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Mechanistic Basis for the Applicability of Animal Models in Diabetic Wound Therapy: A Comparative Analysis of Animal and Human Data

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Abstract | Diabetic foot ulcers (DFUs) are a major clinical and economic burden. Animal models of diabetic wounds are key preclinical tools for developing drugs and therapies. However, their clinical predictive value has long been debated. This review uses leptin receptor-deficient diabetic (db/db) mice and streptozotocin (STZ)-induced diabetes, ischemia, pressure, and infection models as benchmarks. We compared evidence from these models with evidence from human diabetic wound tissue, primary cells, ex vivo skin, and small clinical studies. Evidence was classified as animal causal evidence (A), animal correlative evidence (B), human correlative evidence (C), and human interventional/causal evidence (D). This grading system is a custom evidence-structure framework. It is not a risk-of-bias assessment. Evidence was organized across eight mechanistic axes: advanced glycation end product (AGE)/receptor for AGE (RAGE) and oxidative stress; hypoxia-inducible factor-1α (HIF-1α)/vascular endothelial growth factor (VEGF); inflammation and immunity; cellular senescence; nerve; extracellular matrix (ECM); infection; and epigenetic and metabolic reprogramming. The comparative analysis showed that animal and human evidence was more consistently aligned in direction for AGE/RAGE, HIF-1α/VEGF, NETosis, and CXCR2-related senescence. However, human causal/interventional evidence was limited. No human wound intervention study has targeted AGE/RAGE. Most human HIF-1α/VEGF studies used nonselective interventions. Human CXCR2 evidence came mainly from small ex vivo studies. For macrophage polarization (with conflicting results related to healing stage), nerve repair, reduced fibroblast responsiveness to multiple growth factors, ECM remodeling, infection susceptibility, and epigenetic/metabolic reprogramming, the overall direction of evidence was consistent. However, the strength of evidence varied. Human interventional validation is still lacking for epigenetic/metabolic reprogramming. Therefore, animal models can be used to validate targets and mechanisms of action. However, caution is needed when predicting effect size and clinical endpoints. Model applicability must also be constrained by face validity, construct validity, and predictive validity. On this basis, we propose a tiered validation pathway. This is a narrative mechanistic comparative review. We did not perform a systematic search or quantitative synthesis.

Keywords | diabetic foot ulcer; diabetic wound model; mechanistic comparison; evidence grading; translational research

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1 Introduction

DFUs are among the most severe and costly complications of diabetes. Approximately 15%–25% of patients develop foot ulcers, with markedly increased risks of infection, recurrence, and amputation [1,2]. A DFU is not merely a skin defect. It is a chronic non-healing state caused by the combined effects of neuropathy, peripheral arterial disease, persistent metabolic abnormalities, infection, and biofilm [3].

Animal diabetic wound models bridge mechanistic research and clinical development. However, many growth factor and pro-angiogenic strategies are effective in animals but show limited efficacy or fail in clinical trials. The pro-angiogenic potential of VEGF-A has not consistently translated into functional vessels in early clinical studies. The local growth factor preparation becaplermin for DFUs is also limited by its modest efficacy and safety concerns [4]. This gap should not be attributed simply to differences between animals and humans. It should be examined in the context of model validity, endpoints, disease duration, coverage of comorbidities, wound heterogeneity, and clinical trial design. Unlike previous reviews that mainly describe model types or a single mechanism, this review grades and compares animal causal evidence with human interventional evidence across eight mechanistic axes. We also define the boundaries of model applicability.

This review is a narrative mechanistic comparative review, not a systematic review. It uses animal mechanisms as the benchmark and cross-checks them against human data. The search covered PubMed, Web of Science, CNKI, and Wanfang through October 19, 2026 (before submission). Reference [53] was published online and had not yet been assigned a volume or issue. Search terms included diabetic foot ulcer, diabetic wound healing, animal model, db/db, streptozotocin, ischemia, pressure ulcer, biofilm, AGE/RAGE, HIF-1α/VEGF, NETosis, CXCR2, senescence, extracellular matrix, epigenetics, m6A, and their Chinese equivalents. We included studies of diabetic wound animal models and human DFU mechanisms. We excluded studies without original data and literature not published in Chinese or English. Because the search process was not prospectively recorded as required for a systematic review, we cannot accurately report the numbers of initially identified, deduplicated, and excluded records. Therefore, no PRISMA flow diagram is provided. No post hoc estimates or reconstructed screening counts are reported.

Studies were selected according to the following principles: direct relevance to the eight mechanistic axes; availability of original animal or human data; priority for human DFU tissue, cell, or clinical evidence; and priority for animal causal studies and human intervention studies. Screening was performed by reading full texts and removing duplicate and irrelevant records.

Evidence was graded into four levels. A-level evidence was animal causal evidence, defined as studies involving gene knockout, conditional knockout, pharmacological blockade, or supplementation with functional endpoints. B-level evidence was animal correlative evidence, defined as expression, secretion, cell phenotype, or omics changes in animal models. C-level evidence was human correlative evidence, defined as expression, phenotype, or transcriptomic associations in patient samples, primary cells, or ex vivo skin. D-level evidence was human interventional/causal evidence, defined as intervention studies in humans or clinical evidence that can directly establish causality. Plus and minus signs (C+, D−) indicate stronger or weaker evidence within the same level. This A–D grading was used to structure the evaluation of study design, causal intervention, human relevance, and direction of evidence. It is not equivalent to a formal risk-of-bias assessment tool.

Model applicability was also defined jointly by face validity, construct validity, and predictive validity. Face validity refers to whether the model approximates clinical anatomy, wound site, onset mode, infection, and closure process. Construct validity refers to whether the model truly reproduces the key pathological mechanisms under study. Predictive validity refers to whether intervention effects observed in animals predict human clinical results. We selected db/db mice and STZ-induced diabetes, ischemia, pressure, and infection models because they represent obese type 2 diabetes, acute hyperglycemia, hypoperfusion, mechanical compression, and infection/biofilm, respectively. Pig models were included because they better approximate human skin structure and wound size.

