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Amphibian-derived peptides as novel therapeutics for skin wound healing: Mechanisms, applications, and challenges

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ABSTRACT

Skin injury disrupts the body's primary barrier against pathogens and environmental insults, often leading to infection, delayed healing, and pathological scarring. Therefore, the development of effective wound-healing therapies remains a major clinical priority. Amphibian-derived peptides have attracted growing interest as a diverse group of bioactive molecules for wound repair. In addition to their antimicrobial effects, increasing evidence suggests that these peptides can regulate multiple stages of healing by modulating inflammation, promoting keratinocyte and fibroblast migration and proliferation, enhancing angiogenesis, and supporting extracellular matrix remodeling. This review summarizes current knowledge on the discovery and distribution of amphibian-derived wound-healing peptides and discusses recent findings on their biological activities, mechanisms of action, and potential applications. Rather than simply cataloguing reported peptides, we also assess the strength of the current evidence and discuss major translational challenges in the field, including insufficient mechanistic validation for some candidates, limited use of clinically relevant wound models, peptide instability, toxicity, and barriers related to formulation and delivery. We also discuss strategies that may improve peptide developability, including rational structural modification, biomaterial-based delivery systems, and translation-oriented preclinical evaluation. Overall, amphibian-derived peptides are a valuable source of multifunctional wound-healing agents, but their clinical development will require stronger mechanistic evidence, standardized efficacy assessment, and better translational design.

Keywords: Amphibians; Wound-healing peptides; Skin wound healing; Peptide-based drug discovery; Assessment of wound healing potential

INTRODUCTION

The skin plays a pivotal role in multiple physiological functions, especially in protection from exogenous chemicals and pathogens. Skin wounds can arise from a variety of causes, such as cuts, burns, diabetes, etc. Severe skin wounds can lead to far-reaching complications and even be life-threatening. Burn wounds are one of the most common skin injuries worldwide, ranked as the sixth leading cause of death. According to the latest articles, there were 9 million global burn cases, resulting in 112 billion in economic losses in 2019 (Gerstl et al., 2024; Zhu et al., 2025a). Burn wounds are notoriously intractable due to their diverse etiologies and varying degrees of tissue damage. Furthermore, the healing process is often complicated by systemic inflammation, sepsis, and organ failure (Bahemia et al., 2015; Shimizu et al., 2015). Conservative treatment is insufficient in the case of deep and extensive burn wounds (Esteban-Vives et al., 2016). Another challenging skin condition is diabetes-related skin disorders, such as diabetic ulcers, which are largely refractory to treatment and represent a leading cause of morbidity in diabetic patients (Abate et al., 2025). Considering the wide range of causes of skin wounds, the global burden of this condition is substantial. As such, wounds have been referred to as a "Silent Epidemic" (Ward et al., 2019).

Despite the range of clinical options currently available for skin wound care, achieving satisfactory healing remains challenging, especially in severe acute wounds and chronic non-healing wounds (Falcone et al., 2021; Han & Ceilley,

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2017). Existing treatments mainly involve wound debridement, infection control, conventional or advanced dressings, negative pressure wound therapy, skin grafting, and flap reconstruction (Falcone et al., 2021; Guo & DiPietro, 2010; Han & Ceilley, 2017). Although these approaches can reduce wound burden and improve local healing conditions, their overall effectiveness is still limited. For example, autologous skin grafting, widely regarded as the standard treatment for extensive full-thickness wounds, is restricted by the availability of donor skin and the risk of additional injury at the donor site. By contrast, allogeneic and xenogeneic grafts raise concerns about immune rejection and possible disease transmission (Esteban-Vives et al., 2018; Vig et al., 2017). Conventional dressings also have clear limitations. Although they can protect the wound and maintain moisture, they usually play a largely passive role and do not adequately correct the pathological wound microenvironment, including persistent inflammation, bacterial infection, oxidative stress, ischemia, and insufficient angiogenesis, particularly in chronic wounds such as diabetic ulcers (Falcone et al., 2021; Guo & DiPietro, 2010; Han & Ceilley, 2017). These limitations are particularly pronounced in burn wounds and diabetic ulcers, where complex pathophysiological conditions frequently lead to delayed healing, scar formation, or even treatment failure (Rowan et al., 2015; Wilkinson & Hardman, 2020). Taken together, these shortcomings highlight the need for more effective therapeutic strategies that can simultaneously control infection and inflammation while promoting vascularization and tissue regeneration.

Healing of skin wounds is influenced by multiple factors, including the cause of injury (e.g., burns, cuts, and pressure), the patient's pathological and physiological status (e.g., age, diabetes, autoimmune diseases), wound dimensions (e.g., perimeter, surface area, length, and width), and the skin microbiome (Yang et al., 2024b). Skin wounds can be classified into acute wounds and chronic wounds (Karimi et al., 2017). The process of acute wound healing (e.g., incisions or traumatic injuries) is conventionally categorized into four sequential but overlapping phases: hemostasis, inflammation, proliferation, and tissue remodeling, ultimately leading to the restoration of organized tissue architecture (Fan et al., 2024). In contrast, chronic wounds (primarily categorized as vascular ulcers, diabetic ulcers, and pressure ulcers) (Mustoe, 2004) fail to progress through the normal healing cascade. They are characterized by exudation, persistent inflammation, and necrosis (Widgerow et al., 2015). These "hard-to-heal" wounds, defined as those that fail to heal in an orderly and timely manner with standard therapy (Troxler et al., 2006), often require multidisciplinary care due to their complex pathology.

Healing a skin wound involves not only restoring tissue integrity and anatomical structure but also recovering its pristine function and reestablishing the damaged tissue with a normal appearance. To this end, a growing body of research is focused on developing more effective wound therapies to improve therapeutic outcomes and reduce treatment burden. Emerging therapeutic approaches for wound repair can generally be grouped into several broad categories. Physical and chemical methods, such as ultrasound stimulation (Alavi et al., 2014) and topical chemical agents (Stojadinovic et al., 2008), provide non-invasive or minimally invasive ways to support tissue repair. Biological strategies, including gene therapy (Aragona & Blanpain, 2017; Mavilio et al., 2006) and stem cell-based therapies (Ayavoo et al., 2021), are designed to influence cellular behavior and enhance regenerative potential at the molecular level. Tissue engineering approaches, especially three-dimensional (3D) bioprinting (Singh et al., 2016) and skin organoid technologies (Wang et al., 2025d; Zeng et al., 2025), have created new opportunities for building functional skin substitutes. Additionally, photobiomodulation using red light has also shown potential to accelerate wound closure (Chang et al., 2025). Even with these advances, however, achieving complete functional repair without scar formation remains a major challenge.

Amphibians are a class of vertebrate animals uniquely adapted to exploit both aquatic and terrestrial habitats. Amphibian skin is bare and directly exposed to different environmental challenges, including parasites, microorganisms, predators, and other ambient factors. To compensate for this vulnerability, amphibian skin has evolved specialized defense mechanisms, among which skin secretions play a pivotal role. These secretions contain bioactive peptides that serve dual functions: (i) Defensive offense: Many peptides can irritate mucous membranes, induce pain, or even prove lethal to predators (e.g., through neurotoxic or hemolytic activity). (ii) Immunoprotection: These peptides exhibit antimicrobial properties, effectively inhibiting infections by fungi, bacteria, and viruses (Brunetti et al., 2022). This dual functionality underscores the evolutionary significance of skin secretions in amphibian survival, balancing predation avoidance and microbial defense.

In recent years, the bioactive components of amphibian skin secretions, particularly peptides, have garnered significant attention in scientific research due to their unique biological activities and promising potential for clinical applications (Xu & Lai, 2015; Zhu et al., 2025b, 2025c). Significant advancements in the study of amphibian-derived peptides have been driven by several prominent research groups, including the teams of Professor Xinwang Yang from Kunming Medical University, the team of Professor Ren Lai from Kunming Institute of Zoology, Chinese Academy of Sciences, and Professor Shuyu Zhang from Sichuan University. International contributors include the team of Professor Maria Luisa Mangoni from Sapienza University of Rome, and others. Among these versatile peptides, various wound-healing-promoting peptides have been characterized and summarized in authoritative reviews from different aspects (Assis et al., 2025; Fadilah et al., 2024; Fan et al., 2024; Kröner et al., 2024; Wang et al., 2025a; Zhu et al., 2025c). Unlike previous reviews that primarily catalog these peptides or focus on a single theme, the present review integrates their mechanisms of action, structure–activity relationships, translational potential, and current limitations in wound healing. In this way, we aim to show why amphibian-derived peptides deserve attention as potential candidates for scarless repair and the treatment of chronic wounds.

DISCOVERY AND IDENTIFICATION OF AMPHIBIAN-DERIVED WOUND-HEALING-PROMOTING PEPTIDES

Amphibian-derived peptides provide an invaluable pool for drug discovery, but the methodology for exploring this "treasure house" is crucial for its practical application. In general, peptide-based drug discovery involves candidate identification, activity screening, mechanistic evaluation, and lead optimization. However, the discovery of amphibian skin secretion-derived peptides relies on source-specific workflows

that differ from conventional synthetic peptide development. In most cases, this process starts with the collection of skin secretions, followed by peptidomic or proteomic analysis, *de novo* or transcriptome-assisted sequence identification, dereplication, functional screening and counter-screening, and subsequent optimization (Deng et al., 2019; Proaño-Bolaños et al., 2019; Yang et al., 2012).

Collection and handling of amphibian skin secretions

The discovery of amphibian skin peptides begins with the collection of skin secretions, which contain a complex mixture of host-defense, immunomodulatory, and tissue repair-associated molecules. Because secretion profiles may vary depending on species, physiological state, environmental conditions, and sampling procedures, careful collection and handling are important to preserve the integrity of native peptides. In most studies, secretions are obtained by different stimulation methods, such as mild electrical stimulation (Yang et al., 2012), hand massaging or scratching (Deng et al., 2019; Proaño-Bolaños et al., 2019), and ultraviolet B (UVB) radiation (Yang et al., 2024a). The collected secretions are then immediately transferred into acidified or protease-inhibiting-containing solutions, followed by low-temperature processing, centrifugation, desalting, and lyophilization before further analysis.

