing the number of animals used. To improve reproducibility and translation of preclinical studies using animal models, sample size is an important factor to consider. Too small of a sample size can miss the real effect in an experiment, while a sample size that is larger than necessary will lead to wasting resources and presents ethical issues regarding the euthanized animals[85] and misalignment with the 3Rs that protect animal welfare. Using power analysis is the most scientifically favourable method for sample size calculation[86,87] and free software and calculators are available online for this purpose.[88] To calculate the sample size by power analysis, prior knowledge and information on two main concepts are important: (1) effect size (the minimum difference between two groups that can be considered clinically significant) and (2) standard deviation (the measure of variability within a sample for a quantitative variable).[86]

4.2.3 | Infected wound models

The presence of infection contributes to chronic wound development. Bacterial biofilms are involved in more than 78% of all chronic wound infections.[89] *Pseudomonas aeruginosa* and *S. aureus* are typically found in chronic wounds and commonly used to generate biofilms in different animal infected models, causing a delay in wound closure.[90–93] Of note, commensal bacterial species as well as anaerobic bacteria also colonise human chronic wounds.[94,95] Studies have shown that preformed *P. aeruginosa* biofilm infection in 6 mm wounds in rats leads to delayed wound closure (18–21 days) compared with non-infected wounds, which re-epithelialized within 9–12 days.[90] An increased and prolonged inflammatory response and reduced granulation tissue formation were also observed in *S. aureus* biofilm-infected wounds in rabbit ears.[91] In contrast to established pathogenic species, anaerobic *Alcaligenes faecalis* isolated from patients with healing diabetic foot ulcers promoted wound healing in 8 mm murine wounds.[94]

In these infected models, bacterial cells can be delivered as planktonic culture via a bacterial contaminated implanted material or delivered as preformed biofilm on filter paper. This is followed by occlusive dressings used to cover the infected wounds.[35,94] When *P. aeruginosa* biofilms were added to full-thickness rodent wounds via dressings, healing took longer, and wounds showed reduced re-epithelialization, prolonged inflammation and reduced collagen formation compared with when bacteria were not added.[90] Infected models are also useful to investigate different antibacterial therapies.

Recapitulating the human chronic wound environment in animal models is challenging. Chronic wounds are polymicrobial in nature; however, most infection models are established using a single wound dominant microbial species.[96] Relevant models have now been developed that include polymicrobial biofilms utilising bacterial isolates from patients' wounds to provide relevance to the human wound environment. Another limitation is that infection in these models usually lasts for a short duration, ranging between 2 and 26 days of infection,[97] compared with the prolonged and persistent infection present in chronic wounds, confounding the biofilm and host wound microenvironment.[98] Moreover, *P. aeruginosa* can be motile between wounds, further complicating the use of controls in the same animals. Younger animals can also tolerate bacterial infection well, and infections in wounds are cleared away.[35] This may be circumvented by augmenting bacterial loads to enhance the success of creating biofilm-infected models; however, this may also lead to systemic infection and animal death.[99]

Given the variability of infected models, certain factors to assist with standardisation and translatability to humans may need to be considered when selecting an experimentally induced infection model to study impaired healing and antimicrobial therapy. Hence, the animal wound model checklist also captures important information such as the bacterial culture type (planktonic vs. biofilm) and number of bacteria (in colony forming units), and the route and time of infection.

4.3 | Section III: Wound/animal maintenance and monitoring

Section 4.3 of the animal wound model checklist captures information on analgesic use, medications administered, observational health monitoring methods, the type of wound coverage (e.g., air exposed or dressing), and other materials applied to wound, as well as the frequency of dressing changes. All this relevant information pertains to animal maintenance and monitoring, and outcomes of preclinical wound studies can be greatly affected by these variables. For instance, as part of animal welfare, suitable pain management is important to decrease pain and stress response to surgery. However, careful consideration should be taken as analgesic and anaesthetic choices may impact wound healing and this may be appreciated differentially between models. For example, findings from the Wound Aetiology and Healing study suggest that opioid exposure impedes wound healing in patients with chronic wounds.[100] The impact of analgesics on perfusion and the route of administration must be taken into consideration. Specifically, a pain management plan that requires daily administration with anaesthesia may be detrimental to the animal and alter wound healing. In a rat wound healing study, the anaesthetic drugs lidocaine and prilocaine did not affect wound healing when evaluating tensile strength and collagen ultrastructure as parameters. Conversely, bupivacaine and levobupivacaine had a negative impact on these processes, especially in the late period as evidenced by a significant decrease in wound tensile strength on the 21st day as opposed to 8th and 15th day post-operatively.[101] Other studies reported no significant effects on wound healing using lidocaine and bupivacaine.[102,103] Variation in findings may be attributed to dosage, types of wounds, sex differences, and animal models employed.

The use of occlusive dressings or non-occlusive bandages of varying composition has been shown to lead to changes in the normal wound environment, influencing healing. Dyson et al.[104] compared the effects of moist and dry wound conditions on dermal repair in full thickness excised wounds on porcine skin over a period of 21 days. Moist conditions, achieved with OPSITE dressing (Smith and Nephew, City, State) led to faster wound healing, with a quicker decrease in inflammatory cells (neutrophils and macrophages) and a more rapid increase in proliferative phase cells (fibroblast and endothelial cells) compared with exposure to air through dry gauze dressings. The study showed that moist wounds progressed through the healing phases more efficiently than dry wounds. In another acute, full-thickness biopsy wound model, occlusive dressings increased early epithelial migration of wounds compared with air exposure.[105] On the other hand, though the relationship between the level of moisture and the occurrence of wound-induced hair neogenesis (WIHN) has not been demonstrated conclusively, occlusive dressings may prevent WIHN.

4.4 | Sections IV and V: Wound treatment and wound assessment and analysis

Section 4.4 of the animal model checklist pertains to wound treatment. Key variables, including the number of experimental/treatment groups tested, parameters of control versus treatment groups, and other relevant information concerning treatment, such as dosing, route, timing, and frequency of treatment, are important factors included in the checklist. These factors need careful consideration when designing experiments as they can greatly affect wound healing outcomes.

Wound assessment and analysis, including assessing gross wound size, planimetry, re-epithelialization by histology, transepidermal water loss (TEWL) measurements,[106] presence of scar, tensile/ breaking strength, tensile stiffness, cellular infiltrate, inflammation, fibrosis, microbial composition, granulation tissue formation among others, are some of the methods utilised to determine wound healing.[13] However, there is inconsistency in the reporting of some of this experimental detail in published protocols. Hence, Section 4.4 of the checklist includes vital information to be documented, such as assessment methods, frequency of assessment, time points analysed, scar assessment, signs of regeneration, among others, to improve transparency and allow for reproducibility and translatability to humans.