This article is organized as follows. Section 2 compares animal and human evidence across the eight mechanistic axes. Section 3 discusses the basis and

boundaries of model applicability. Section 4 proposes strategies for model selection, reporting, and translational validation. Section 5 summarizes key challenges and future research directions.

2 Comparison of Animal and Human Evidence Across Eight Mechanistic Axes

2.1 AGE/RAGE and oxidative stress.

Is AGE/RAGE signaling in diabetic wounds a modifiable therapeutic target that is relevant to human wounds? In wounds of db/db diabetic mice, AGE and its receptor RAGE are elevated. Soluble RAGE (sRAGE) acts as a decoy that binds AGE and blocks AGE–RAGE signaling. This reduces tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and matrix metalloproteinases (MMPs). It also increases platelet-derived growth factor-B (PDGF-B) and VEGF and accelerates wound closure. Inhibition of cytochrome P450 1A1 (CYP1A1) or treatment with valsartan also improves oxidative stress and mitochondrial metabolism (A-level) [5-7]. In human DFUs, MMP/proteolytic imbalance and upregulation of S100 calcium-binding protein A8/A9 (S100A8/A9) and oxidative stress-related genes are observed. However, no human intervention study targeting wound RAGE is available (C-level) [8-11]. Therefore, RAGE is a candidate target with human data consistent in direction, but it has not been therapeutically validated in humans.

AGE/RAGE and oxidative stress have relatively strong causal evidence in animal models. Human DFUs also show MMP/proteolytic imbalance, S100A8/A9 changes, and oxidative stress-related gene changes. The direction is consistent [5-11]. Unmet need: Human evidence is mainly correlative. No human intervention study has targeted wound RAGE. The downstream effects of RAGE in different cell types and wound stages remain unclear [8-11]. Therefore, the RAGE pathway should be regarded as a candidate target with directionally consistent human data. It cannot yet be called a therapeutically validated target in humans.

In summary, AGE/RAGE and oxidative stress have strong causal evidence in animals. However, human interventional evidence is still lacking. The next axis discusses the intersecting HIF-1α/VEGF angiogenic mechanism.

2.2 HIF-1α/VEGF and angiogenesis.

Can restoring HIF-1α/VEGF-mediated angiogenesis translate the pro-healing effects observed in animals into clinical benefit for human DFUs? Topical administration of deferoxamine (an iron chelator) stabilizes and restores HIF-1α and VEGF and promotes wound closure. Silencing prolyl hydroxylase domain protein 2 (PHD2) also accelerates wound healing in db/db mice. When βII spectrin (SPTBN1) is deleted, angiogenic and inflammatory changes are inconsistent. This indicates mechanistic branching of this pathway in vivo (A-level) [12-14]. Small human intervention studies show that negative-pressure wound therapy increases basic fibroblast growth factor (bFGF), phosphorylated extracellular signal-regulated kinase 1/2 (pERK1/2), and collagen in wound tissue (D− level) [15]. Electrical stimulation increases HIF-1α and VEGF in wound fluid. Antibiotic bone cement increases VEGFR2, CD31, α-smooth muscle actin (α-SMA), and Ki67 expression in wound-edge tissue and shortens healing time (D− level) [16,17]. However, none of these interventions selectively targets HIF-1α/VEGF. Adiponectin improves blood flow recovery after ischemia in wild-type mice but is ineffective in STZ diabetic mice and db/db mice [18]. This suggests that diabetes itself can make some pro-angiogenic interventions ineffective. Their effects should not be extrapolated directly from healthy animals to diabetic wounds.

In animals, the HIF-1α/VEGF axis can be restored by upstream interventions such as deferoxamine or PHD2 silencing. Small human studies also show that negative-pressure wound therapy, electrical stimulation, and antibiotic bone cement can improve angiogenesis-related markers. However, these human interventions do not selectively target HIF-1α/VEGF [12-17]. Unmet need: Selective HIF-1α/VEGF interventions and high-quality clinical endpoint evidence are still lacking. Diabetes can also blunt some pro-angiogenic responses [18]. Therefore, this axis can support mechanistic validation. It is not yet sufficient to predict clinical effect size.

In summary, the HIF-1α/VEGF axis has strong mechanistic evidence in animals. However, human selective and causal interventional evidence is still limited. The next axis discusses the intersecting inflammatory and immune microenvironment.

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2.3 Inflammation and immune imbalance.

Is inflammation in diabetic wounds simply "excessive inflammation," or is it a repair barrier caused by an imbalance among neutrophils, neutrophil extracellular traps (NETs), and macrophage subsets? In wounds of db/db mice, macrophage inflammatory protein-2 (MIP-2) and monocyte chemoattractant protein-1 (MCP-1) remain highly expressed [19]. Diabetic mice show increased NETs. Padi4 deficiency (loss of PAD4 function), DNase 1 treatment, and the PAD4 inhibitor Cl-amidine each accelerate healing (A-level) [20,21]. Tibial cortex transverse transport (TTT) mobilizes non-classical monocytes from bone marrow and promotes macrophage polarization from the proinflammatory M1 phenotype to the reparative M2 phenotype. ON101 promotes M2c macrophages associated with repair/remodeling. When monocytes/macrophages are depleted, the pro-healing effects of both therapies disappear. This indicates that macrophages are a key component (A-level) [22,23]. NETs can also promote endothelial–mesenchymal transition through the Toll-like receptor 9 (TLR9)–p21-activated kinase 2 (PAK2)–Hippo/Yes-associated protein (YAP) pathway [24]. In humans, neutrophils from both type 1 and type 2 diabetes patients are more prone to NETosis. Wound NET proteins and serum markers are associated with wound deterioration, infection, delayed healing, and amputation [20,21,25,26]. Single-cell and biopsy studies show that the relationship between M1/M2 and healing outcomes varies with healing stage. These findings do not support a simple model in which M1 is always harmful and M2 is always beneficial [10,11,27].