Peptidomics and proteomics workflows

After collection, crude skin secretions are typically subjected to chromatographic separation and analytical characterization. Reverse-phase high-performance liquid chromatography (RP-HPLC) is widely used to fractionate these complex peptide mixtures, and the resulting fractions can then be analyzed by mass spectrometry (MS). Together, peptidomic and proteomic approaches make it possible to identify peptide masses, fragmentation patterns, and relative abundance. These methods are often combined with activity-guided fractionation to connect specific secretion components with antimicrobial, immunomodulatory, or wound-healing activities (Fu et al., 2022a; Proaño-Bolaños et al., 2019).

***De novo* sequencing and transcriptome-assisted peptide identification and dereplication**

Because amphibian skin secretions often contain multiple homologous peptides and low-abundance components, peptide identification usually depends on tandem mass MS-based *de novo* sequencing rather than simple mass matching alone. In parallel, sequence information derived from precursor cDNAs or transcriptomic analysis of skin glands provides complementary evidence for peptide annotation, including conserved signal peptides, acidic spacer regions, and mature peptide domains. Combining peptidomic and transcriptomic data improves sequence confidence, facilitates the mapping of precursors to mature peptides, and helps accelerate the discovery of new peptide families (Jiang et al., 2017; Liu et al., 2012, 2025). This precursor-guided strategy is particularly important in amphibian peptide research, where conserved prepropeptide organization coexists with substantial diversification of mature bioactive sequences. After sequence assignment, dereplication is necessary to distinguish genuinely novel peptides from previously reported homologs or minor sequence variants. This is especially important in amphibian peptide research because many peptides belong to conserved families that recur across related taxa. Comparison with curated peptide databases can reduce redundant characterization and strengthen the reliability of novelty claims (König et al., 2015; Liu et al., 2025).

Functional screening and counter-screens

Once candidate peptides have been identified, their biological properties need to be evaluated through a range of assays. At the same time, early counter-screening is important for judging their developability. Many amphibian peptides possess membrane-active properties, meaning that antimicrobial potency may be accompanied by hemolysis or broader mammalian cytotoxicity. For this reason, hemolysis assays, mammalian cell compatibility testing, and selectivity assessment should be introduced at an early stage of the discovery process to distinguish wound-healing candidates with therapeutic potential from peptides likely to cause unacceptable side effects (König et al., 2015).

Current approaches to drug screening: The major screening strategies used in contemporary drug discovery are depicted in Figure 1, which highlights the distinct but complementary workflows of target-based drug discovery (TBDD) and phenotype-based drug discovery (PBDD). In the TBDD route, drug discovery usually starts with target selection, followed by assay development and biochemical screening to identify compounds that directly modulate a predefined molecular target, such as a protein or nucleic acid. This pathway is increasingly supported by fluorescent screening methods, display technologies, computer-aided drug design (CADD), and artificial intelligence (AI) to improve screening efficiency and hit identification (Tauro et al., 2025). By contrast, the PBDD route starts with assay development and cell-based screening, focusing on drugs that induce phenotypic changes (e.g., proliferation, death, migration, and differentiation) within cell-based assays. One advantage of this strategy is that biologically relevant effects can be detected without prior definition of a molecular target (Tauro et al., 2025). The molecular basis of the observed phenotypes is then investigated through target deconvolution. As shown in Figure 1, both strategies ultimately converge on lead optimization, preclinical evaluation, clinical trials, and eventual large-scale production.

High-throughput screening (HTS) describes a method for rapidly identifying bioactive compounds from compound libraries within either strategy, which was established in the 1980s (Pereira & Williams, 2007). HTS has significantly transformed the drug discovery process, particularly in scenarios where limited information is available regarding the target. Various HTS formats have been developed, encompassing biochemical assays, binding-based assays, and cell-based assays (Cronk & Shearer, 2021; Janzen, 2014).

Biochemical screening is used to test large numbers of compounds or peptides against a specific and purified target, such as enzymes. These assays are usually executed in microplates and read out using an optical method (Stoddart et al., 2016), such as fluorescence, absorbance, and luminescence, among which the fluorescent method is frequently used because of its high sensitivity and ease of handling (Fang et al., 2019; Jager et al., 2003; Kumar et al., 2024). Binding-based assays are used to measure the interaction between a drug and its specific target, which is useful for determining binding affinity, hit validation, and optimization (Bergsdorf & Ottl, 2010), such as fragment-based drug design (Xu & Kang, 2025), nuclear magnetic resonance (Shortridge & Varani, 2021), surface plasmon resonance (Das et al., 2024), and X-ray crystallography (Günther et al., 2021;

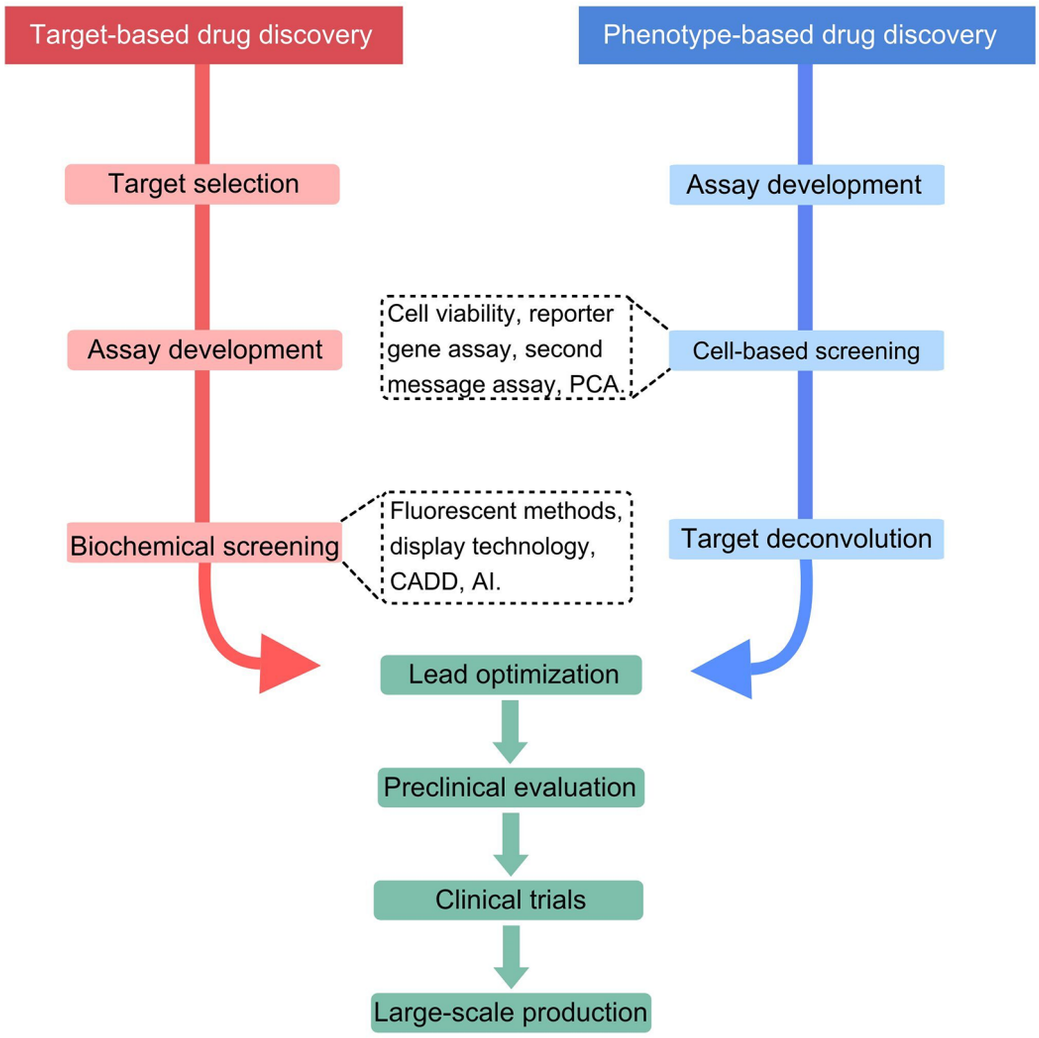

Figure 1 Screening methods used in the major drug discovery processes

Schematic overview of the principal screening approaches employed in two major drug discovery paradigms. The target-based strategy generally proceeds through target selection, assay development, and biochemical screening, whereas the phenotype-based strategy involves assay development, cell-based screening, and subsequent target deconvolution. Representative techniques applied during these stages include cell viability assays, reporter gene assays, second messenger assays, protein-fragment complementation assay (PCA), fluorescent methods, display technology, computer-aided drug design (CADD), and artificial intelligence (AI). Both strategies ultimately support lead optimization, preclinical evaluation, clinical trials, and large-scale production (Created with MedPeer (https://user.medpeer.cn/user/index/login/?url=https%3A%2F%2Fwww.medpeer.cn)).

Lee et al., 2022). Cell-based assays are used to measure the functional effects of a compound or peptide on living cells, providing physiological data by measuring cellular processes like viability, differentiation, and apoptosis (Larsson & Parris, 2023; Sazonova et al., 2022). In addition, reporter gene assays, secondary messenger assays, and protein-fragment complementation assays (PCA) are also frequently used cell-based screening methods in drug discovery (Wei et al., 2021).

Display technologies exhibit excellent performance in TBDD, enabling researchers to screen libraries of peptides against specific targets. Display technologies can be broadly categorized into two main types: cellular display systems and acellular display systems. Cellular display systems encompass phage display, bacterial display, yeast display, and mammalian cell display, while acellular display systems include ribosome display, mRNA display, and cDNA display (Jaroszewicz et al., 2022). Phage display technology was established in 1985 to present peptides on the surface of lysogenic filamentous bacteriophages (Smith, 1985). In phage display, exogenous DNA is inserted into the gene encoding the phage coat protein, which is then expressed and presented on the phage surface. In a phage library representing a repertoire of unique peptides exposed to selected targets, a panning process is executed for multiple rounds to obtain a highly enriched pool of clones; the specifically bound phages are eluted and amplified for subsequent sequencing to identify the corresponding peptides (Mimmi et al., 2019; Panagides et al., 2022). Several peptide-based drugs, such as ecallantide (Markland et al., 1996), pegcetacoplan (Sahu et al., 1996), and peginesatide (Wrighton et al., 1996), were discovered via phage display. Consequently, phage display technology has emerged as a highly effective methodology for the identification and selection of target-specific peptides. Genomics and peptidomics technologies also enable the discovery of new peptide drugs (Dogiparthi et al., 2025; Romanova & Sweedler, 2015).