Studies have shown that time points of assessment may influence wound healing outcomes. For example, in a STZ-DM induced rat model, 3 weeks post-STZ induction of diabetes was too early to observe all wound healing delay parameters whereas by 6 weeks, more parameters such as planimetry, re-epithelialization and macrophage count could be determined, showing an impaired healing phenotype.[26]

Wound assessment methods, both non-invasive and invasive, can be performed before or until complete wound closure, depending on the wound parameter(s) being investigated. Non-invasive methods include planimetry measurements, such as wound tracing and photographic analysis, used to determine wound reduction over time.[24] With digital photography and image assessment, analysis software such as ImageJ is widely used to provide accuracy in monitoring wound healing outcomes. These methods are useful for assessing re-epithelization, are an important compliment to histologic evaluation, and can be reproduced when planimetry analysis standards are maintained. A variety of other image analysis software is also available for histological analysis.[24] Additionally, other non-invasive methods for wound healing measurements include biophysical techniques such as optical coherence tomography (OCT),[107] confocal laser scanning microscopy,[108] and near-infrared and diffuse reflectance spectroscopy,[109] among others. A conventional method used for the characterisation of wound healing is the measuring of TEWL, which is used for the analysis of re-epithelialization and restoration of barrier function.[110] Non-invasive methods such as evaporimetry have been used to measure TEWL.[106]

Invasive methods include biopsy or full excision followed by histology, which provide more detailed microscopic information on the healing progression using methods such as haematoxylin and eosin (H&E) stain, as well as special non-H&E stains like trichome stains, picrosirius red, and immunohistochemical markers using immunohistochemistry. An important variable to record is the wound and biopsy location. The best site for biopsy has been reported to be at the wound edge as it allows for comparison between the ulcerated area and the surrounding skin to be made[111]; however, limitations of this selection should be taken into account. For histopathological analysis, the ideal biopsy will encompass the whole wound, including the edges,[24] that is, a full excision. Factors to be considered when taking

one or more biopsies is that this procedure creates a fresh wound and can be a confounding factor in the overall wound healing process in small animals like mice, as well as in pig studies, depending on the methodology used. The collection of a wound biopsy may impact subsequent healing and later assessments conducted.

Scar formation, an important aspect of the wound healing process, necessitates assessment. Various incisional and excisional animal wound models can be used to generate different scar types depending on investigation, including normal scarring, pathological scarring, transplantation of patient-derived scar tissue, and reconstitution of human scars in animal skin (i.e., tissue engineered skin constructs).[112,113] Scar assessment employs similar wound assessment invasive methods, such as general histology and immunohistochemical analysis utilising biopsies, as well as mRNA sequencing and quantitative polymerase chain reaction for further analyses to investigate cellular and molecular mechanisms of scar tissue. Non-invasive methods such as monitoring instruments, quantitative devices, and computational methods are also typically used. For example, in a rodent model, the use of polarisation-sensitive optical frequency domain imaging, an advanced variant of polarisation-sensitive OCT to facilitate 3D collagen image evaluation, has been reported.[114] Furthermore, visual assessment scales, such as the modified Vancouver Scar Scale and the Manchester Scar Scale, have been adapted for use in animal models, to assess scar characteristics.[115]

De novo hair follicle regeneration in the centre of large, full-thickness murine wounds, a phenomenon known as WIHN, and characterised by the formation of hair placodes, hair germs, and hair pegs has been previously documented.[116,117] However, studies on WIHN have reported mixed results due to the varied wounding protocols and interpretations of different models. Hence, it is important to document all experimental variables when presenting findings related to WIHN. For example, in preclinical studies in mice, it is important to specify the timepoint of assessment and provide a rationale for the timepoint selection. In the case of large full-thickness excisional wounds (e.g., 1 cm^2) on the dorsum of 3-week-old wild-type mice, WIHN initiation in the centre of the re-epithelialized wound as indicated by K17 staining and the WNT pathway marker LEF1, typically does not occur before 10 days post-re-epithelialization. This timeframe is determined by scab detachment, which usually occurs 10–12 days post-re-epithelialization.[116,117] Therefore, assessing WIHN at earlier timepoints may not accurately reflect its occurrence.

Due to the variety of assessment methods available, careful consideration should be taken when selecting the best method for wound analysis. Planimetry provides macroscopic examination of the wound, while histological analysis provides microscopic examination and gives detailed information about the wound's cellular composition, tissue architecture, inflammation levels, and other specific characteristics. However, animals need to be culled thereby preventing serial measurements from being recorded.[63] Results obtained from both methods can be variable depending on the type of wound and the planimetry and histology measurements used. Furthermore, tissue handling and processing are factors that can significantly affect the quality of histological analysis and staining of wound samples. Both measurements may also generate inconsistent results even within the same type of wounds. For example, Ansell et al.[63] attempted to optimise a mouse acute wound model and methods of evaluation by profiling secondary intention healing of incisional and excisional wounds in the same animal and assessing different parameters using planimetric and histological methods. Both types of wounds displayed different healing profiles when assessed planimetrically, with excisional wounds being more suited to planimetric analysis, while incisional wounds displayed a greater correlation between planimetric and histological parameters. Moreover, excisional wounds showed no correlation between macroscopic (planimetry) and microscopic (histology) measurements at Days 3 and 7 time points. Histology was also found to be the least variable assessment of healing and was able to detect rapid healing compared with planimetry.[63] The authors concluded that simple changes in experimental designs and analysis can help guide and refine future studies, yield reproducible results, and permit more efficient screening of wound phenotypes.[63] In another STZ-DM rat wound model study, the same authors reported that planimetry (wound photographs) was a more reliable measure of overall healing delay in the STZ-DM model. However, histological analysis was able to show a statistically significant delay in re-epithelialization (i.e., delayed wound closure) after 6 weeks of DM.[26]

4.5 | ISO 10993 Standards and FDA guidelines

In relation to wound care products classified as medical devices, both ISO 10993-2:2022 Biological Evaluation of Medical Devices – Part 2: Animal Welfare Requirements 2022[6] and ISO 10993-6: 2016 Biological Evaluation of Medical Devices – Part 6: Tests for Local Effects after Implantation[5] should be considered, as they describe relevant methods for the assessment of local effects after implantation of biomaterials, specify animal welfare requirements and outline the principles of animal species, size of implants, test duration, among others. The Guidance for Industry ICH S6 (R1) Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals[7] provides a basic framework for the preclinical safety evaluation of biotechnology-derived pharmaceuticals. In addition, the first part of FDA's published Guidance for Industry Chronic Cutaneous Ulcer and Burn Wounds – Developing Products for Treatment[8] provides preclinical considerations to sponsors developing drugs, biologics, and device products to treat chronic cutaneous ulcer and burn wounds. The above guidelines offer valuable information for sponsors considering preclinical studies and reporting for regulatory submissions.