NETs, PAD4, and macrophage subsets have causal evidence in animals. In human DFUs, NET markers and stage-related M1/M2 changes also show concordant associations [10,11,19-27]. Unmet need: The roles of NETosis and macrophage subsets are clearly stage- and cell-type-dependent. Human causal evidence that can directly guide clinical intervention is still lacking [10,11,27]. Therefore, M1/M2 cannot be used as simple therapeutic target labels. Related interventions must be evaluated in the context of healing stage and cell source.

In summary, inflammation and immune imbalance have strong causal evidence in animals. However, human interventional evidence is still limited. The next axis discusses the intersecting cellular senescence and CXCR2 signaling.

2.4 Cellular senescence.

Are cellular senescence and its senescence-associated secretory phenotype (SASP) directly reversible therapeutic targets, or are they stage-specific responses during wound repair? In wounds of db/db mice, senescent cells increase. The CXCL1/2–CXCR2-mediated SASP is enhanced. The CXCR2 antagonist SB265610 promotes healing and reduces inflammation (A-level) [28]. In human ex vivo skin, CXCL1/2 delays wound closure, and SB265610 improves closure. However, that study used few donors. The effect also depends on the ligand combination of CXCL1 and CXCL2 (C-level) [28]. The cellular senescence/CXCR2 pathway can be used for mechanistic comparison between animals and humans. However, it cannot yet support direct recommendations to use an agonist or antagonist for diabetic wounds. It is first necessary to clarify the ligand, the target cell type, and the healing stage. Only then can activation or blockade be selected. Otherwise, inflammation may improve while angiogenesis or re-epithelialization is impaired.

Both animal and human ex vivo skin data show that CXCL1/2–CXCR2 is involved in SASP and delayed healing. SB265610 improves closure. The direction is consistent [28]. Unmet need: Human evidence comes only from ex vivo studies with a small number of donors. The effect depends on the ligand combination of CXCL1 and CXCL2. It is not yet possible to choose an agonist or antagonist strategy on this basis [28]. Therefore, this axis is suitable for mechanistic comparison. It cannot yet be directly translated into clinical drug recommendations.

In summary, the cellular senescence/CXCR2 axis has strong causal evidence in animals. However, human interventional evidence is still insufficient. The next axis discusses the intersecting neuropathy and nerve repair signals.

2.5 Neuropathy and nerve repair.

Clinical question: Can neuropathy and neurogenic repair signals be sufficiently reproduced in animal models and become therapeutic targets for human DFUs? Diabetic mice show reduced epidermal nerves. Local supplementation with substance P, nerve growth factor (NGF), or transforming growth factor-β3 (TGF-β3) promotes closure (A− level) [29-31]. However, the typical long-duration plantar neuropathy in clinical practice is difficult to fully reproduce in animals [32].

Long-duration ZDF rats develop slowed motor and sensory nerve conduction velocities, selective mechanical hyperalgesia, distal myelinated and unmyelinated axon atrophy, and loss of skin nerve terminals after 4 months of hyperglycemia [33]. STZ rats subjected to 3 h ischemia followed by 7 d reperfusion develop endoneurial edema and ischemic fiber degeneration at 1 and 4 months. The changes are more severe at 4 months [34]. In human evidence, among 8 amputation specimens from DFUs, PGP9.5-positive nerves in ulcers and adjacent skin were 0/8, versus 5/5 in controls (C-level) [30]. However, no chronic wound model currently combines neuropathy, ischemia, infection, and plantar load.

In animals, supplementation with substance P, NGF, or TGF-β3 promotes healing. Human DFU specimens also show reduced epidermal nerves. The direction of nerve repair is consistent [29-31]. Unmet need: Current models cannot simultaneously reproduce long-duration neuropathy, ischemia, infection, and plantar load. Human evidence comes mainly from amputation specimens. Targeted intervention trials are still lacking [30,32-34]. Therefore, this axis can only support validation of nerve-related mechanisms. It cannot yet predict clinical efficacy.

In summary, neuropathy can be partially reproduced in animals. However, human evidence for nerve-related interventions is still lacking. The next axis discusses the intersecting ECM and fibroblast responses.

2.6 ECM, fibroblasts, and MMP/tissue inhibitor of metalloproteinases (TIMP).

Clinical question: Among ECM degradation, reduced fibroblast responsiveness, and abnormal collagen quality, which is the key modifiable target in human DFUs? Porcine diabetic wounds show downregulation of ECM genes (B-level) [35]. sRAGE and isovitexin improve collagen remodeling. Diabetic skin shows decreased mechanical properties and a reduced collagen I/I:III ratio (A/B-level) [5,36,37]. Human ulcer fibroblasts show reduced responses to multiple growth factors [38]. Non-healing wounds show disturbed ECM signaling [8,11]. Protease-modulating dressings can reduce the MMP-9/TIMP-2 ratio. Collagen scaffold randomized controlled trials (RCTs) and systematic reviews show improvements in closure and histological indicators [39,40]. Local application of fibroblast growth factor family members also shows pro-angiogenic and fibroblast-proliferative effects (review) [41]. These trials support matrix-based therapies. However, they have not directly proven the MMP/TIMP causal chain or long-term collagen quality.

Reduced growth factor responsiveness (relative resistance) is directionally consistent with insufficient growth factor signaling, limited fibroblast proliferation, and limited matrix synthesis in animal models. However, human evidence comes mainly from cultured ulcer fibroblasts in vitro. It cannot yet show that this is an independent causal mechanism of DFU non-healing.