Once the target and HTS format are selected, compounds

are assayed against the target to elucidate the interaction between them, such as inhibition, activation, or a lack of interaction. In practice, HTS for peptide binders or modulators is typically implemented through very large peptide libraries, display-based platforms, and increasingly through computational or virtual screening that ranks candidate sequences against a known target structure before experimental validation (Lionta et al., 2014; Macarron et al., 2011). In these settings, the molecule most commonly labeled is the molecular target itself, such as a purified receptor, enzyme, antibody, or an intact target-expressing cell, while in competitive assays, the peptide ligand, a known reference ligand, or a secondary detection reagent may be labeled instead. The main labeling strategies include fluorescent labels (e.g., FITC, Cy3/Cy5, Alexa Fluor dyes, fluorescent proteins, or lanthanide-based TR-FRET probes), radiolabels (e.g., ^{3}H, ^{125}I, or ^{35}S) for highly sensitive binding or receptor-occupancy assays, and affinity-based tags (e.g., biotin, His_6, FLAG, or DIG) that enable capture or detection through streptavidin surfaces or tag-specific antibodies (Hall et al., 2016; Inglese et al., 2007). “Positive hits” are usually defined by assay-specific readouts such as enrichment across iterative selection rounds in display systems, increased fluorescence or radiometric signal relative to negative controls, competitive displacement of a known ligand, dose-dependent binding or activity, and confirmation in orthogonal assays such as flow cytometry, enzyme-linked immunosorbent assay (ELISA), surface plasmon resonance (SPR), fluorescence polarization, FRET/TR-FRET, or IC_{50}/Kd measurements (Hall et al., 2016; Inglese et al., 2007). In well-controlled HTS campaigns, statistical criteria such as signal-to-background ratio, Z’-factor, Z-score, and reproducibility across replicates are also used to distinguish true hits from noise (Macarron et al., 2011).

If a compound demonstrates the intended biological activities, a hit-to-lead optimization process is initiated to generate a drug candidate for further research and development. Employing automated screening systems, these screening programs can achieve throughput rates exceeding 100 000 assays per day, a methodology referred to as ultra-high-throughput screening (uHTS) (Sundberg, 2000).

Computational and AI-driven innovations in drug discovery: Traditional drug screening methods consistently suffer from drawbacks in efficiency, success rates, and cost (Dowden & Munro, 2019; Sertkaya et al., 2024; Singh et al., 2023). PBDD approaches are largely conducted empirically (Wang et al., 2022), with primary detection methods relying on observing morphological changes in cells. Although these readouts are simple and direct, they are not always sufficiently precise. Moreover, the probability of technical success at the primary screen is lower for PBDD, and hit validation and target identification remain major challenges. By contrast, TBDD depends on prior knowledge of specific targets that play crucial roles in a disease, which makes it challenging for poorly understood diseases, such as neurodegenerative disorders, or for developing multi-target drugs. Furthermore, this reliance may lead to the neglect of novel therapeutic modalities not linked to current knowledge.

To overcome these bottlenecks, innovative methods have increasingly emerged. CADD and virtual screening provide effective approaches to addressing these challenges, offering efficient methodologies for drug screening. CADD is generally categorized into structure-based and ligand-based drug discovery (Yang et al., 2022). The former screens drug candidates based on the structures of the target and compounds through a docking process, which is adopted to predict the binding mode and affinity of compounds within the binding site of specific receptors (Guedes et al., 2014). The latter is used to identify compounds that could bind to a known target, hinging on compounds with similar properties to existing drugs (Wu et al., 2024a). A typical CADD process includes identifying a specific target, using computers to identify “hits”, and selecting the most desirable “leads” (Niazi & Mariam, 2023). This approach allows for the efficient screening of numerous compounds. Another vital contribution of CADD to drug discovery is targeting “undruggable” proteins. These targets are characterized by highly dynamic structures, a lack of ligand-binding sites, or the presence of conserved active sites, all of which pose obstacles for conventional drug discovery methods (Sun et al., 2025).

The development of AI further advances CADD by enabling the rapid processing of large and complex datasets through advanced algorithms and high-performance computing (Brogi & Calderone, 2021; Wu et al., 2024b). In addition to improving virtual screening and lead identification, AI can also support data integration, decision-making, and multi-parameter optimization throughout the drug discovery process (Paul et al., 2021; Vijayan et al., 2022). These strengths are especially useful for peptide-based therapeutics, where large numbers of structurally related sequences often need to be screened and prioritized. In the context of amphibian-derived peptides, AI-assisted approaches may complement conventional peptidomic, transcriptomic, and structure-based strategies by facilitating sequence mining, candidate prioritization, and prediction of biologically relevant properties such as antimicrobial activity, wound-healing potential, cytotoxicity, hemolysis, and stability. Therefore, beyond its general role in modern drug discovery, AI may provide a practical means of enriching the discovery pipeline for amphibian-derived wound-healing peptides.

ASSESSMENT MODELS FOR WOUND HEALING AND CHALLENGES IN CROSS-STUDY COMPARABILITY

Wound healing is a complex process due to the multifaceted wound environment and diverse causes of injury, particularly the varied underlying etiology of chronic wounds. Therefore, discovering drugs with wound-healing potential requires diverse methods and models. Selecting an appropriate model can improve the validity and reliability of experimental results and provide deeper insights into the mechanisms of action, thereby promoting candidate discovery and clinical translation. Currently, numerous methods and models are being developed to boost the discovery and evaluation of new candidates. These models are usually divided into *in vitro* and *in vivo* models, each with its own advantages and disadvantages (Stephens et al., 2013).

The *in vitro* models of wound-healing potential evaluation

In vitro models are easily established and have been widely utilized in disease research for decades. However, their value in preclinical assessment depends not only on model selection but also on the use of standardized endpoints, assay conditions, and appropriate controls. The cell monolayer scratch assay is one of the most common *in vitro* wound models. In this assay, a wound gap is created in a confluent cell monolayer, and the subsequent proliferation and migration of cells at the wound edge into the gap are monitored (Rodriguez et al., 2005). By periodically assessing the wound,

the healing rate can be quantified. This model is ideal for studying the effects of various treatments or pathological conditions. For example, many amphibian-derived peptides accelerate the proliferation and migration of human skin keratinocytes (HaCaT) and human skin fibroblasts (HSFs), thereby promoting wound healing. Because scratch assays are sensitive to cell density, serum conditions, scratch width, and imaging time points, these parameters should be standardized or clearly reported to enable comparison across studies.

Hypertrophic scarring affects 32%–72% of post-injury cases (Mony et al., 2023), often leading to itching, anxiety, and depression. This condition is associated with the excessive deposition of extracellular matrix (ECM, mainly collagen and fibronectin) and connective tissue at the wound site, a process that entails the interaction between the matrix and fibroblasts. The fibroblast-populated collagen lattice (FPCL) was designed to study this interaction *in vitro* (Bell et al., 1979). The FPCL is established by mixing fibroblasts with collagen and casting in a petri dish to form a cell-encapsulated 3D lattice. The degree of FPCL organization is quantified by measuring the reduction in lattice area over time to demonstrate FPCL contraction and fibroblast-mediated collagen reorganization. This model can be used to study various wound healing scenarios by using different cells to form FPCLs. A previous study demonstrated that chronic wound fibroblasts (CWFs) exhibit a significantly reduced initial rate of ECM reorganization and contraction in CWF-encapsulated FPCLs (Cook et al., 2000). Another study showed that silk fibroin microneedles suppressed hypertrophic scarring via mechanical remodeling in hypertrophic scar-derived fibroblast-encapsulated FPCLs (Zhang et al., 2022a).

Since the first organoid was developed in 2009 (Sato et al., 2009), various organoid models have been established to study human physiology and pathology. These organoids have gained increasing attention because they represent the actual physiopathology of native organs more accurately. Skin organoids are 3D culture models that closely mimic the structure and function of human skin. They can be used not only to accelerate wound healing but also to study drug activity and toxicity, providing an alternative to animal testing and facilitating high-throughput drug screening. For example, skin organoid transplantation promotes tissue repair in frostbite (Wang et al., 2025c), as well as in full-thickness skin incision and burn models (Thai et al., 2023; Zhang et al., 2024). Additionally, a novel reepithelialization mechanism was revealed using a 3D wounded-skin model (Safferling et al., 2013), thereby enriching basic theoretical knowledge. Furthermore, diabetic foot ulcer-derived fibroblasts were used to establish a chronic wound model that exhibits key hallmarks of the disease (Maione et al., 2015). A 3D-bioprinted diabetic skin model was also developed, which represents the pathological features of diabetes (Kim et al., 2021). Moreover, several melanoma organoids have been developed for studying cancer pathology and performing drug testing (Cho et al., 2022; Li et al., 2011; Müller & Kulms, 2018; Vörsmann et al., 2013). A recent study introduced a novel, cost-effective method for generating uniform microspherical skin organoids, enabling high-throughput drug screening applications (Xie et al., 2025). Given the rapid advancement of skin organoid technology, this approach will significantly advance the treatment of skin wounds.

While monolayer cell cultures and skin organoids can simulate certain physiopathological features, they cannot fully recapitulate the entire structure and function of living skin. In contrast, skin explant culture represents a superior *ex vivo* model due to its close resemblance to living skin. It is widely acknowledged that excised human skin is the gold standard for *in vitro* studies on permeation and novel skin formulations (Moniz et al., 2020; Planz et al., 2016). The skin explant model was established by culturing excised human skin in *vitro* for research purposes. These explants preserve the native 3D architecture of the tissue, encompassing the epidermis, the dermis, and various endogenous components such as resident immune cells, vasculature, and skin appendages (e.g., hair follicles and sweat glands) (Cousin et al., 2023; Wang et al., 2023). In addition, patient-derived skin explants retain information regarding age and living patterns. Zhou et al. (2023) developed an improved skin explant culture method capable of maintaining healthy skin tissue viability for up to 14 days, providing an efficient and cost-effective platform for evaluating and testing personal care products and novel formulations. Bauer et al. (2021) utilized a skin explant model to investigate thymic stromal lymphopoietin expression following barrier insult. Ultimately, this valid *ex vivo* model authentically recapitulates native skin and facilitates the study of wound healing with distinct advantages.