5 | WRAHPS CHECKLIST AND REPORTING TEMPLATE FOR HUMAN EX VIVO MODELS

The WRAHPS checklist and reporting template for human ex vivo models (Checklist 2) highlight the key items that should be included when reporting the experimental design in preclinical wound studies involving the human ex vivo model. Similar to the animal wound model checklist, this comprehensive checklist encompasses vital variables, including the storage and shipping of donor skin, donor site,

sex, age, wounding type, simulated wound aetiology, patient comorbidities, type of tools used for wound generation, wound size, number of wounds, experimentally induced infection, culture conditions and details of wound treatment. This information includes the number of wounds per experimental/treatment groups, differentiation between control and treatment groups, and other relevant treatment-related aspects, such as dosing, route, timing, and frequency of administration. Additionally, the checklist covers assessment methods, frequency of assessment, time points analysed, and scar evaluation. In Section 6.4, we describe these models and discuss their relevance and application in human ex vivo studies. Capturing the checklist items will further facilitate transparent reporting, ensuring that all important aspects of the study are disclosed to enable reproducibility of experiments in future human ex vivo studies.

6 | SPECIFIC MODELS

This section discusses the most commonly used animal models in preclinical studies, along with their relevance, strengths, and limitations (Table 3).

6.1 | Rodent models

Rodent models are among the most frequently used in vivo models for wound healing studies. In addition to ease of use and maintenance, small size, and lower costs compared with other animal models, rats and mice are over 99% genomically similar to humans, making results from these models reasonably translatable to clinical medicine. Nonetheless, it is critical to understand the advantages and disadvantages of the rodent model when extrapolating to human wound healing. Mouse models can be standardised by sex, age, genetics, and history, are amenable to genetic manipulation to simulate impaired human conditions, and enable use of a high number of animals for statistical analyses.[118,119] The advantages of rodent models are not exclusive to wound healing studies and have contributed extensively to the advancement of medicine. As such, there is a broad availability of rodent-specialised biological and chemical reagents (antibodies for immunohistochemistry, primers for gene expression analysis, etc.) for robust data collection. They also provide versatility to investigate a variety of factors that contribute to wound physiology. One way this is done is by creating genetically modified strains of these animals. A number of molecular tools have been developed to easily create

TABLE 3 Key advantages and limitations of preclinical models.

Models	Advantages	Limitations
Rodent	Over 99% genomically similar to humans	May fail to capture genetic diversity in the human population
	Ease of use, maintenance, small size, and lower costs	Rapid wound healing by contraction, unlike humans
	Amenable to genetic manipulation to simulate impaired human conditions	Thinner epidermal and dermal layers, loose skin, dense hair, different viscoelastic properties compared with human skin
	Broad availability of rodent-specific reagents	Differing inflammatory responses compared with humans
	Low genomic variation between animals within the same strain	
Pig	Skin is anatomically and physiologically more representative of human skin	High costs associated with the model
	Partial-thickness wounds largely repaired through granulation and re-epithelialization, similar to humans	Requires specialised facilities and surgical/technical/veterinary expertise
	Large surface area allows for the analysis of numerous test groups	Relative lack of porcine-specific reagents compared with other models
	Often required in regulatory submissions	Wound resolution time often inconsistent with observed healing rates in humans
Rabbit	Ear skin wounds heal by re-epithelialization and granulation, similar to humans	Limited genetic tractability and lack of species-specific reagents
	Can be used to study wound healing in the context of ischaemia, infection, and metabolic disorders	Rabbit ear skin architecture differs from human skin (dermis tightly attached to cartilage, avascular wound base)
	Relatively inexpensive compared with larger animal models	
	Large ear surface area allows for several biopsy replicates	
Human ex vivo	Closely mimics normal human skin	Lacks blood supply, innervation, and immune cell migration
	Cost-effective, eliminates the need for in vivo models	Limited availability of donor skin
	Can be used for both normal and pathological skin	Logistical challenges in storing, shipping, and processing fresh skin
	Enables assessment of tissue morphology, inflammation, protein expression, signalling, and gene expression	Variations in methodologies (culture conditions and media)
	Multiple replicates can be generated from a single skin sample, reducing interpatient variability	
	Gene expression patterns closely resemble those observed in vivo	

genetically modified rodents. These modifications are broadly useful, from visualising protein expression[120] to mimicking human disease states (e.g., the db/db diabetic model). Laboratory rodents, while not isogenetic, also have very low genomic variation between animals, allowing for high reproducibility of physiological conditions between groups of animals in the same strain.[121]

Rodent models also have notable limitations that should be considered when choosing an in vivo model for wound healing. Despite their high genomic similarities to humans, inbred rodent strains may fail to capture important elements of genetic diversity in the human population that are crucial to the success of wound care therapies. Rodents have also evolved to have extraordinarily quick wound healing via contraction.[122] This is not entirely mimetic of human wound healing responses and can reduce the effect size of an experimental treatment. External interventions (wound splinting,[123] drug treatments,[124] or genetic modification) slow rodent wound healing and create human-like wound closure via re-epithelialization and granulation. Rodent skin is also quite different structurally and mechanically from that of humans, having thinner epidermal and dermal layers and different viscoelastic properties.[125] Additionally, inflammatory responses, which are crucial in wound healing, are well reported to differ significantly between rodents and humans.[28]

6.2 | Porcine models

Many similarities exist between porcine and human skin making porcine wound models an often-preferred model for preclinical assessment. Pigs have been used for decades to develop specific porcine models for different pathologies, such as chronic non-healing wounds, diabetic wounds, infected wounds, burns, hypertrophic scars, infected burn wounds, diabetic infected wounds, and chronic ischaemic wounds.[126–129] Typically, domestic farm breeds, including Yorkshire, Red Duroc, and Landrace pigs have been implemented in these studies. The choice of breeds utilised in studies depends on the aspects of wound healing being studied. For example, Red Duroc pigs have been used for studying hypertrophic scarring,[130,131] whereas Yorkshire pigs are often used for wound healing studies.[132–134]

Even though small mammal models, such as rodents, offer several advantages, porcine studies are often required in regulatory submissions as porcine skin is anatomically and physiologically more representative of human skin.[10] Additionally, pig skin has less epidermal appendages and is more comparable in thickness of the epidermis and dermis to humans than other animals, such as rodents. The method of skin repair in pigs is also more consistent with humans. Partial-thickness wounds are largely repaired through granulation and re-epithelialization[135] and circular full-thickness wounds heal mainly through contraction, dependent on site and wounds size.[136] Given the sampling requirements from regulatory agencies, the large surface area available for wounding allows for the analysis of numerous test groups and thus may be advantageous for certain studies.