ECM genes are downregulated in porcine diabetic wounds. In animals, sRAGE, isovitexin, and collagen-related interventions improve matrix remodeling. Human DFU fibroblasts show reduced responses to growth factors such as EGF and IGF-I. Non-healing wounds show disturbed ECM signaling. The overall direction is consistent [5,8,11,35-38]. Unmet need: Human evidence comes mainly from cultured ulcer fibroblasts in vitro. The MMP/TIMP causal chain and long-term collagen quality have not been confirmed. “Reduced growth factor responsiveness” cannot be directly equated with in vivo FGF resistance [38-41]. Therefore, this axis supports validation of matrix and fibroblast mechanisms. It is not yet sufficient to predict clinical efficacy.

In summary, ECM and fibroblast abnormalities are directionally consistent in animals and humans. However, human causal interventional evidence is still insufficient. The next axis discusses the intersecting infection and biofilm mechanisms.

2.7 Infection and biofilm.

Clinical question: Can single-species animal infection models represent the multispecies biofilms and dynamic microbiota changes of human DFUs? Animal models have expanded from single-species to multispecies models. In a mouse 4-species biofilm model, polymicrobial infection causes more marked healing delay and higher antimicrobial tolerance than single-species infection. In STZ diabetic rats with a dual-species biofilm of methicillin-resistant Staphylococcus aureus (MRSA) and Acinetobacter baumannii, triple therapy reduced bacterial load by more than 4 log10 CFU/g. It downregulated icaA and bap genes by about 3-fold and 4-fold, respectively. It also achieved complete re-epithelialization on day 14. A porcine dorsal 8-week biofilm model showed that wounds could close by area, but skin barrier function was not restored. This was termed the “invisible

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wound" [42-44]. Human longitudinal studies show that microbiota changes dynamically with outcome and infection stage. In 16S rRNA analysis of 30 patients, healing wounds were enriched in Alcaligenes and Corynebacterium, while deteriorating wounds had increased Enterococcus and Serratia. Amputees had high abundance of Escherichia and reduced Staphylococcus. Follow-up of 8 patients for 10 weeks showed decreased diabetic skin microbiota diversity, but genus abundance was not significantly associated with healing. Metagenomic analysis of 6 patients before and after infection found that pathogen abundance actually decreased and obligate anaerobes increased at infection [45-47]. Therefore, infection assessment should be stratified by stage, anaerobes, and barrier function.

Multispecies biofilm models and human longitudinal microbiota studies both suggest that infection is stage-specific, involves anaerobes, and is associated with impaired barrier function. The direction is consistent between animals and humans [42-47]. Unmet need: Current animal models still cannot fully reproduce the microbiota heterogeneity, recurrent infection, and barrier function changes of human DFUs. Results from single-species models cannot be directly extrapolated [42-47]. This axis therefore supports stratified infection assessment but remains mainly for mechanism and model validation.

In summary, infection models have advanced from single-species to multispecies biofilms. However, human evidence for infection intervention and barrier repair is still limited. The next axis discusses the intersecting epigenetic and metabolic reprogramming.

2.8 Epigenetic and metabolic reprogramming.

Have epigenetic and metabolic reprogramming become modifiable targets for human DFUs, or are they still at the mechanistic validation stage?

2.8.1 Animal causal evidence

Lactate-driven lactylation of histone H3 lysine 18 (H3K18la) upregulates methyltransferase-like 3 (METTL3). METTL3 stabilizes DNA methyltransferase 1 (DNMT1) mRNA through the m6A–heterogeneous nuclear ribonucleoprotein A2/B1 (HNRNPA2B1) axis. This promotes keratinocyte proliferation [48]. Chromatin immunoprecipitation sequencing (ChIP-seq) shows that H3K18la is enriched at the Acvr1c locus. H3K18la promotes angiogenesis through Acvr1c [49]. Brahma-related gene 1 (BRG1) binds the promoter of collagen type XVI alpha 1 chain (COL16A1). This binding was validated by ChIP-qPCR and luciferase reporter assays. It can promote wound healing [50]. A hydrogel loaded with an FTO inhibitor achieved wound closure rates of about 98% on day 14 in streptozotocin (STZ) rats and about 93% on day 20 in leptin receptor-deficient diabetic (db/db) mice. Control groups achieved about 77% and 60%, respectively [51].

2.8.2 Human direct measurement evidence

Human evidence has expanded from inferred gene expression to direct measurement. DFU wounds show decreased m6A levels and increased FTO [51]. Multi-omics and functional experiments suggest that methylation-related genes such as METTL16 are involved in DFU pathogenesis [52]. After negative-pressure therapy, 46 DFU patients and 16 patients with non-diabetic chronic ulcers showed increased WTAP/m6A levels. These levels were positively correlated with 4-week healing outcomes [53]. Wound biopsies from 20 patients per DFU grade showed upregulation of HDAC1/3/4/11 and SIRT3/5/6/7. HDAC2/8 and SIRT1/2/4 were downregulated. HDAC4 was negatively correlated with Nrf2 and angiogenesis markers [54].

2.8.3 Consistency assessment

The animal causal evidence above and the human direct measurement evidence are generally consistent in direction. In animals, intervention in the H3K18la–METTL3/m6A, BRG1–COL16A1, or FTO pathways promotes wound healing. Human DFUs also show epigenetic changes in m6A, FTO, WTAP, and HDAC/SIRT [48-54]. However, most human evidence is expression association, ex vivo experiments, or small-sample observations. Human interventional evidence with strength equal to animal causal experiments is still lacking.

2.8.4 Unmet need

No human intervention trial has targeted these epigenetic targets. Therefore, these pathways cannot be regarded as established human therapeutic targets. Future work should validate key molecules in stratified DFU cohorts. It should also conduct mechanistic intervention studies with wound closure, angiogenesis, and histological improvement as endpoints. This axis is a candidate but still unvalidated therapeutic direction.