In vivo models of wound healing potential evaluation

Common laboratory animals, including mice, rats, rabbits, and pigs, are widely used to establish skin wound models. Additionally, genetically modified animals are employed to mimic human pathologies, such as diabetic ulcers. Among these *in vivo* models, full-thickness excision wound models (Galiano et al., 2004), which are created by removing all skin layers, are frequently used to assess the pro-inflammatory, anti-inflammatory, granulation, re-epithelialization, angiogenesis, and tissue remodeling activities of potential candidates. The wound area can be periodically recorded to evaluate the wound healing rate, while histopathological analysis is performed to elucidate the underlying mechanisms of the candidates. This method has been adopted in numerous studies (Fu et al., 2022a; Li et al., 2023, 2025). The punch wound model is another full-thickness wound model, typically generated in the ears of rabbits and mice (Bos et al., 2001; Clark et al., 1998). Compared to full-thickness wounds, partial-thickness wounds are generated using dermatomes, suction blisters, or scald/thermal burns to separate the dermis and epidermis, serving as effective models for studying re-epithelialization (Stephens et al., 2013).

Furthermore, chronic wounds are more complex due to their impaired healing capacity and severe clinical consequences. Therefore, there is an urgent need to develop appropriate methods to discover potential treatments. Chemically induced or high-fat diet-induced diabetic mice, along with genetically diabetic mice, have been used to model human diabetes; these models consistently exhibit impaired wound healing compared to control animals (Kwon et al., 2009; Lau et al., 2008; Michaels et al., 2007). In addition, as the formation of vascular and pressure ulcers is closely linked to tissue ischemia, ischemic models of the back and ear are widely used to study these conditions (Buemi et al., 2004; Mogford et al., 2006). Cross-study comparison is further complicated by variation in wound size, anatomical site, splinting methods, diabetic induction strategy, infection status, and treatment schedule. The translational relevance of these models therefore depends not only on their biological relevance but also on greater consistency in study design and outcome reporting.

These evaluation methods have been widely used in peptide-based wound-healing therapeutics. For example, JH015, OA-GL17d, and OA-GP11d significantly promoted cutaneous wound healing in mice. Their activities were further supported by *in vitro* findings showing enhanced proliferation and migration of HaCaT cells and HSFs, together with improved re-epithelialization and granulation tissue formation *in vivo* (Chen et al., 2025; Fu et al., 2022a; Zhang et al., 2022b). These studies highlight the value of combining *in vitro* and *in vivo* approaches during peptide screening and validation. In diabetic wound research, RL-QN15 has been evaluated in diabetic mouse and diabetic foot ulcer models, where wound closure, histological examination, angiogenesis-related markers, and signaling pathway analyses were used to verify its therapeutic potential (Sun et al., 2023). Taken together, these examples suggest that current evaluation methods are useful not only for mechanistic investigation but also for the screening, optimization, and translational development of peptide-based wound-healing therapeutics.

Limitations of current models and challenges in cross-study comparability

Although a range of *in vitro*, *ex vivo*, and *in vivo* models is available for evaluating the wound-healing potential of amphibian-derived peptides, important limitations still remain. *In vitro* models are convenient and widely used, but their shortcomings are well recognized. Cultured primary cells or immortalized cell lines differ substantially from their *in vivo* counterparts and lack the multicellular interactions and microenvironmental complexity present in real wounds. As a result, these models are often unable to adequately simulate prolonged inflammation, infection, ischemia, or chronic exposure conditions (Tice et al., 2013). Skin organoids, although more physiologically relevant than monolayer cultures, still do not fully recapitulate native skin complexity. Current organoid systems often lack resident immune cells, functional vascular and lymphatic networks, neural components, and stromal heterogeneity, all of which are crucial for regulating inflammation, angiogenesis, matrix remodeling, and tissue regeneration (Lee et al., 2020; Wang et al., 2025d). Skin explant cultures preserve native tissue architecture more effectively, yet their short lifespan limits their usefulness for modeling chronic wounds or long-term healing outcomes. Moreover, compared with living organisms, explants lack systemic circulation, immune recruitment, and neural regulation (Cho et al., 2013).

In vivo models provide a more integrated physiological context, but their translational relevance is still limited. A major concern is the biological difference between animals and humans, which contributes to the well-known gap between preclinical success and clinical outcomes (Van Norman, 2020). For instance, rodent wound healing relies heavily on contraction, whereas human wound healing depends more on re-epithelialization and granulation tissue formation. In diabetic wound studies, chemically induced, diet-induced, or genetically diabetic mice generally show delayed healing, but these models more closely resemble impaired acute wounds than the persistent, multifactorial chronic wounds observed in patients (Stephens et al., 2013). Similarly, many existing animal models do not adequately reflect the combined effects of ischemia, biofilm-associated infection, neuropathy, recurrent trauma, and systemic comorbidities that shape human chronic wounds. Thus, although these models are valuable for mechanistic investigation and early efficacy testing, they cannot fully reproduce the pathological complexity of clinical wound-healing disorders.

In addition to conventional wound-healing readouts, skin microbiomes should also be considered in the design of assessment models. In cutaneous wounds, microbial dysbiosis and biofilm formation may exacerbate inflammation, impair re-epithelialization, and delay tissue repair. Therefore, screening pipelines for amphibian-derived peptides may be strengthened by incorporating biofilm-related assays and infection-associated wound models, particularly those involving clinically relevant wound pathogens (Kang et al., 2018; Shi et al., 2020). Advanced skin-relevant systems, such as human skin explants or reconstructed skin models, may further improve the physiological relevance of these evaluations (Shannon et al., 2022). Combining antimicrobial and anti-biofilm assessments with traditional pro-healing endpoints would allow for a more comprehensive evaluation framework that better reflects the complexity of the wound microenvironment.

Beyond the intrinsic shortcomings of each model, a major challenge in this field is the poor comparability across studies. This problem stems largely from substantial variation in assay design, dosing regimens, control settings, and outcome measures (Ansell et al., 2014; Liang et al., 2007; Wong et al., 2011). *In vitro* wound-healing assays are highly sensitive to variables such as cell type, serum conditions, scratch width, peptide concentration, and treatment duration, all of which can markedly affect migration and closure readouts (Jonkman et al., 2014; Liang et al., 2007). Likewise, *in vivo* wound models differ considerably in animal species and strain, wound size and location, splinting methods, diabetic or infectious background, route of administration, dosing frequency, and follow-up duration, thereby limiting direct cross-study comparisons (Dorsett-Martin, 2004; Falanga, 2005; Wong et al., 2011).

Taken together, these limitations indicate that progress in this field depends not only on the development of more representative models but also on the establishment of a more standardized assessment framework. At minimum, future studies should clearly report model characteristics, peptide dose and administration schedule, control groups, and predefined primary and secondary endpoints. *In vivo*, wound closure rate may serve as a basic primary endpoint, but it should ideally be complemented by histological and mechanistic readouts such as re-epithelialization, granulation tissue formation, collagen remodeling, angiogenesis, inflammatory status, microbial burden, and scar quality. *In vitro* and *ex vivo* studies should also include appropriate safety and selectivity assessments, such as cytotoxicity and hemolysis when relevant. Greater standardization in study design and outcome reporting would substantially improve reproducibility, cross-study comparison, and the preclinical evaluation of amphibian-derived wound-healing peptides.

REPRESENTATIVE AMPHIBIAN-DERIVED WOUND-HEALING PEPTIDES

The order Anura (frogs and toads) represents the most diverse group of amphibians, with species numbers substantially exceeding those of the two other extant orders: Urodela (salamanders and newts) and Gymnophiona (caecilians). According to the Amphibian Species of the World database (https://amphibiansoftheworld.amnh.org/) maintained by the American Museum of Natural History,

Anura accounts for 7 864 of the 8 919 known amphibian species as of September 2025. An authoritative review cataloged approximately 2 000 peptides derived from amphibian skin (Xu & Lai, 2015). These peptides are stored in granular glands (also known as poison glands) and can be released into skin secretions when the amphibian is stressed or injured (Brunetti et al., 2018).

These peptides exhibit diverse biological activities, with several demonstrating significant potential in promoting skin wound (acute and chronic wounds) healing. Despite their structural diversity in sequence, length, and physicochemical properties, these peptides converge functionally on several major aspects of tissue repair, including antimicrobial defense, modulation of inflammation, promotion of HaCaT cells and HSFs migration and proliferation, enhancement of angiogenesis, antioxidant protection, radioprotection, and regulation of ECM remodeling (Figure 2). Rather than acting through a single mechanism, many amphibian peptides appear to exert multifunctional effects within the wound microenvironment, which may contribute to their therapeutic potential. The major reported peptides are summarized in Supplementary Table S1.

Beyond acute and chronic wounds, UVB-induced skin lesions represent another critical area where amphibian-derived peptides show immense potential. UVB radiation triggers a cascade of detrimental effects, including oxidative stress, DNA damage, and the overproduction of pro-inflammatory cytokines, leading to photoaging and skin barrier dysfunction (Zhao et al., 2021). Recent studies have identified several amphibian peptides with potent antioxidant and anti-inflammatory properties that can effectively scavenge reactive oxygen species (ROS) and inhibit DNA damage, thereby mitigating UVB-induced skin lesions (Figure 3).

Peptides with prominent antimicrobial and anti-inflammatory activities

A major group of amphibian-derived wound-healing peptides promotes repair by combining direct antimicrobial activity with regulation of excessive inflammation, a particularly relevant property for contaminated or infection-prone wounds. These peptides display broad-spectrum antimicrobial activity against common wound-associated pathogens, including *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, consistent with the membrane-disruptive behavior typical of cationic amphibian peptides. Additionally, these peptides appear to involve attenuation of inflammatory mediators such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), thereby promoting wound healing.