Despite the advantages associated with porcine preclinical models, several limitations also exist. The substantial cost associated with these models, the facilities and surgical/technical/veterinary expertise required to perform and monitor the studies, and the relative lack of porcine-specific reagents compared with other available preclinical models, are often prohibitive factors in their use. Another limitation of porcine models, as well as other animal models, is the wound resolution time, as the return to natural healing rates are often inconsistent with observed healing rates in humans presenting similar pathologies.[42,92,137,138] For example, in a porcine diabetic wound model described by Velander et al.,[42] diabetic wounds healed after 18 days, which is considerably faster than healing rates observed in diabetic wounds in humans. This is most likely due to the inability to mimic the chronicity of human diabetes. These discrepancies can make comparisons between control and treatment groups difficult, and further complicate the assessment of the translational potential of the product. To overcome these discrepancies, genetically modified pigs have been generated to increase their utility.[139,140] For example, Renner et al.[139] created transgenic pigs as a model for human Type 2 DM that express a human dominant-negative glucose-dependent insulinotropic polypeptide receptor mutant (GIPRdn) in pancreatic islets under the control of the rat insulin promoter. 11-week-old pigs displayed decreased glucose tolerance as a result of delayed insulin secretion, and decreased insulin secretion and pancreatic β-cell mass with advancing age.[139]

Important factors to consider when using porcine preclinical models extend to the selection, setup, and maintenance of the test animals. Standards, such as ISO and ICH guidelines, as well as expertise and testing facilities offered by external contract research organisations present extensive guidelines and know-how to support preclinical studies. Sample size, number of samples per animal, and number of treatment and control groups are examples of study details that should be considered. Additionally, the size, weight, and age of the pigs should be assessed when selecting an implant site to ensure that placement of the treatments does not increase the likelihood of health problems or complications that could affect the results of the study. Additionally, when measuring wound area in pigs, another factor to consider is their growth rate, as they frequently increase in overall size during the on-study period. The size of the wound relative to the overall size of the pig is important, and the change in pig size/weight over the study period should be factored into wound area calculations.

6.3 | Rabbit models

Rabbit models have commonly been used to study wound healing in the context of ischaemia[56,141] and infection,[91,142] as well as metabolic disorders such as diabetes.[40,143] Since infection and ischaemia are known to play major roles in chronic wound development, the rabbit ear model serves as a useful model to study chronic wound pathogenesis. The rabbit ear shares some similarities with humans in the wound healing physiology context.[9] The dermis of rabbit ear skin is attached to the underlying cartilaginous layer, which hinders contraction of the wound. Hence, wounds in the rabbit ear model heal by re-

epithelialization and granulation tissue formation, similar to humans.[9] The model can also be used to study hypertrophic scar formation.[33,144] Additionally, the ischaemia created in the rabbit ear model is reversible as the rabbit ear tends to form collateral circulation,[10] a trait also observed in humans.

The rabbit ear model has been used to study growth factors and proteoglycans, highlighting its usefulness in therapeutic testing that could be translated to the clinic.[145–147] The effects of bacteria on wound healing have also been explored using the rabbit ear model. Rabbit ear wounds infected with *P. aeruginosa*[148,149] and *S. aureus*[91] resulted in impaired healing through biofilm formation.

These models are relatively inexpensive compared with larger animal models. The rabbit ear excisional model is established by creating punch biopsies in the ear, and due to its large surface area, several biopsy replicates can be created. Although the rabbit model has many benefits, there are also some limitations due to its genetic tractability and lack of species-specific reagents.[10] Moreover, the rabbit ear is dissimilar to human skin in terms of architecture, as the dermis is tightly attached to the cartilage layer below and the wound base is avascular.[10] These aforementioned factors need to be taken into consideration when using rabbit models for wound healing studies.

6.4 | Human ex vivo wound models

Human ex vivo models, based on organ culture, were first developed over two decades ago.[150] They are cost-effective models that are useful for assessing various biological and pathophysiological processes and evaluating a variety of molecules and novel therapeutic agents on epithelialization. These models closely mimic normal skin and can be used for full or partial thickness skin biopsies, reducing the need for in vivo models.[9,11]

Human ex vivo models are created from skin that is routinely discarded during surgery such as abdominoplasty and breast reduction. The underlying fat is removed, and a 3–4 mm punch is used to create an excisional wound to the depth of the papillary dermis. A wider 6–10 mm biopsy punch is then made surrounding the newly created wound. The resulting donut-shaped ex vivo wound is maintained at an air–liquid interface with culture medium, enabling full wound healing through epithelialisation.[3,11] A large skin sample provides more wound replicates from the same donor, improving reliability of results.

Human ex vivo models utilise both normal and pathological skin.[12] They can also include 3D organotypic cultures from primary cells derived from either healthy donors, actual patient tissue, or from genetically modified cells.[151–154] As the induced pluripotent stem cell technology advances more complex skin 3D equivalents that include more than keratinocytes and fibroblast such as neurons or melanocytes are being developed.[155–157] Treatments can be applied directly to the donut-like wound to test topical effects or to the media to test systemic effects.[158] For example, in an ex vivo wound infection model to test topical and systemic treatment with antibiotics, *S. aureus* biofilms grown either on a polycarbonate membrane or explanted skin were treated either topically, by placing antibiotic-loaded electrospun matrices atop the biofilms, or systemically by adding antibiotics in the growth medium that flowed beneath the membrane or skin. The findings demonstrated that microbial viability in the biofilms was reduced with topical treatment compared with systemic treatment.[158] Alternatively, treatment may also be injected.[3] In these models re-epithelialization is assessed using histomorphometric analysis and keratin immunostaining.[3] Ex vivo models have also been utilised to study the effects of candidate molecules such as growth factors, microRNAs, and pharmacological agents on wound healing, as well as other pathologies and molecular mechanisms and allow higher throughput than individual animals.[159–165] For example, miR193b-3p knockdown was shown to accelerate wound reepithelialisation in a human ex vivo wound model to study impaired healing.[153] In another study, Yoon et al.[166] described a simplified ex vivo human skin model of infection in which wounds were inoculated with *S. aureus* strain UAMS-1, or under aseptic conditions. The infected wounds displayed biofilm formation and impaired re-epithelialization compared with the control. In addition, pro-inflammatory genes were significantly upregulated while pro-migratory and pro-reparative genes were significantly downregulated, demonstrating molecular characterizations of impaired healing translatable to chronic wounds. Furthermore, human ex vivo models enable the assessment of tissue morphology under normal and pathological conditions, including scarring,[113,167] as well as inflammation, protein expression, signalling, and gene expression.[9]