In summary, epigenetic and metabolic

reprogramming has advanced from expression association to direct modification measurement and animal causal intervention. However, human targeted interventional evidence is still lacking. The next part discusses the overall evidence, counterexamples, and boundaries of animal model applicability.

Table 1. Evidence levels and consistency across Sections 2.1–2.8

Mechanistic axis	Animal evidence level	Human evidence level	Directional consistency	Key counterexample	Unmet need
AGE/RAGE and oxidative stress	A	C	Direction consistent; human interventional evidence lacking [5-11]	Targeted evidence such as sRAGE comes mainly from animals; no human wound RAGE intervention [5,8-11]	Need human RAGE-targeted intervention and validation of dose and safety window
HIF-1α/VEGF and angiogenesis	A	D−	Overall direction consistent, but human interventions are nonselective [12-18]	SPTBN1 deletion shows in vitro/in vivo divergence [13]; adiponectin is ineffective in STZ and db/db models [18]	Need selective HIF-1α/VEGF drugs and trials with closure and re-epithelialization endpoints
Inflammation and immune imbalance	A	C	Partially consistent; varies with healing stage and cell subsets [10,11,19-27]	M1/M2 dichotomy is unstable; NET effects are stage- and context-dependent [20,21,24,27]	Need human anti-NET or macrophage subset interventions, stratified by healing stage
Cellular senescence/CXCR2	A	C	Direction consistent, but limited by ligand combination and sample size [28]	Human ex vivo studies have few donors; CXCL1/2 ligand combination determines effect [28]	Need human intervention after defining ligand, cell type, and healing stage
Neuropathy and nerve repair	A−	C	Partially consistent [29-34]	Models cannot simultaneously reproduce long disease duration, ischemia, infection, and plantar load; human evidence comes mainly from amputation specimens [30,32-34]	Need multifactorial chronic wound models and nerve-targeted interventions
ECM, fibroblasts, and MMP/TIMP	A/B	C/D	Direction consistent, but causal chain incomplete [5,8,11,35-41]	Reduced fibroblast responsiveness comes mainly from in vitro data; MMP/TIMP causal chain and long-term collagen quality are unproven [38-41]	Need in vivo human causal validation and long-term matrix quality endpoints
Infection and biofilm	A/B	C	Partially consistent; clear stage and individual heterogeneity [42-47]	Single-species and multispecies models differ; pig model shows area closure but barrier not restored; pathogen abundance can decrease during human infection [42-47]	Need standardized multispecies models, barrier function, and longitudinal causal studies
Epigenetic and metabolic reprogramming	A	C	Direction consistent, but human intervention lacking [48-54]	Human evidence is mostly expression, ex vivo, or small-sample observation; no targeted intervention [48-54]	Need stratified DFU intervention trials to validate key modification sites and cell types

Note: A, animal causal evidence; B, animal correlative evidence; C, human correlative evidence; D, human interventional/causal evidence; D−, weaker D-level human interventional evidence.

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3 Mechanistic Basis and Qualifying Conditions for Using Animal Models in Human Diabetic Wound Therapy Development

3.1 Mechanistic basis

Animal models and human DFUs share pathological components such as hyperglycemia, oxidative stress, chronic inflammation, ischemia, and infection. Therefore, animal models can answer two questions: whether a target is involved in diabetic wound healing, and whether the direction of intervention is correct. Gene knockout, pharmacological blockade, cell depletion, and functional rescue studies in animals can establish causal chains. Human wound tissue, single-cell transcriptomics, and ex vivo skin can provide concordant or discordant validation. However, this basis supports mechanistic validation only. It cannot automatically provide effect size, optimal dosing timing, or clinical endpoints. This section focuses on the basis and boundaries of model applicability. Specific model selection, reporting, and validation pathways are discussed in Section 4.

3.2 Evidence for applicability and counterexamples

Applicability comes mainly from the following points. (1) Animal targets can be corroborated in the same direction by human data. (2) Models can reproduce some treatment-relevant pathological phenotypes. (3) Genetic, pharmacological, and cellular causal manipulations can be performed in animals. (4) CXCR2, NETosis, and other targets have partial bidirectional human–animal validation [20,21,28]. (5) Humanized mice, ex vivo human skin, immune-integrated organ chips, and pig models can provide additional cross-species support. Their specific positioning and use are discussed in Section 4.3. At the same time, human evidence is mostly expression association or indirect intervention. Wound contraction, short disease duration, single-species infection, and endpoint differences limit direct extrapolation from animal results to human DFU patients [32-34,41,43,44,55-67].

Counterexamples further show that applicability is not unconditional. The contribution of contraction to wound closure in unsplinted mice is debated [61,62]. The biological effect of CXCR2 depends on which CXCL ligands are present, their relative concentrations, and the cell types involved. Therefore, simple CXCR2 blockade does not necessarily produce consistent pro-healing effects [28]. In vitro inhibition of angiogenesis by SPTBN1 did not translate into decreased in vivo vascular density [13]. STZ and db/db models show opposite directions of bone marrow mobilization. With the same mobilization stimulus, STZ models show reduced mobilization, while db/db models show increased or unchanged mobilization [68]. Adiponectin is effective in wild-type mice but ineffective in both diabetic models [18]. Polymicrobial biofilms and microbiota changes during infection also differ from single-species models [43,46]. Animal efficacy with clinical failure is also related to mismatched disease duration and comorbidity scale, different endpoint meanings, multispecies biofilm differences, compensatory pathways, dosing timing, and population heterogeneity [4,69]. Therefore, positive animal results cannot be directly interpreted as clinical efficacy. Clinical failure cannot be simply attributed to target ineffectiveness.