MSI-1 and Pse-T2 represent well-characterized examples of this functional class. MSI-1 exhibits potent bactericidal activity

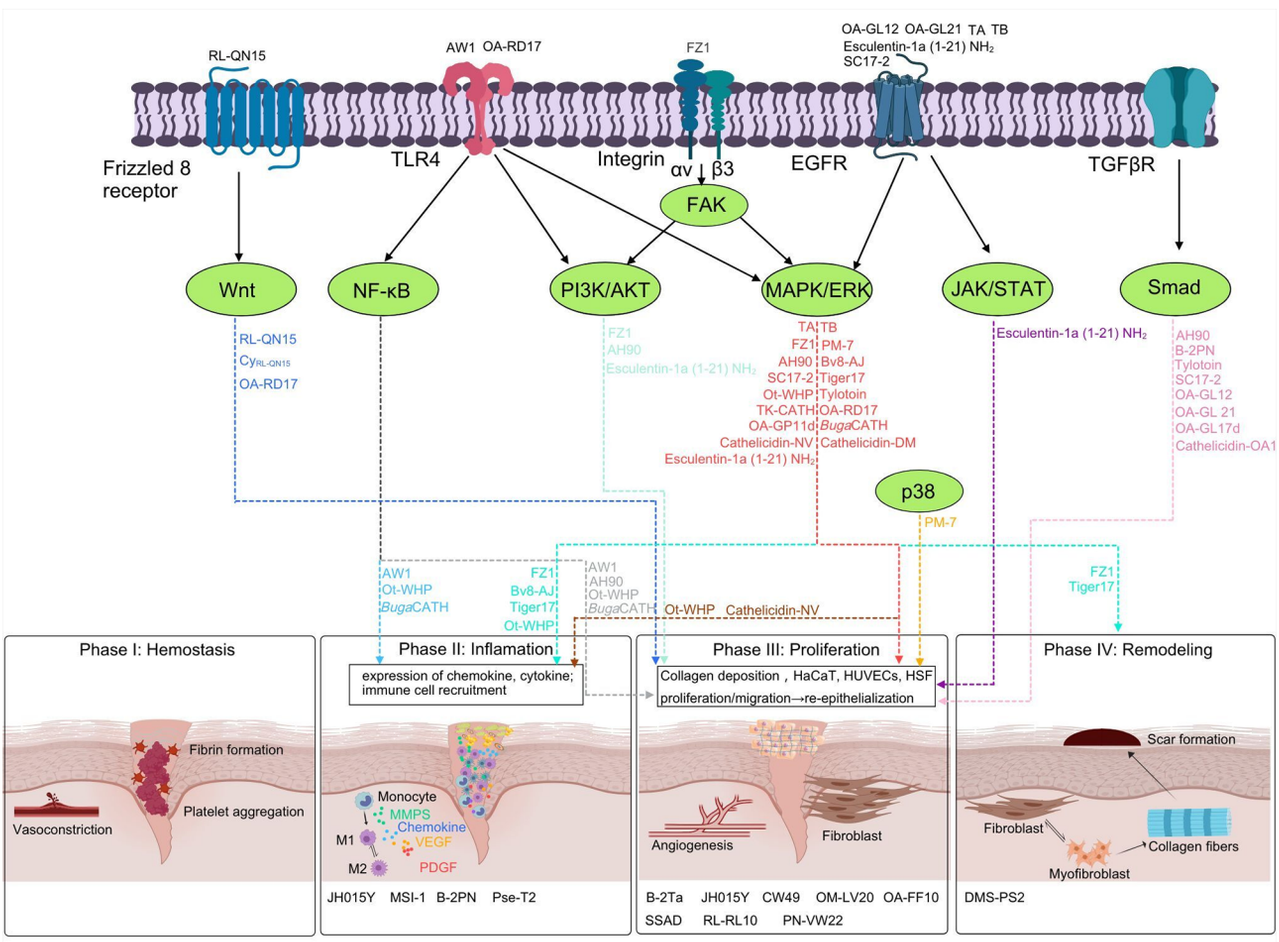

Figure 2 Mechanisms of reported amphibian-derived wound-healing-promoting peptides

This figure summarizes the major mechanisms by which amphibian-derived peptides facilitate wound repair. Reported peptides regulate multiple signaling pathways, including Wnt, NF-κB, PI3K/AKT, MAPK/ERK, JAK/STAT, and Smad pathways, thereby influencing key wound-healing events such as inflammatory modulation, cell proliferation and migration, angiogenesis, collagen deposition, re-epithelialization, and remodeling. The lower panel shows the four stages of wound healing and lists peptides reported to be involved in specific stages for which precise molecular mechanisms remain unclear (Created with MedPeer).

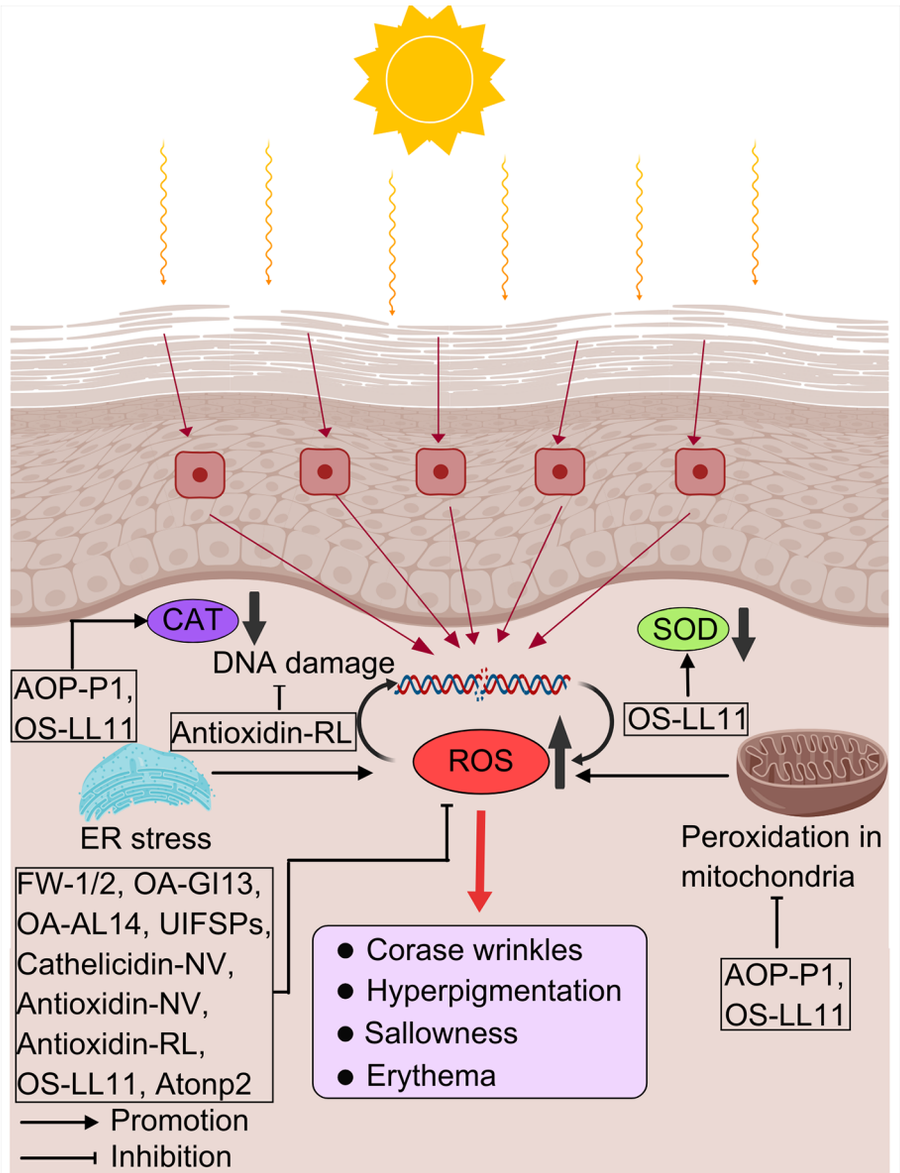

Figure 3 Reported amphibian-derived peptides that alleviate ultraviolet B (UVB)-induced skin lesions

This schematic summarizes the protective effects of reported amphibian-derived peptides against UVB-induced skin damage. UVB irradiation increases reactive oxygen species (ROS) production, leading to DNA damage, endoplasmic reticulum stress, mitochondrial lipid peroxidation, and changes in antioxidant defense systems, including catalase (CAT) and superoxide dismutase (SOD), which ultimately contribute to coarse wrinkles, hyperpigmentation, sallowness, and erythema. The indicated peptides alleviate UVB-induced skin lesions mainly by reducing oxidative stress and cellular damage and by modulating antioxidant and stress-response pathways (Created with MedPeer).

and significantly improves survival rates and wound closure in murine models infected with penicillin-resistant *E. coli*. This is achieved primarily through the elimination of bacterial colonization in both internal organs and subeschar tissue, thereby effectively preventing systemic bacterial dissemination. Furthermore, in a standardized scald injury model, MSI-1 significantly downregulates the expression of pro-inflammatory cytokines, specifically TNF-α and IL-6, in skin homogenates (Ma et al., 2020). Pse-T2, a truncated and Lys-substituted analog of pseudin-2, exhibits superior antimicrobial efficacy and markedly lower cytotoxicity compared to the parent peptide. Additionally, Pse-T2 significantly enhances wound healing in multidrug-resistant *Pseudomonas aeruginosa*-infected models by exerting anti-inflammatory effects, as evidenced by the suppression of key pro-inflammatory cytokines including interleukin-1β (IL-1β), IL-6, and TNF-α (Kang et al., 2018). This combination suggests that MSI-1 and Pse-T2 act at the interface of host defense and regeneration, rather than functioning as simple antimicrobial agents.

Peptides with broader pro-regenerative and immunomodulatory effects

A second group of amphibian peptides exhibits more direct pro-regenerative activities, including stimulation of cell migration and proliferation, modulation of inflammatory cytokine production, enhancement of angiogenesis, and regulation of extracellular matrix remodeling. Andersonin-W1 (AW1), isolated from *Odorrana andersonii*, is a representative example of this functional class. AW1 exhibits potent antimicrobial activity against Gram-positive, Gram-negative, and fungal strains (Yang et al., 2012). Beyond its antimicrobial properties, AW1 demonstrates remarkable efficacy in promoting skin wound healing (Li et al., 2024). At the cellular

level, AW1 enhances HaCaT cells proliferation, migration, and scratch repair, stimulates macrophage proliferation, and promotes tube formation in human umbilical vein endothelial cells (HUVECs). These effects contribute to re-epithelialization, granulation tissue regeneration, and angiogenesis, leading to accelerated healing of full-thickness wounds, deep second-degree burns, and diabetic skin wounds in mice. Mechanistically, AW1 directly binds to the extracellular domain of toll-like receptor 4 (TLR4), modulating the downstream nuclear factor kappa-B (NF-κB) signaling pathway to facilitate inflammatory cytokine secretion while suppressing excessive inflammation induced by lipopolysaccharide (LPS) in macrophages. Additionally, AW1 promotes macrophage polarization, facilitating the transition from the inflammatory to the proliferative phase of wound healing.