Human ex vivo models offer several advantages, primarily the use of the human as the model, but also the ability to maintain uniformity in wound size and standardise experimental conditions. Additionally, multiple replicates can be generated from a single skin sample, reducing interpatient variability. These models contain cellular elements of the skin and also preserve the basement membrane zones, mimicking natural skin morphology,[168] unlike organotypic cultures that lack basement membrane structures. Moreover, the gene expression patterns observed in these ex vivo wound models closely resemble those observed in vivo,[159,169,170] enhancing the translational potential of ex vivo data in clinical care. However, it is important to note that these models have limitations, including the absence of blood supply, innervation, immune cell migration, and the limited availability of donor skin.[9,12] There is also the logistical challenge of storing and shipping skin from a remote site and, the laboratory must also remain prepared to receive and process fresh skin, even in the face of unpredictable operating room and shipping/delivery schedules. Variations in methodologies include different conditions used for culture and culture mediums.[12] These factors should be taken into consideration when utilising human ex vivo models.

7 | CONCLUSIONS

In summary, standardised reporting in preclinical testing for wound healing disorders is critical to ensure accurate and transparent research and determine likelihood of success in human clinical trials. Existing guidelines provide valuable insights, but they may not offer specific recommendations for individual studies. We have addressed this gap by creating the WRAHPS checklists and reporting templates as a guide to optimise and refine future studies and to assist with

standardising preclinical testing. Careful consideration to select the appropriate model is necessary to accurately determine both the wound response to treatment and provide further understanding of the mechanism(s) of action of novel therapeutic strategies. Specific details of the model should be included in publications to allow for better extrapolation, interpretation, and comparative analyses of data. Additional variables included in the checklists should be considered and incorporated into the experimental design. It is also worth noting that innovative technologies such as 'organs-on-chips', which create miniature models of human organs on microengineered chips, and other technologies, are being actively developed and evaluated by the FDA as potential alternative methods to reduce or replace animal testing in the future.[171,172] By addressing this gap, researchers, sponsors, and funding agencies considering preclinical studies can improve the selection of preclinical models, enhance study design, and improve the consistency of experimental parameter reporting. Overall, the incorporation of clear reporting standards and adherence to guidelines will benefit the wound healing field by promoting robust research, to support the evaluation and development of effective products and therapies.

1. WRAHPS Checklist and Reporting Template for Animal Wound Models

Section I. Animal Wound Model:

1. Animals Used and Biological Variables
   - ☐ Rat
     - Species: _____: Strain/breed/genotype: _____
     - Age _____; Sex_____; Weight_____
     - Genetically modified
       - ☐ None
       - ☐ Yes (Specify:_____)
     - Identification Method;_____
   - ☐ Mouse
     - Species: _____: Strain/breed/genotype:
     - Age _____; Sex_____; Weight_____
     - Genetically modified
       - ☐ None
       - ☐ Yes (Specify:_____)
     - Identification Method;_____
   - ☐ Guinea Pig
     - Species: _____: Strain/breed/genotype: _____
     - Age _____; Sex_____; Weight_____
     - Genetically modified
       - ☐ None
       - ☐ Yes (Specify:_____)
     - Identification Method;_____
   - ☐ Rabbit
     - Species: _____: Strain/breed/genotype: _____
     - Age _____; Sex_____; Weight_____
     - Genetically modified
       - ☐ None
       - ☐ Yes (Specify:_____)
     - Identification Method;_____
   - ☐ Pig
     - Species: _____: Strain/breed/genotype: _____
     - Age _____; Sex_____; Weight_____
     - Genetically modified
       - ☐ None
       - ☐ Yes (Specify:_____)
     - Identification Method;_____
   - ☐ Other
     - Species: _____: Strain/breed/genotype: _____
     - Age _____; Sex_____; Weight_____
     - Genetically modified
       - ☐ None
       - ☐ Yes (Specifiy:_____)
     - Identification Method;_____

2. Type of wound simulated
   - ☐ Chronic
   - ☐ Acute

3. Wound etiology simulated
   - ☐ Surgical/traumatic
   - ☐ Diabetic
   - ☐ Ischemic
   - ☐ Ischemia/reperfusion
   - ☐ Surgical
   - ☐ Burn
   - ☐ Pressure
   - ☐ Infection
     - ☐ Local
     - ☐ Systemic
   - ☐ Other (Specify:_____)

4. Induction of diabetes
   - ☐ None (*proceed to* ***Section II***)
   - ☐ Chemical
     - ☐ Streptozotocin (Specify dose & duration:_____)
     - ☐ Alloxan (Specify dose & duration:_____)
     - ☐ Dithizone (Specify dose & duration:_____)
     - ☐ Gold Thioglucose (Specify dose & duration:_____)
     - ☐ Monosodium Glutamate (Specify dose & duration:_____)
     - ☐ Other (Specify dose & duration:_____)
   - ☐ Genetic
     - ☐ Db/db
     - ☐ Ob/ob
     - ☐ KK-A^y
     - ☐ Goto-Kakizaki rat

- ☐ Otsuka Long Evans Tokushima fatty rat
- ☐ Spontaneous Diabetic Torii rat
- ☐ Fa/fa rat (Zucker Diabetic Fatty Rat)
- ☐ Other (Specify:_____)

☐ Diet induced (Specify diet type & duration:_____)
☐ Virus induced (Specify:_____)
☐ Hormone induced (Specify:_____)
☐ Surgically induced (Specify:_____)
☐ Other (Specify:_____)

5. Did the animal receive special treatment prior to wound generation (i.e., diet, topical, systemic, environmental) for diabetes induction?
   ☐ No
   ☐ Yes
      i. Specify: _____
      ii. Duration: _____

6. Confirmation of diabetes
   ☐ None
   ☐ Yes (Specify method of diabetes confirmation:_____)

7. Monitoring of diabetes
   ☐ None
   ☐ Yes (Specify method of monitoring: _____)

8. Management of diabetes
   ☐ None
   ☐ Insulin (Specify dosage: _____)
   ☐ Other (Specify type and dosage:_____)

9. Time between diabetes induction and wounding
   ☐ 0-1 days
   ☐ 2-3 days
   ☐ 3-4 days
   ☐ 5-6 days
   ☐ 7-10 days
   ☐ 11-14 days
   ☐ 30 days
   ☐ More than 30 days, please specify_____