3.3 Applicability boundaries, sources of controversy, and limits of conclusions

Controversies between current animal and human evidence should not be simply attributed to “animal models are ineffective” or “species differences.” They are better analyzed item by item according to six factors: data bias, label definitions, model differences, endpoint differences, dosing timing, and population heterogeneity.

3.3.1 Data bias

Some human studies have small sample sizes. Sampling sites and sex composition are inconsistent. Single-cell studies may also be affected by cell capture rate, sequencing depth, and sampling time [10,11,27]. Negative and null results are underreported in animal studies. This can overestimate effect sizes [70-72]. Differences in microbiome studies are also related to sampling site, infection stage, and sequencing method. Therefore, microbiota changes in different studies should not be directly regarded as contradictory [45-47].

3.3.2 Label definitions

M1/M2 are simplified labels for macrophage activation states. They do not fully represent the continuum and functional heterogeneity of macrophages in vivo [73,74]. Single-cell transcriptomics

reflects only the transcriptional state at the sampling time. It cannot alone prove the causal role of a cell subset. Differences in healing stage, sampling site, sex composition, and label definitions may all cause contradictions in the relationship between M1/M2 and healing outcomes [10,11,27].

3.3.3 Model differences

db/db, STZ, ischemia, pressure, and infection models simulate different diabetic and wound pathological components. Their metabolic states, immune backgrounds, disease durations, and healing modes are not the same [55,56,66,67,75,76]. For example, STZ and db/db models can show opposite directions of bone marrow mobilization [68]. Unsplinted mouse wounds also have obvious contraction confounding [61,62]. Therefore, when the same mechanism shows inconsistent results in different models, the first step is to determine whether the difference comes from the model structure itself.

3.3.4 Endpoint differences

Wound area reduction, re-epithelialization, complete closure, closure time, and histological indicators reflect different biological processes [57-60]. If animal studies use area reduction as the primary endpoint, while clinical studies use complete closure or long-term non-recurrence as the primary endpoint, the two cannot be directly compared for effect size even if the direction is consistent. Differences in endpoint definitions, follow-up duration, and blinding can themselves create the appearance of “animal efficacy, clinical failure.”

3.3.5 Dosing timing

Animal studies often give preventive treatment before wound formation or start intervention early after wound formation. Clinical DFU patients are usually already in a chronic stage with long-term hyperglycemia, neuropathy, ischemia, and repeated infection. Treatment often starts long after wound formation [4,69]. The same pathway may have different effects in the inflammatory, proliferative, and remodeling phases. Preventive, early, and late dosing may also produce opposite results. Therefore, dosing timing is a key variable for explaining animal–human differences.

3.3.6 Population heterogeneity

Human DFU patients differ in age, sex, diabetes type, disease duration, peripheral arterial disease, neuropathy, infection, plantar load, comorbidities, and clinical subtypes [45-47,77-83]. Animal experiments often use young animals of a single sex, specific pathogen-free status, and controlled comorbidities. These animals cannot fully cover the above heterogeneity. Therefore, results from animal models should be regarded as mechanistic evidence under specific model and population conditions. They cannot be directly extrapolated to all DFU patients.

In summary, contradictions such as those involving M1/M2 and the microbiome should not be simply classified as “animal–human inconsistency.” Priority should be given to checking data bias, label definitions, model differences, endpoint differences, dosing timing, and population heterogeneity. Only after these six factors are recorded, stratified, and corrected as much as possible can animal–human consistency judgments be comparable. Therefore, the applicability conclusions of this article are limited to mechanistic validation and direction judgment. They cannot be directly used to predict clinical effect size or replace human intervention trials [4,59,60,69]. These six factors define the boundary for extrapolating the conclusions of this article. Specific gaps in human interventional evidence for targets are discussed in Section 3.4. Specific operations for model selection, reporting, and tiered validation are discussed in Sections 4.1 and 4.3.

Table 2. Reasons for and qualifying conditions for using animal models in human diabetic wound therapy development

No.	Reason for applicability	Corresponding mechanistic axis	Boundary/counterexample	Key evidence	Applicability conclusion
1	Animal targets can be corroborated in the same direction by human data	1, 2, 3, 7	Human evidence is mostly expression, cell, or indirect intervention evidence, not causality	Human DFU tissue, primary-cell, and clinical evidence in Sections 2.1–2.8	Supports continued human bridging validation; do not directly call it a clinically effective target

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Table 2.(continue) Reasons for and qualifying conditions for using animal models in human diabetic wound therapy development

No.	Reason for applicability	Corresponding mechanistic axis	Boundary/counterexample	Key evidence	Applicability conclusion
2	Models can reproduce some treatment-relevant pathological phenotypes	1–8	Wound contraction confounding, short disease duration, opposite directions across models, incomplete comorbidities, interindividual microbiota differences, inconsistent endpoints, publication bias	[83–90; see Sections 2.1–2.8 and 3.3]	Used for mechanistic screening and surrogate endpoints; does not replace clinical closure endpoints
3	Genetic, pharmacological, and cellular causal validation can be performed in animals	1, 2, 3, 4, 6	Knockout or pharmacological net effects may be compensated; SPTBN1 shows mechanistic divergence in vitro and in vivo	[13]	Used to exclude ineffective targets and confirm mechanism direction; does not estimate effect size
4	CXCR2, NETosis, and others have partial bidirectional human–animal validation	3, 4	Human samples are small; M1/M2 results are inconsistent; human anti-NET interventions are still lacking	[24-28]	Demonstrates therapeutic relevance of targets; direction must be confirmed by context
5	Humanized, ex vivo, organ-chip, and pig models can provide tiered bridging	3, 4, 6, 8	Immunodeficient background, donor differences, and in vitro environment limit extrapolation	[55,75,76,84-89]	Used for direction verification, cell source confirmation, and dose calibration; does not replace prospective human trials

Overall judgment: Animal models should be trusted for target and mechanism validation and used cautiously for predicting effect size and clinical endpoints.