Peptides with antioxidant activities

Antioxidant peptides represent a functionally distinct subgroup of amphibian-derived wound-healing agents that act primarily through protection against oxidative stress and modulation of redox-sensitive signaling pathways. Antioxidin-RL, isolated from *Odorrana livida*, is a well-characterized example of this class. Cell-based assays demonstrate that Antioxidin-RL effectively inhibits UVB-induced ROS production and prevents oxidative damage to DNA and proteins in HaCaT cells and HSFs. Mechanistically, Antioxidin-RL downregulates the expression of matrix metalloproteinase-1 (MMP-1) (which degrades collagen), COX-2 (a pro-inflammatory mediator), and pro-inflammatory cytokines, while upregulating fibroblast growth factor (FGF), collagen type I, and transforming growth factor-beta (TGF-β), thereby promoting a pro-regenerative phenotype. In mouse models, Antioxidin-RL ameliorates UVB-induced erythema, skin thickening, and inflammation, demonstrating protective effects against UV-induced photodamage (Qin et al., 2018).

Similarly, UV-induced frog skin peptides (UIFSPs), identified from dark-spotted frogs (*Pelophylax nigromaculatus*) following constant UVB exposure, exhibit free radical scavenging capacity and improve UVB-induced oxidative stress by neutralizing ROS (Yang et al., 2024a). Moreover, UIFSPs significantly decrease the death of WS1 cells and HaCaT cells after UVB exposure, indicating protection against UVB-induced death and senescence in skin cells. To enhance intracellular peptide concentration, the trans-activating transcriptional (TAT) protein was fused to UIFSPs. TAT, the first identified protein transduction domain (PTD) (Green & Loewenstein, 1988), enables its conjugated cargo to penetrate cellular membranes efficiently. These TAT-conjugated peptides notably accelerate membrane penetration and potentiate the protective effects of UIFSPs. *In vivo*, TAT-UIFSPs prevent and attenuate UVB-induced skin damage in mice by negatively regulating the Ras/Akt and Ras/MAPK signaling pathways. A newly identified peptide, OA-AL14, was found to significantly ameliorate UVB-induced erythema, pigmentation, epidermal hyperplasia, and skin barrier disruption in a mouse model via activation of the Nrf2 antioxidant pathway and restoration of cellular redox homeostasis (Peng, et al., 2026).

Beyond skin protection, certain amphibian-derived antioxidant peptides also display therapeutic potential in vascular aging. AL-VI10 is a novel amphibian skin-derived bioactive peptide with potent anti-vascular aging activity. It acts as a safe and effective agent by targeting key molecular pathways, particularly through activation of the CD44-mediated SIRT1-Nrf2 signaling axis. Studies have shown that AL-VI10 possesses excellent biosafety and antioxidant capacity, reducing ROS accumulation while enhancing antioxidant enzyme activity. In parallel, it significantly attenuates vascular aging-related phenotypes, including inflammation and medial thickening. By directly binding to the CD44 receptor, AL-VI10 promotes endothelial recovery and improves cellular homeostasis, highlighting its promise as a candidate for the prevention and treatment of age-related cardiovascular diseases (Liu et al., 2026).

Peptides with radioprotective activities

Radioprotective peptides represent another specialized functional category that addresses radiation-induced skin damage, a clinically important concern in contexts such as radiotherapy, occupational exposure, and nuclear accidents. RIFSP-2 is one of the dark-spotted frog (*Pelophylax nigromaculatus*) skin-derived peptides following electron beam exposure. It exhibits membrane permeability, maintains cellular homeostasis, and reduces pyroptosis in irradiated cells (Geng et al., 2024). Ionizing radiation induces the expression of type I interferons and pro-inflammatory cytokines by activating the stimulator of interferon genes (STING) and TANK-binding kinase 1 (TBK1) (Yu et al., 2023). In contrast, RIFSP-2 reduces the phosphorylation of STING and TBK1 by targeting stearyl-CoA desaturase 1 (SCD1). This restrains the biogenesis of monounsaturated fatty acids (MUFAs) in irradiated skin cells, thereby ameliorating radiation-induced inflammation. In addition, RIFSP-2 also promotes the degradation of TBK1 in a ubiquitin-dependent manner and SCD1-regulated autophagy, hinting that RIFSP-2 protects from ionizing radiation through the SCD1/STING pathway. This conclusion is further supported by the fact that RIFSP-2 treatment had little effect on healing in *STING*$^{-/-}$ mice (Yu et al., 2023). The identification of RIFSP-2 illustrates that amphibian skin peptides can provide protection not only against microbial threats and oxidative stress but also against radiation-induced damage, expanding the potential therapeutic applications of these molecules to contexts involving environmental or medical radiation exposure.

Peptides with therapeutic activity for diabetic foot ulcers

Beyond their evaluation in standard acute wound models, a subset of amphibian-derived peptides has been investigated in more clinically relevant or challenging wound contexts, which provides stronger evidence for their therapeutic potential and helps define their possible clinical niches. Diabetic wounds represent one of the most important clinical challenges in wound management, characterized by impaired angiogenesis, chronic inflammation, increased susceptibility to infection, and delayed healing.

A recent cover story from the *Journal of Medicinal Chemistry* reported a cyclic heptapeptide, FZ1, which exerts highly potent activity in promoting skin regeneration under hyperglycemic conditions (Wang et al., 2025b). *In vivo*, FZ1 strikingly accelerates epidermal re-epithelialization and collagen deposition and upregulates the expression of angiogenesis-related genes CD31 and α-smooth muscle actin (α-SMA) in a full-thickness skin injury diabetic mouse model, consequently promoting wound healing and neovascularization. To unravel the underlying mechanisms, single-cell RNA sequencing and cell-cell interaction analyses were performed and revealed that FZ1 plays a pivotal role in different phases of wound healing. The transcriptomic analysis

also reveals that FZ1 activates FAK/ERK/AKT signaling cascades and upregulates the expression of vascular endothelial growth factor isoform c (VEGFC), as confirmed in HUVECs. Molecular docking analysis and surface plasmon resonance analysis both demonstrate that FZ1 regulates FAK phosphorylation by targeting integrin αvβ3. Notably, FZ1 is the first reported peptide agonist targeting integrin αvβ3 to promote diabetic wound healing. Recently, an engineered peptide, (RADA)4-FZ1, formed by covalently conjugating FZ1 to RADA16-I (cell scaffolding), was reported. This multifunctional hydrogel, (RADA)4-FZ1, exhibits excellent biocompatibility, antioxidant and anti-inflammatory properties, and a strong capacity to promote epidermal regeneration, angiogenesis, and collagen deposition. These combined properties resulted in significantly accelerated healing of diabetic wounds within 14 days, demonstrating superior efficacy to the commercial agent rh-bFGF (Fu et al., 2026). Peptides with similar activity also include RL-QN15 (Sun et al., 2023), $Cy_{RL\text{-}QN15}$ (Wu et al., 2024c), and AW1 (Li et al., 2024), etc.

Peptides with activity in reducing scar formation

Some studies have explored the potential of amphibian peptides in surgical wound healing and scar reduction. For example, certain peptides have been reported to modulate collagen deposition and organization in ways that may reduce excessive scarring or promote more organized tissue remodeling. For instance, OA-GL21 was identified from the odorous frog *Odorrana andersonii*. In vitro, OA-GL21 promotes wound healing in a full-thickness skin wound model in dose- and scar-free manners without hemolytic or acute toxic activity *in vivo* (Bian et al., 2018). However, the evidence in this area remains preliminary, and more detailed studies are needed to determine whether amphibian peptides can meaningfully influence scar formation.

Overall, while the evaluation of amphibian peptides in intractable wound contexts remains limited compared to standard acute wound models, the available evidence suggests that these peptides may offer advantages in managing complex or challenging wounds characterized by infection, chronic inflammation, oxidative stress, radiation damage, or impaired healing. However, more rigorous validation in clinically relevant models, including assessment of long-term outcomes, safety, and comparison with standard-of-care treatments, will be essential for determining their true therapeutic potential in these contexts.

INTEGRATIVE ANALYSIS AND MECHANISMS OF AMPHIBIAN WOUND-HEALING-PROMOTING PEPTIDES

Structural and physicochemical determinants

Amphibian-derived peptides are primarily classified into families such as the Bombinins, Temporins, Tigerinins, Magainins, Brevinins, Dermaseptins, Cathelicidins, and Prokineticins, based on their amino acid sequences, spatial structures, functional characteristics, and evolutionary origins. An integrative analysis of these wound-healing peptides reveals several conserved structural and physicochemical signatures that are crucial for their bioactivity. Most identified tissue-repairing peptides possess distinctive structural motifs that fundamentally determine their biological efficacy.

Amphipathic α-helical conformation: Many amphibian-derived wound-healing peptides exist as random coils in aqueous solutions but adopt an amphipathic α-helical structure in membrane-mimetic environments. This conformation is vital for their dual functionality. The helical structure physically separates the positively charged hydrophilic amino acids from the hydrophobic ones along the longitudinal axis, enabling more efficient recognition and insertion into microbial membranes, increasing membrane permeability, and ultimately causing leakage of cellular contents and microbial cell death. Moreover, the structural scaffold enables the peptide to activate intracellular signaling pathways (Chai et al., 2021; Conlon et al., 2009; Ezema et al., 2025; Mangoni et al., 2000, 2006; Matsuzaki, 1998; Mor et al., 1991; Pouny et al., 1992).

Cationic charge and electrostatic interactions: The presence of basic residues (Lys and Arg) gives these peptides a net positive charge. This cationicity drives the initial electrostatic attraction to negatively charged phospholipids (such as phosphatidylglycerol) prominently displayed on microbial membranes. This interaction ensures that the peptide selectively targets pathogens over the zwitterionic (neutral) membranes of mammalian cells, resulting in a favorable therapeutic index (Arnab et al., 2023). Moreover, the cationicity facilitates interactions with anionic components of the ECM and cell surface heparan sulfate proteoglycans. These interactions can concentrate growth factors at the site of injury, thereby accelerating the transition from the inflammatory phase to the proliferative phase of wound healing (Mangoni et al., 2016; Mu et al., 2014).

Cyclic motifs and stability (The "Rana Box"): Several amphibian-derived wound-healing peptides contain a C-terminal disulfide-bridged loop known as the "Rana box". This cyclic motif provides structural rigidity and protects the peptide from rapid proteolytic degradation in the enzyme-rich environment of a wound. Peptides such as tylotoin, OM-LV20, and RL-QN15 utilize this cyclic structure to stimulate the proliferation of HaCaT cells and HSFs to induce the expression of TGF-β, which is crucial for collagen synthesis (Mu et al., 2014). FZ1 and $Cy_{RL\text{-}QN15}$ are Rana box-retaining derivative peptides, exhibiting enhanced activity compared to their native counterparts (Ru et al., 2025; Wang et al., 2025b).