Section II. Wounding Process:

10. Anesthesia used (check all that apply)
    ☐ Inhaled (Specify type and %: _____)
    ☐ Injectable (Specify type, dosage, route:_____)

11. Location of wound
    ☐ Cheek
    ☐ Ear
    ☐ Tail
    ☐ Dorsum
    ☐ Other (Specify: _____)

12. Depilation Technique
    ☐ Shaving
    ☐ Clipping
    ☐ Chemical (Nair/Veet)
    ☐ Wax
    ☐ None
    ☐ Other (Specify:_____)

13. Type of Wounding
    ☐ Incisional
       ☐ Healing by primary intention (surgical closure)
          ☐ Method of surgical closure (Give details: _____)
       ☐ Healing by secondary intention
    ☐ Excisional
       ☐ Partial-Thickness Excisional
       ☐ Full-Thickness Excisional
    ☐ Pressure injury / Ulcer
       ☐ Presence of necrotic tissue
          ☐ Yes (Specify %: ____)
          ☐ No
       ☐ Presence of undermining or tunneling
          ☐ Yes
             ☐ Undermining location (Specify clock face: ____)
             ☐ Tunneling location (Specify clock face: _____)
          ☐ No
       ☐ Presence of exudate
          ☐ Yes
             ☐ Amount (Specify None, Scant, Moderate, or Large: ___)
          ☐ No
       ☐ Periwound skin condition (Specify Intact, Macerated, Erythematous, or Other:_____)
    ☐ Burn
       ☐ Water scalding
          ☐ Time (Specify:_____)
          ☐ Temperature (Specify:_____)
          ☐ Percentage burn surface area (Specify____)
       ☐ Contact burn
          ☐ Time (Specify:_____)
          ☐ Pressure/weight (Specify:_____)
          ☐ Temperature (Specify:_____)
          ☐ Methods of heating (Specify :_____)
          ☐ Material used (Specify:_____)
          ☐ Percentage burn surface area (Specify_____)
          ☐ Were wound debrided after injury?
             ☐ Yes
             ☐ No
          ☐ Other (Specify:_____)
       ☐ Chemical
    ☐ Other (Specify:_____)

14. Tool used to generate wound(s)
    ☐ Specify: _____

15. Wound splint used
    ☐ None
    ☐ Static

- ☐ Silicone ring
  - ☐ How was splint applied?
    - ☐ Sutured. Number of stiches____
    - ☐ Glued, Type of glue _____
    - ☐ Other
- ☐ Steel ring
- ☐ Polydimethylsiloxane (PDMS) device
- ☐ Mechanical
- ☐ Other type of device (Specify:_____)
- ☐ Provide additional relevant details (_____)

16. Size of wounds made (mm):

- ☐ Incisional wounds:
  - ☐ Length: _____
  - ☐ Width (if applicable): _____
  - ☐ Depth (if applicable): _____
- ☐ Excisional wounds:
  - ☐ Length: _____
  - ☐ Width: _____
  - ☐ Depth (if applicable): _____
- ☐ Other wound shapes (Specify_____)
  - ☐ Longest length: _____
  - ☐ Widest width (perpendicular to length): _____
  - ☐ Depth (if applicable): _____
- ☐ Additional measurements (e.g. distance between wounds):_____
- ☐ Other (Specify:_____)

17. Number of wounds per animal
    - ☐ 1-2
    - ☐ 3-4
    - ☐ 5-8
    - ☐ More than 8 (Specify:_____)
18. Number of animals per experimental / treatment group
    - ☐ 1-2
    - ☐ 3-4
    - ☐ 5-6
    - ☐ More than 6
    - ☐ Other (Specify if more than one experimental /treatment group per animal:____)
19. Power analysis performed
    - ☐ Yes
    - ☐ No
20. Experimentally induced infection
    - ☐ None
    - ☐ Planktonic (Specify)
      - ☐ Species and strain:_____
      - ☐ Growth media used:_
      - ☐ Logarithmic phase__
      - ☐ Stationary phase_____
      - ☐ Amount (CFUs):_____
      - ☐ Route of infection: _____
      - ☐ Duration of infection:_____
    - ☐ Biofilm (Specify)
      - ☐ Species and strain: _____
      - ☐ Amount of CFUs: _____
      - ☐ Route of infection: _____
      - ☐ Duration of infection: _____
      - ☐ Method of validation of biofilm formation:__
      - ☐ Duration of biofilm formation for pre-formed biofilms: _____
    - ☐ Other (Specify:_____)

Section III. Wound/Animal Maintenance and Monitoring:

21. Analgesia provided during treatment
    - ☐ Yes (Specify type, intervals, dosage, timing): _____)
      - ☐ Provide all other information (e.g. if timing was preemptive or after observation, if all animals were given analgesics, decisions for dosing etc.:_____)
    - ☐ No (If not, explain rationale:_____)
22. Other medications administered
    - ☐ None
    - ☐ Specify type, intervals, and dosage:_____
23. Observational health monitoring methods (Describe): _____
24. Wound coverage
    - ☐ Air exposed
    - ☐ Dressing (Specify type): _____
      - ☐ Specify primary: _____
      - ☐ Specify secondary: _____
25. Frequency of dressing changes:
    - ☐ N/A
    - ☐ Daily
    - ☐ 2 days
    - ☐ 3 days
    - ☐ Weekly
    - ☐ Other* (Specify:_____)

    *include details on all dressings utilized

Section IV. Wound Treatment:

26. Number of experimental / treatment groups tested
    - ☐ 1-2
    - ☐ 2-4
    - ☐ More than 4
27. Control Group(s): _____
    - ☐ Substance(s) applied: _____
    - ☐ Amount/Dosage: _____
    - ☐ Frequency of application: _____
    - ☐ Route and timing of administration: _____
    - ☐ Vehicle/Carrier used: _____

- ☐ Other materials applied to wound (e.g., hydrogel, cadexomer iodine, etc.):
  - ☐ None
  - ☐ Yes (Specify, including amount/concentration: _____)

Treatment Group(s): _____

- ☐ Substance(s) applied: _____
- ☐ Amount/Dosage: _____
- ☐ Frequency of application: _____
- ☐ Route and timing of administration: _____
- ☐ Vehicle/Carrier used: _____
- ☐ Other materials applied to wound (e.g., hydrogel, cadexomer iodine, etc.):
  - ☐ None
  - ☐ Yes (Specify, including amount/concentration: _____)