3.4 Asymmetry between animal causal evidence and human interventional evidence

In this article, “directionally consistent” mainly means that animal mechanisms and human expression, cell, or clinical observations point in the same direction. It does not mean that the target has been causally validated in humans. The most obvious current evidence gaps are concentrated in targets such as RAGE, CXCR2, S100A8/A9, and PAD4/NETosis.

Table 3. Animal causal evidence and human interventional evidence gaps for key targets in diabetic wounds

Target	Animal causal evidence	Current human evidence	Main intervention gap
RAGE	sRAGE or RAGE blockade reduces inflammation and MMPs and promotes wound closure in db/db mice [5]	Human DFUs show changes in RAGE-related ligands, MMPs, and oxidative stress, but evidence is mainly expression and omics association [8-11]	No human intervention study has targeted wound RAGE. Local RAGE blockade needs validation for target engagement, dose, safety, and closure endpoints

Table 3.(continue) Animal causal evidence and human interventional evidence gaps for key targets in diabetic wounds

Target	Animal causal evidence	Current human evidence	Main intervention gap
CXCR2	The CXCR2 antagonist SB265610 promotes healing and reduces inflammation in db/db mice [28]	Human ex vivo skin and small-sample experiments suggest that CXCL1/2–CXCR2 is involved in delayed healing. However, donors are few, and the effect depends on ligand combination [28]	No human CXCR2 intervention trial is available. Specific ligands, cell types, healing stages, and patient subtypes need to be defined
S100A8/A9	S100A8 can affect diabetic wound healing through the TLR4/NF-κB axis [90]	Human DFU single-cell and transcriptome studies show upregulation of S100A8/A9-related inflammatory signals [8,10,11]	Human interventional evidence targeting S100A8/A9 or the TLR4/NF-κB axis is lacking. It is necessary to test whether local blockade improves healing without impairing antimicrobial immunity
PAD4/NETosis	Padi4 deficiency, DNase 1, or PAD4 inhibitors reduce NETs and accelerate wound healing in diabetic mice [20,21,24]	In human DFUs, NET proteins and serum markers are associated with deterioration, infection, delayed healing, and amputation [20,21,25,26]	Human DFU trials of PAD4 inhibitors, DNase, or other anti-NET strategies are still lacking. Whether NET burden can serve as a pharmacodynamic marker remains unvalidated

Therefore, these targets can currently be regarded only as candidate targets with relatively strong animal causal evidence and human correlative evidence. They cannot be described as therapeutically validated targets with completed human causal validation. Human interventional validation should prioritize target engagement, dose, dosing timing, wound closure, and safety endpoints listed in Table 3. It should also connect with the tiered human–animal validation pathway in Section 4.3.

4 Strategies for Model Selection, Reporting, and Translational Validation

This section provides operational strategies based on the applicability basis and boundaries described above. It does not repeat the mechanistic consistency argument in Section 3.

4.1 Select models by mechanism and define endpoints in advance

For AGE/RAGE, oxidative stress, or metabolic–inflammatory mechanisms, db/db mice are preferred. For acute hyperglycemia combined with infection, the STZ model can be used. For hypoxia and vascular mechanisms, ischemic or pressure models should be used. For closure mode and dressing evaluation, splinted mice or pig models should be used. For chronic multifactorial wounds, a multi-hit model is preferred [55,61-63,65-67]. Porcine skin flaps are closer to chronic ischemia than hindlimb ligation [91]. When selecting a model, the primary endpoint should be defined in advance: wound area reduction or re-epithelialization. Blood glucose control, degree of ischemia, splinting method, and microbiota status should be recorded. Area reduction cannot be equated with true re-epithelialization [59,60].

4.2 Reporting, preregistration, and regulation

The Animal Research: Reporting of In Vivo Experiments 2.0 (ARRIVE 2.0) guidelines require reporting of the Essential 10 items: randomization, blinding, sample size, animal characteristics, procedures, and results [92,93]. Animal studies should be preregistered in the Animal Study Registry or preclinicaltrials.eu. Preregistration can be integrated into ethics review [94-98]. Systematic review protocols can be registered on PROSPERO4animals [99]. For regulation, animal data usually support safety, dose, and mechanism. The US Food and Drug Administration Animal Rule is a rare exception in which animal efficacy data support approval. It applies only when human efficacy studies are not feasible or not ethical [100]. Diabetic wound products generally still require human efficacy trials. Animal data are used mainly to support safety, dose, and mechanism.

The primary efficacy endpoint for wound products should be complete closure: 100% re-epithelialization, no exudate, and no need for dressings [59]. Wound area reduction alone is usually insufficient as a primary efficacy endpoint. The DFU core outcome set has

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proposed eight core outcomes [60]. Regulatory qualification is limited to a specific context of use. Most nonclinical qualifications accepted to date are safety biomarkers with histopathology as a reference [101-103]. The same framework has been suggested for evaluating new technologies that may replace whole-animal models [101]. Publications should report negative results, effect sizes, confidence intervals, and blinding. They should also consider funnel plots and publication bias [70,104-111].

4.3 Tiered human–animal validation and evidence integration

We recommend completing animal causal validation first. Then, humanized mice, ex vivo human skin, immune-integrated organ chips, and pig models can be used for tiered bridging [55,75,76,84-89]. Humanized mice can combine diabetes with wound healing on transplanted human skin. However, the immunodeficient background alters inflammatory and vascular responses. Ex vivo human skin and immune-integrated organ chips are suitable for direction validation and dose screening. However, they lack systemic metabolic and neural factors. Pig models are closest to human skin and wound size. However, they rarely form truly chronic ulcers naturally. Therefore, these platforms should be used complementarily, not as replacements for one another.