Sequence length and the "Minimal Bioactive Moti": Structure-activity relationship studies have revealed that the full-length sequence of native amphibian peptides is often not necessary and can sometimes be too toxic for mammalian cells. By identifying the critical structural domains, researchers have found that shorter, truncated peptide segments, such as the first 21 amino acids of Esculentin-1a, preserve the α-helical active center necessary for receptor binding while eliminating the severe membrane-disrupting toxicity (hemolysis) associated with the full-length peptide (Di Grazia et al., 2015).

In addition to these conserved structural features, some peptides also possess their own unique structural characteristics. Bv8-AJ, an analogue of Bv8, belongs to the nonmammalian prokineticin family. Prokineticins from both mammalian and nonmammalian species contain the conserved N-terminal sequence AVITGA and are therefore collectively termed AVIT proteins (Kaser et al., 2003). Another shared characteristic is the presence of 10 cysteines with identical spacing defining five disulfide bridges (Negri & Ferrara, 2018). Studies have shown that deletion of the first A of AVITG in Bv8 results in significantly lower receptor affinity compared with native Bv8, and deletion of the first two amino acids of Bv8 abolishes its biological activity (Negri et al., 2005); substitution of the first A with methionine or the addition

of a methionine to AVITG inhibits the activation of prokineticin receptors; and mutations in selected cysteine residues in the C-terminal domain result in loss of biological activity (Bullock et al., 2004).

Cathelicidins are defined by a highly conserved N-terminal "cathelin" pro-region and a highly variable C-terminal domain that, once cleaved and released, exerts potent biological activity (Zanetti et al., 1995). The conserved N-terminal cathelin domain is thought to function as a molecular chaperone and a protease inhibitor, preventing the active C-terminal peptide from damaging the host cell during storage in granules. Biological activity is only triggered when the C-terminal domain is enzymatically cleaved, for example, by proteinase 3 in human releasing the active antimicrobial peptide (Gennaro & Zanetti, 2000; Zanetti et al., 1995). The cathelin domain typically contains 4 highly conserved cysteine residues, which form 2 intramolecular disulfide bonds, playing a crucial role in maintaining peptide structural stability.

Magainins are a class of cationic antimicrobial peptides (AMPs) originally isolated from the skin of the African clawed frog (*Xenopus laevis*). Magainins tend to form "toroidal pores" to disrupt the integrity of the membrane. Once a critical concentration of peptides accumulates on the membrane surface, they dive into the lipid bilayer, inducing the membrane to bend continuously through the pore. The peptides and lipid head groups together line the water-filled pore, leading to the leakage of intracellular contents, collapse of the transmembrane potential, and ultimately cell death (Matsuzaki, 1998, 1999).

Dermaseptins are a highly potent family of linear, polycationic AMPs originally isolated from the skin of South American tree frogs belonging to the genus *Phyllomedusa* (Mor et al., 1991). A distinct structural signature of the dermaseptin family is their highly conserved N-terminal sequence, uniquely featuring a tryptophan (Trp) residue at position 3. This Trp residue plays a crucial structural role acting as a hydrophobic "anchor". Because of its preference for the interfacial region of lipid bilayers, it facilitates the initial insertion and stabilizes the binding of the peptide to the membrane (Feder et al., 2000; Pouny et al., 1992). Dermaseptins primarily exert their activity via the "carpet" mechanism rather than forming static transmembrane pores. The amphipathic helices accumulate parallel to the membrane surface (like a carpet). Once a critical threshold concentration is reached, they disrupt the membrane by causing a detergent-like micellization, leading to the collapse of the bilayer structure and rapid cell death (Pouny et al., 1992).

Evolutionary patterns in distribution and mechanisms

Amphibian-derived wound-healing peptides display notable evolutionary patterns that likely reflect the unique selective pressures acting on amphibian skin. As a multifunctional organ responsible for barrier defense, respiration, osmoregulation, and chemical protection, amphibian skin is continuously exposed to mechanical injury and dense microbial challenge. Under such conditions, amphibians have evolved a remarkably rich repertoire of skin-secreted peptides, many of which were initially characterized as antimicrobial molecules but are now increasingly recognized for their additional wound-healing-promoting activities (Conlon et al., 2009; Li et al., 2007), suggesting that wound-healing activity may have emerged through diversification of ancestral host-defense peptides that were originally selected to prevent infection at damaged epithelial surfaces.

One prominent evolutionary pattern is the coexistence of family-level conservation and species-level diversification. As previously mentioned, amphibian-derived peptides are commonly grouped into different families. Within these families, conserved sequence scaffolds, physicochemical traits, or structural frameworks are frequently retained, including cationicity, amphipathicity, moderate hydrophobicity, and, in some cases, conformationally stabilizing motifs such as the "Rana box" disulfide-bonded loops. These conserved features probably enhance peptide stability and membrane or receptor interactions in the protease-rich wound microenvironment, while substantial amino acid variation accumulates among species or even among closely related peptide isoforms. This pattern is consistent with repeated gene duplication followed by sequence diversification, allowing maintenance of a core defensive framework while generating new or refined biological activities (Conlon et al., 2009; Li et al., 2007).

A second evolutionary feature is the apparent association between ecological adaptation and peptide diversification. Amphibians inhabit moist and microbe-rich environments in which skin damage can rapidly lead to infection, osmotic imbalance, and impaired physiological homeostasis. Therefore, selective pressure would be expected to favor molecules capable not only of direct antimicrobial defense but also of rapid restoration of epithelial integrity. The recurrent discovery of wound-healing peptides in anuran skin secretions supports this interpretation, particularly in frogs and toads. A typical example is the peptide SC17-2 from the plateau frog *Nanorana parkeri*, which lacks antibacterial activity but promotes angiogenesis and wound healing, reflecting adaptations to the extreme environment of the Qinghai-Xizang Plateau, characterized by intense UV radiation and low microbial diversity (Cao et al., 2026).

The third pattern is functional expansion from antimicrobial defense to immunomodulation and tissue regeneration. Increasing evidence indicates that amphibian wound-healing peptides promote skin wound healing through various mechanisms of action, such as stimulating HaCaT cells and HSFs proliferation and migration, enhancing collagen deposition, promoting re-epithelialization and angiogenesis, reducing microbial burden, quenching ROS, and modulating relevant signaling pathways and immune responses (Figure 2; Figure 3). These findings support the concept that, during evolution, some amphibian skin peptides underwent functional co-option, whereby molecules originally selected as anti-infective effectors acquired regulatory roles in host tissue repair. In this context, wound-healing-promoting peptides may represent specialized descendants of broader host-defense peptide lineages that have acquired the ability to regulate inflammation, cell migration, proliferation, angiogenesis, and extracellular matrix remodeling. A better understanding of their evolutionary history will not only clarify how multifunctional peptide systems arise in vertebrate skin but may also facilitate the discovery of new peptide templates for regenerative medicine and wound therapy.

Shared and distinct mechanisms of amphibian-derived wound-healing peptides

Despite substantial diversity in sequence and source, these peptides share several mechanistic similarities. Most notably, they accelerate wound closure by promoting the migration and proliferation of HaCaT cells, HSFs, and, in some cases, HUVECs, thereby facilitating re-epithelialization, granulation

tissue formation, and angiogenesis. In addition, many of these peptides modulate the inflammatory microenvironment in a temporally coordinated manner, promoting early immune-cell recruitment while limiting excessive or persistent inflammation that impairs repair. For instance, tylotoin, AH90, and SC17-2 enhance wound healing by promoting cell motility and proliferation, TGF-β1 release, collagen deposition, and neovascularization, mainly via activation of the MAPK and TGF-β/Smad signaling pathways (Cao et al., 2026; Liu et al., 2014; Mu et al., 2014). Esculentin-1a(1-21)NH_2 exerts both direct antimicrobial activity and host-directed reparative effects, while also stimulating keratinocyte migration through EGFR-dependent signaling (Di Grazia et al., 2015). In addition, FZ1 promotes wound healing across three distinct phases of the repair process (Wang et al., 2025b) (as summarized in Figure 2). Therefore, although amphibian wound-healing peptides differ in their dominant molecular targets and signaling pathways, their shared functional hallmark is a multifactorial mode of action that integrates antimicrobial defense, inflammation control, cell migration and proliferation, ECM remodeling, and vascular regeneration. This coordinated, multi-target activity likely underlies their superior capacity to promote efficient wound repair and highlights their translational potential as templates for next-generation wound therapeutics (Mangoni et al., 2016).

Insights into wound healing biology: refinement and challenges

The integrative analysis of amphibian-derived wound-healing peptides has significantly advanced our understanding of wound healing biology. On one hand, these peptides refine our understanding of wound healing mechanisms. They have revealed the complexity and multi-faceted nature of the wound healing process beyond traditional models. For example, investigations into peptides like RL-QN15 provide profound insights into tissue remodeling by dynamically regulating the balance of transforming growth factor isoforms. By modulating the ratio of TGF-β3 (which is crucial for anti-fibrotic, scarless tissue regeneration) to TGF-β1 (which contributes to wound repair and scar formation), these peptides offer a compelling biological strategy for achieving high-quality, scarless wound healing (Wang et al., 2021). Moreover, the observation that amphibian peptides can target multiple downstream signaling pathways simultaneously, such as the MAPK, Smad, and Wnt/β-Catenin pathways, challenges the traditional single-target pharmacological paradigm. It underscores that multi-targeted properties are highly effective in orchestrating comprehensive tissue repair (Liu et al., 2014; Wang et al., 2025b).

On the other hand, these peptides challenge existing paradigms in wound healing research. The therapeutic application of certain amphibian bioproducts contradicts the conventional belief that scar formation is an inevitable outcome of wound healing. Specifically, research on OA-GL21 and RL-QN15 has demonstrated their potential to induce regenerative responses and promote scarless wound healing. This finding opens major possibilities for developing true regenerative therapies in human medicine. Furthermore, the demonstrated efficacy of amphibian peptides (such as RL-QN15 and FZ1) in chronic, non-healing wounds challenges the prevailing notion that pathological wound environments, such as diabetic foot ulcers, are irreversibly stalled. This implies that biologically evolved multi-target peptides may overcome the inherent limitations of current treatments in severe chronic wounds.