28. Microbial laboratory parameters (describe): _____
29. Microbiological evaluation (describe): _____
    i. Wound sampling method (describe):
30. Microbiology culture conditions for bacterial enumeration:
    - ☐ Temperature (°C)
      - ☐ Specify: ____
    - ☐ Humidity (%)
      - ☐ Specify: _____
    - ☐ Culture medium
      - ☐ Specify: ____
    - ☐ Other conditions
      - ☐ Specify: ____

Section V. Wound Assessment and Analysis:

31. Gross analysis assessment (describe): _____
    - ☐ Clinical signs of infection (e.g. redness, swelling, discharge etc.) (describe): _____
    - ☐ Microbial analysis of infection (describe wound sampling): _____
      - ☐ Specify methods for microbiological enumeration_____
32. Were wound assessors blinded to experimental groups?
    - ☐ No
    - ☐ Yes
    - ☐ Other (if blinding was done for some analyses, please specify which:_____)

(Note: for nonclinical studies, the pathology evaluation (gross and histopathology) should typically be conducted unblinded)

33. Frequency of assessment
    - ☐ Daily
    - ☐ Weekly
    - ☐ Monthly
    - ☐ Other (Specify:_____)
34. Timepoints of assessment (Select all that apply)
    - ☐ Single timepoint (Specify:_____)
    - ☐ Multiple timepoints
      - ☐ <1 Day (Specify:_____)
      - ☐ Day 1
      - ☐ Day 2
      - ☐ Day 3
      - ☐ Day 4
      - ☐ Day 5
      - ☐ Day 6
      - ☐ Day 7
      - ☐ Day 8
      - ☐ Day 9
      - ☐ Day 10
      - ☐ Day 11
      - ☐ Day 12
      - ☐ Day 13
      - ☐ Day 14
      - ☐ Day 15
      - ☐ Day 16
      - ☐ Day 17
      - ☐ Day 18
      - ☐ Day 19
      - ☐ Day 21
      - ☐ Day 28
      - ☐ >28 Days (Specify:_____)
35. Assessment Method? (Select all that apply)
    - ☐ Non-invasive/Minimally invasive
      - ☐ Wound tracing
      - ☐ Photographic analysis
      - ☐ Trans-epidermal water loss measurement
        - i. Describe tools used: _____
      - ☐ Blood / wound fluid sampling
        - i. Specify: _____
      - ☐ Other
        - i. Specify: _____
      - ☐ Describe tools and/or software used: _____
    - ☐ Invasive
      - ☐ Biopsy/Excision
        - i. Location of wound: _____
        - ii. Size / depth (mm): _____
        - iii. Partial thickness: _____
        - iv. Full thickness: _____
      - ☐ Histology
        - i. Tissue fixation method: _____
        - ii. Embedding medium:_____
        - iii. Sectioning technique and thickness (μm):_____
        - iv. Staining method(s):_____

v. Other histological techniques (Specify:_____)
- ☐ Blood / wound fluid sampling
  - i. Specify: _____
- ☐ Other
  - i. Specify: _____
- ☐ Describe tools and/or software used: _____

36. Was Percent (%) Wound Area Reduction measured?
- ☐ No
- ☐ Yes, externally
  - i. Specify method(s) used: _____
- ☐ Yes, internally
  - i. Specify method(s) used: _____

37. Time to wound closure (defined as 100% epithelialized where wound is fully covered by keratinocyte layer)
- ☐ Not measured
- ☐ Measured externally (e.g., visual assessment, photography)
  - i. Specify method(s) used: _____
- ☐ Measured histologically
  - i. Specify histological method(s) used: _____
- ☐ Measured using other methods
  - i. Specify method(s) used: _____

38. Signs of regeneration
- ☐ Hair follicles
  - ☐ Timepoint analyzed (Specify days/weeks post-injury: _____)
  - ☐ Rationale for selected timepoint (Specify: _____)
  - ☐ Method (Specify Histology, Visual inspection, or Other: _____)
- ☐ Pigment changes
  - ☐ Timepoint (Specify days/weeks post-injury: _____)
  - ☐ Method (Specify Histology, Visual inspection, or Other: _____)
  - ☐ Rational for selected timepoint (Specify: _____)
- ☐ Wound-Induced Hair Neogenesis (WIHN)
  - ☐ Assessed (Yes/No): _____
  - ☐ If Yes:
    - ☐ Timepoint (Specify days/weeks post-injury: _____)
    - ☐ Method (Specify Histology, Visual inspection, or Other: _____)
    - ☐ Rationale for selected timepoint (Specify: _____)
- ☐ Other (Specify: _____)

39. Scar assessment
- ☐ Not assessed
- ☐ Assessed
  - ☐ Describe parameters: _____
  - ☐ Methods and tools used: _____

40. Wound breaking strength measured
- ☐ No
- ☐ Yes
  - ☐ Describe parameters: _____
  - ☐ Methods and tools used: _____

2. WRAHPS checklist and Reporting Template for Human *Ex Vivo* Models

Skin

1. Time lapse between harvest, donation and testing:
- ☐ Less than 24 hours
- ☐ 1-2 days
- ☐ Other
  - ☐ Specify: ____
- ☐ Unknown

2. Storage condition of skin before use/testing:
- ☐ Fresh (not stored)
- ☐ Refrigerated
  - ☐ Storage liquid medium/buffer (Specify type, and include antibiotics and nutrients used:_____)
  - ☐ Duration (Specify:_____)
- ☐ Other
  - ☐ Specify: ____
- ☐ Unknown

3. Any treatment, such as trimming of fat, before use/testing:
- ☐ Yes
  - ☐ Specify: _____
- ☐ No
- ☐ Unknown

4. Any type of quality control, before starting the experiment:
- ☐ Yes
  - ☐ Specify: _____
- ☐ No
- ☐ Unknown

5. Biological sex of the donor(s):
- ☐ Single donor:
  - ☐ Female
  - ☐ Male
  - ☐ Unknown
- ☐ Multiple donors:
  - ☐ Number of female donors: _____
  - ☐ Number of male donors: _____
  - ☐ Number of donors with unknown biological sex: _____

6. Age of the donor(s):
- ☐ Child

- ☐ Newborn
- ☐ Specify: ____
- ☐ Adult
  - ☐ Specify age: ____
- ☐ Elderly
  - ☐ Specify age: ____
- ☐ Unknown

7. Donor Site
   - ☐ Abdomen
   - ☐ Breast
   - ☐ Axilla
   - ☐ Back
   - ☐ Arm
   - ☐ Other
     - ☐ Specify: ____

8. Wound etiology simulated
   - ☐ Surgical/traumatic
   - ☐ Diabetic
   - ☐ Ischemic
   - ☐ Surgical
   - ☐ Burn
   - ☐ Pressure
   - ☐ Infection
   - ☐ Other
     - ☐ Specify: _____