After platform validation, single-cell or spatial transcriptomics can be used to locate key cell populations. Finally, validation should be performed in stratified human cohorts and small-sample mechanistic trials [11,20,25,74]. In the evidence integration stage, Bayesian hierarchical models, systematic reviews, and replication success metrics should be used. Effect size, confidence intervals, heterogeneity, and publication bias should be evaluated together [69,104-108,112-114]. The core of this pathway is tiered validation and dynamic correction, not one-time extrapolation of animal results to the clinic.

5 Challenges and Future Research Directions

5.1 Key challenges

Current major challenges include model heterogeneity, sex/age/comorbidity bias, microbiota differences, inconsistent endpoint definitions, missing negative results, incomplete registration and reporting, and the lack of unified preclinical efficacy guidelines [45,47,57-60,70-72,77-83,94-98]. First, different diabetes models differ markedly in disease duration, metabolic state, immune background, and wound healing mode. Experimental animals also often have sex, age, and comorbidity structure bias [77-83]. Second, microbiota composition and infection routes in animal models cannot fully represent the multispecies biofilms and dynamic microbiota changes of human DFUs [45,47]. Third, definitions of endpoints such as wound area reduction, re-epithelialization, closure time, and histological indicators are not uniform. This affects comparisons across studies [57-60]. In addition, negative and null results are underreported. Animal study registration and reporting are incomplete. Unified preclinical efficacy evaluation guidelines are lacking. These issues also reduce the interpretability and translational value of preclinical evidence [70-72,94-98].

5.2 Future research directions

We suggest advancing future research along four levels: mechanistic research, model development, reporting standards, and regulatory translation. Priorities should be set according to how well each level supports animal–human translational evidence.

5.2.1 Mechanistic research: highest priority

Priority should be given to building an animal–human mechanistic comparison database that includes positive, negative, and null results. This database should link mechanistic findings, animal models, intervention methods, human tissue or single-cell evidence, and clinical outcomes [70]. At the same time, small-sample mechanistic trials targeting CXCR2 and NETosis should be prioritized in DFUs. These trials should test whether ligand combination, NET formation level, cell source, and healing stage can predict intervention response [20,21,25-28]. For evidence integration, Bayesian hierarchical models are used mainly to handle heterogeneity across animal models, species, and human studies. They bridge animal and human effect sizes rather than simply pooling effect values [112,113]. Systematic reviews and replication success metrics are used to evaluate evidence consistency, effect size, and publication bias [69,104-108,114].

5.2.2 Model development: high priority

A model selection decision tree should be established. Example dimensions should include at

least diabetes type, disease duration, wound site, presence of ischemia, presence of infection, weight-bearing status, and primary endpoint type. Sex pairing, age stratification, and comorbidity stratification should also be included. This avoids evaluating treatment effects only in young animals of a single sex without comorbidities [79-83]. For complex mechanisms, a tiered bridging strategy can be used: animal causal model, humanized or ex vivo model, immune-integrated organ chip, and pig model [55,75,76,84-89]. Humanized mice are used to validate targets in a human skin background. Ex vivo skin and organ chips are used for direction validation and dose screening. Pig models are used to evaluate wound size, skin structure, and closure mode. However, none of these three can replace prospective human trials.

5.2.3 Reporting standards: high priority as infrastructure for translational research

Animal experiments should be preregistered before study initiation and should follow ARRIVE 2.0 reporting standards. Randomization, blinding, sample size, animal characteristics, exclusion criteria, primary endpoints, and statistical analysis should be reported completely [92-98]. Negative results, null results, effect sizes, and their confidence intervals should be systematically reported. This reduces publication bias and improves the interpretability of evidence [70-72].

5.2.4 Regulatory translation: medium-high priority

Animal data mainly support safety, dose, and mechanism. They cannot alone provide sufficient evidence of clinical efficacy for diabetic wound products. Human studies should use complete closure rate as the primary efficacy endpoint: 100% re-epithelialization, no exudate, and no need for dressings. Reporting only wound area reduction is usually insufficient to support efficacy conclusions [59]. At the same time, the DFU core outcome set should be used to standardize reporting of wound healing, recurrence, infection, amputation, quality of life, and death [60]. For new models or replacement technologies, regulatory qualification can be advanced for a specific context of use. However, the scope of application and evidence boundaries must be clearly defined [100-103].

In summary, implementation should first prioritize building the mechanistic database and conducting CXCR2/NETosis mechanistic trials. Next, the model decision tree and tiered bridging platforms should be improved. Preregistration and ARRIVE reporting standards should be admission requirements for all studies. Finally, regulatory translation should be promoted within the framework of the FDA wound product guidance and the DFU core outcome set [59,60,70,92-98].

5.3 Limitations of this review

This article is a narrative mechanistic comparative review. We did not perform a systematic search, independent dual screening, or quantitative pooling of effect sizes according to systematic review procedures. Therefore, the conclusions are a mechanistic evidence synthesis, not an efficacy estimate (①). Literature inclusion was limited to Chinese and English, which may cause language bias (②). The A–D evidence grading used here is a custom framework. We did not perform independent dual grading, and we did not conduct a formal risk-of-bias assessment for each included study (③). In addition, we did not systematically assess publication bias (④). Some human studies had small sample sizes and short follow-up (⑤). We did not cover all diabetic wound animal models or all DFU subtypes. Extrapolation of the conclusions therefore requires caution.

Author contributions

Haoyuan Chen: Writing–review & editing, Writing–original draft, Visualization, Investigation. Lin Xu: Writing–review & editing, Writing–original draft, Visualization, Investigation, Funding acquisition. Runmin Li: Writing–review & editing, Investigation, Funding acquisition. Yuanke Chen: Writing–review & editing, Investigation. Chao Cheng: Writing–review & editing, Supervision, Project administration. Jingguo Li: Writing–review & editing, Supervision, Conceptualization, Project administration.

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Conflict of interest

The authors declare that they have no conflict of interest.

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