9. Patient Comorbidities
   - ☐ Specify: _____

10. Cellular composition organotypic 3D model
    - ☐ Keratinocytes
      - ☐ Primary
      - ☐ Immortalized, specify_____
      - ☐ Passage number (or range), specify _____
      - ☐ Genetically modified, specify _____
      - ☐ Culture medium used, specify_____
      - ☐ Scaffold used, specify _____
    - ☐ Fibroblasts
      - ☐ Primary
      - ☐ Immortalized, specify_____
      - ☐ Passage number (or range), specify _____
      - ☐ Genetically modified, specify _____
      - ☐ Culture medium used, specify_____
      - ☐ Scaffold used, specify _____
    - ☐ Other cell types, specify_____
      - ☐ Primary
      - ☐ Immortalized, specify_____
      - ☐ Passage number (or range), specify _____
      - ☐ Genetically modified, specify _____
      - ☐ Culture medium used, specify_____
      - ☐ Scaffold used, specify _____

11. Type of Wounding
    - ☐ Incisional
      - ☐ Healing by primary intention (surgical closure)
      - ☐ Healing by secondary intention
    - ☐ Excisional
      - ☐ Partial-Thickness Excisional
      - ☐ Full-Thickness Excisional
    - ☐ Pressure injury / Ulcer
    - ☐ Burn
      - ☐ Water scalding
        - ☐ Time (Specify:_____)
        - ☐ Temperature (Specify:_____)
        - ☐ Percentage burn surface area (Specify:____)
      - ☐ Contact burn
        - ☐ Time (Specify:_____)
        - ☐ Pressure/weight (Specify:_____)
        - ☐ Temperature (Specify:_____)
        - ☐ Methods of heating (Specify :_____)
        - ☐ Material used (Specify:_____)
        - ☐ Percentage burn surface area (Specify:_____)
        - ☐ Other (Specify:_____)
      - ☐ Chemical
    - ☐ Other
      - ☐ Specify: ____

12. Tool used to generate wound(s)
    - ☐ Specify: _____
13. Size of wounds made (mm)
    - ☐ Incisional (length) : _____
    - ☐ All others: _____
14. How many wounds were made?
    - ☐ 6-8
    - ☐ More than 8
      - ☐ Specify:_____

15. How many wounds per experimental / treatment groups??
    - ☐ 1-2
    - ☐ 3-4
    - ☐ 5-6
    - ☐ More than 6
      - ☐ Specify: _____

16. Experimentally induced infection
    - ☐ None
    - ☐ Planktonic (Specify)
      - ☐ Species and strain:_____
      - ☐ Growth media used:_
      - ☐ Logarithmic phase__
      - ☐ Stationary phase
      - ☐ Amount (CFUs):_____
      - ☐ Route of infection: _____
      - ☐ Duration of infection:_____

- ☐ Biofilm (Specify)
  - ☐ Species and strain: _____
  - ☐ Growth media used:_
  - ☐ Age of culture _____
  - ☐ Amount of CFUs: _____
  - ☐ Route of infection: _____
  - ☐ Duration of infection: _____
  - ☐ Method of validation of biofilm formation:_____
- ☐ Other (Specify:_____)

17. Control Group(s): _____

☐ Substance(s) applied: _____
☐ Amount/Dosage: _____
☐ Frequency of application: _____
☐ Route and timing of administration: _____
☐ Vehicle/Carrier used: _____

Treatment Group(s): _____

☐ Substance(s) applied: _____
☐ Amount/Dosage: _____
☐ Frequency of application: _____
☐ Route and timing of administration: _____
☐ Vehicle/Carrier used: _____

18. Microbiological evaluation (describe): _____
    - ☐ Wound sampling method (describe):
19. Microbiology culture conditions for bacterial enumeration:
    - ☐ Temperature (°C)
      - ☐ Specify: ____
    - ☐ Humidity (%)
      - ☐ Specify: _____
    - ☐ Culture medium
      - ☐ Specify: ____
    - ☐ Other conditions
      - ☐ Specify: ____
20. Timepoints of assessment (Select all that apply)
    - ☐ Single timepoint
      - ☐ Specify: _____
    - ☐ Multiple timepoints
      - ☐ Day 1
      - ☐ Day 2
      - ☐ Day 3
      - ☐ Day 4
      - ☐ Day 5
      - ☐ Day 6
      - ☐ Day 7
      - ☐ Day 8
      - ☐ Day 9
      - ☐ Day 10
      - ☐ Day 11
      - ☐ Day 12
      - ☐ Day 13
      - ☐ Day 14
21. Frequency of assessment
    - ☐ Daily
    - ☐ 2 days
    - ☐ 3 days
    - ☐ 4 days
    - ☐ Other
      - ☐ Describe: _____
22. Was Percent (%) Wound Area Reduction measured?
    - ☐ No
    - ☐ Yes
      - ☐ Assessment Method:
        - ☐ Photographic analysis
        - ☐ Other (Specify:_____)
        - ☐ Described tools and/or software used: _____
        - ☐ Histology
          - ☐ Method(s): _____
          - ☐ Other (Specify: _____)
      - ☐ Describe tools and/or software used: ___
23. Time to wound closure (defined as 100% epithelialized where wound is fully covered by keratinocyte layer)
    - ☐ Not measured
    - ☐ Measured externally (e.g., visual assessment, photography)
      i. Specify method(s) used: _____
    - ☐ Measured histologically
      i. Specify histological method(s) used: _____
    - ☐ Measured using other methods
      i. Specify method(s) used: _____
24. Scar assessment
    - ☐ Not assessed
    - ☐ Assessed
      - ☐ Describe parameters: _____
      - ☐ Methods and tools used: _____

AUTHOR CONTRIBUTIONS

Conceptualization: L.G., V.R.D., and M.T.-C. *Drafting initial article*: N.O., N.M.V., and M.T.-C. *Substantial editing of multiple drafts*: N.O., N.M.V., I.P., S.V., T.W., S.G., A.R.-W., L.A.D., and M.T.-C.

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The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ORCID

Irena Pastar https://orcid.org/0000-0003-0197-6198
Allison N. Ramey-Ward https://orcid.org/0009-0007-9502-1531
Vickie R. Driver https://orcid.org/0000-0002-5148-5365
Lisa J. Gould https://orcid.org/0000-0001-5167-4679
Marjana Tomic-Canic https://orcid.org/0000-0002-9341-0193

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SUPPORTING INFORMATION

Additional supporting information, a fillable PDF checklist form, can be found online here: https://onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1111%2Fwrr.13232&file=wrr13232-sup-0001-supinfo.pdf.

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