CHALLENGES IN CLINICAL TRANSLATION AND FUTURE DIRECTIONS

Amphibian-derived peptides represent a unique reservoir of bioactive molecules with broad wound-healing-promoting activities. Although numerous studies have demonstrated their experimental efficacy in wound healing, progress toward clinical translation remains limited. One notable indicator of this translational gap is the surprisingly weak patent landscape. A recent analysis of the Derwent World Patents Index (DWPI) showed that, among 188 identified patents related to anuran antimicrobial peptides, only six explicitly claimed wound-healing-promoting activity, and four of these were already “dead” (García et al., 2024). To date, PD-DP-008 gel (also known as JIJ02 gel, whose active ingredients are derived from frog skin secretion) is the only reported peptide drug for the treatment of skin lesions (acne) currently in phase I clinical trial (http://www.chinadrugtrials.org.cn/clinicaltrials.searchlist.dhtml). This discrepancy between robust laboratory evidence and sparse patent representation underscores a significant “translational gap”. It suggests that while biological activity is frequently reported in academic literature, the progression toward commercialization, which requires stringent patenting of specific therapeutic indications, stable formulations, and industrial scalability, is still in its infancy.

A confluence of factors contributes to this challenging context, with a primary driver being the insufficient research on amphibian-derived peptides. According to the Amphibian Species of the World, there are 8 919 known amphibian species as of September 14th, 2025. However, the Antimicrobial Peptide Database (APD) (https://aps.unmc.edu/) records only 1 112 membrane-active peptides from amphibians, while the Database of Anuran Defense Peptides (DADP) (http://split4.pmfst.hr/dadp/) contains 2 571 entries. Considering the overlap between these databases and the fact that only a few peptides are typically isolated from a single species, the vast majority of amphibian species remain unexplored. For instance, no wound-healing peptides have been reported from the order Gymnophiona (caecilians) to date. As illustrated in Figure 4, amphibians known to secrete wound-healing peptides account for only a small fraction of the total. This discrepancy underscores the urgent need for systematic studies of amphibian-derived peptides and the development of new technologies for peptide-based drug discovery.

The inadequate research is also evident in the fact that studies of amphibian-derived peptides in wound healing have mainly focused on severe wounds, such as incisions, burns, and ulcers, while relatively little attention has been paid to other skin lesions like acne and rosacea. Acne is a chronic inflammatory skin disease characterized by skin changes such as seborrhea, non-inflammatory lesions (comedones), and inflammatory lesions (papules and pustules) (Melnik, 2015). Although some mechanisms of *Propionibacterium acnes*-induced pathogenesis have been discussed (Thiboutot et al., 2009), and certain amphibian peptides are claimed to possess anti-acne activity (Huang et al., 2025; Urbán et al., 2007), there is still a notable lack of corroborative evidence from *in vivo* experiments.

Beyond these research gaps, the intrinsic properties of peptides themselves create additional obstacles for

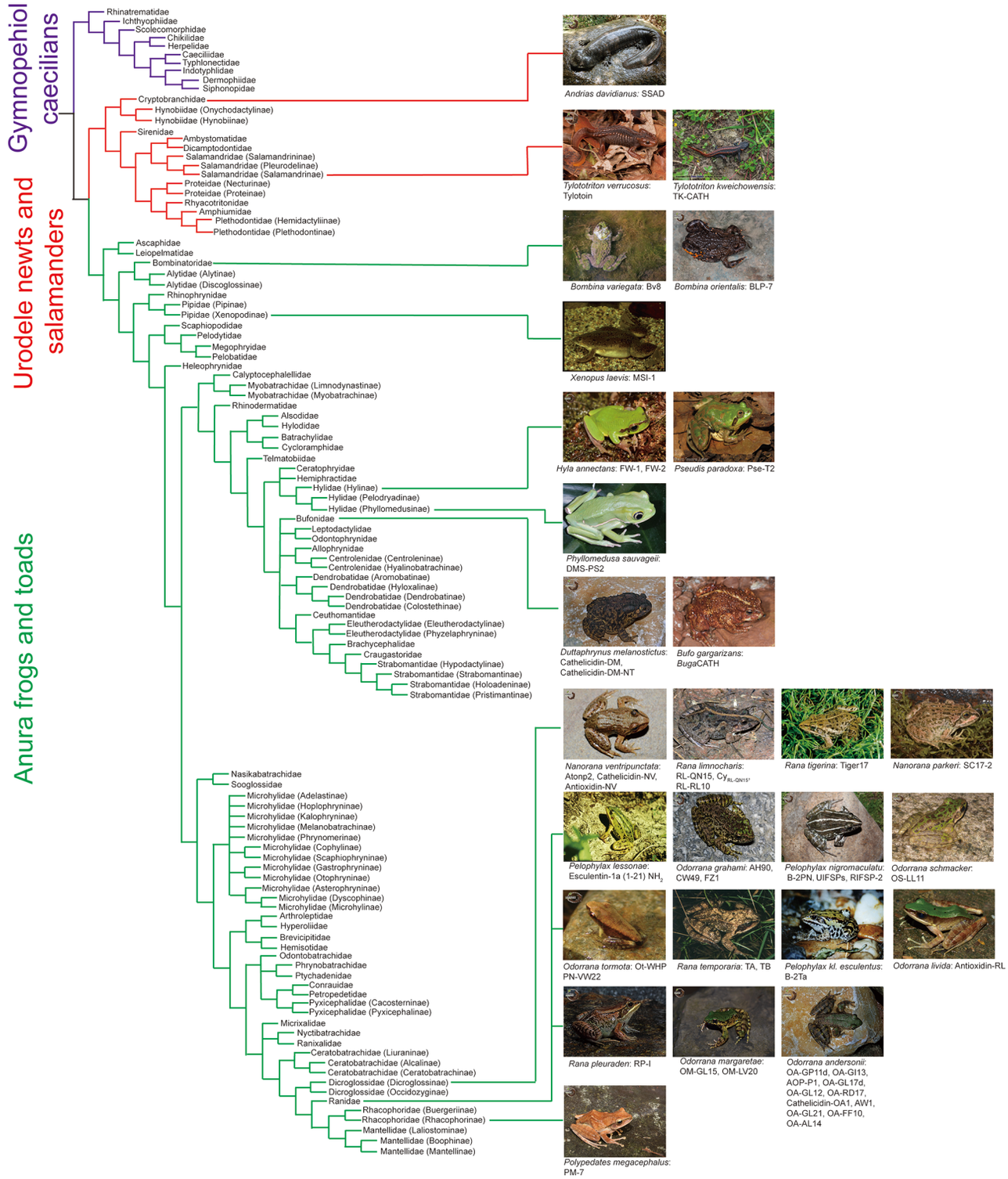

Figure 4 Reported amphibian-derived wound-healing peptides

This figure presents the family-level phylogeny of amphibians and the taxonomic distribution of reported amphibian-derived wound-healing peptides across different species. Representative species from the three major extant amphibian groups, namely Gymnophiona (caecilians), Urodela (newts and salamanders), and Anura (frogs and toads), are shown together with the corresponding peptides that have been reported to promote wound healing. The amphibian family phylogeny was adapted from AmphibiaWeb (https://amphibiaweb.org/?o=0), and the animal images were adapted from AmphibiaWeb and Amphibia China (https://www.amphibiachina.org/).

therapeutic development. For instance, peptides are susceptible to enzymatic hydrolysis, inactivation by fluctuations in temperature, pH, or osmotic pressure, and may even act as nutrients that promote bacterial proliferation, which markedly reduces systemic bioavailability (Fosgerau & Hoffmann, 2015; Werle & Bernkop-Schnürch, 2006). To improve stability while preserving bioactivity, several optimization strategies have been explored in peptide development more broadly, including D-amino acid substitution, backbone cyclization, N-terminal acetylation, C-terminal amidation, PEGylation, lipidation, and the elimination of protease-sensitive residues or motifs (Lau & Dunn, 2018;

Muttenthaler et al., 2021; Vlieghe et al., 2010).

The backbone cyclization optimization strategy is exemplified by RL-QN15 and its cyclic analogue $Cy_{RL\text{-}QN15}$. RL-QN15 is a linear 15-amino-acid peptide, whereas $Cy_{RL\text{-}QN15}$ was developed as a structurally constrained derivative designed to overcome some of the inherent limitations of linear peptides. Cyclization reduces backbone flexibility and stabilizes the bioactive conformation, an approach widely recognized in peptide drug development because cyclic peptides often exhibit enhanced proteolytic resistance, improved conformational stability, and prolonged biological activity relative to their linear counterparts (Sharma et al., 2023; Zorzi et al., 2017). Thus, the conversion of RL-QN15 into $Cy_{RL\text{-}QN15}$ represents a rational strategy to improve peptide stability and therapeutic persistence while preserving or enhancing wound-healing efficacy. Consistent with these anticipated advantages, $Cy_{RL\text{-}QN15}$ has shown strong pro-healing potential and has been further incorporated into multifunctional hydrogel-based delivery systems to maximize both regenerative and antimicrobial performance (Fu et al., 2022b; Jia et al., 2024). Similarly, Andersonin-D1 (AndD1), a natural peptide isolated from *Odorrana andersonii*, has demonstrated potent antimicrobial activity but exhibits a tendency to aggregate *in vitro*, which hinders its ability to reach and bind to its target, resulting in decreased antimicrobial activity and increased toxicity. By removing the Phe residue of AndD1 at its N-terminus and amidating the C-terminus of AndD1, a new synthetic peptide named dF-AndD1 was created, which significantly overcame the shortcomings of AndD1 and exhibited superior activity. This synthetic peptide is currently at the preclinical stage (Technology Readiness Level 3-4) and is expected to enter clinical trials within the next decade, offering a promising new strategy to combat multidrug-resistant bacterial infections (Ageitos et al., 2025).

Chemical modification represents another practical and widely adopted approach to circumventing these limitations. The intrinsic physicochemical properties of peptides, which dictate their bioactivity, absorption, metabolism, and stability, are the primary determinants of their application. Therefore, by investigating structure-activity relationships, researchers can identify active sites and apply appropriate chemical modifications to enhance strengths while mitigating weaknesses. Frequently used approaches for peptide modification are summarized in a comprehensive review (Sharma et al., 2023). For example, MSI-1 was designed by truncating 14 amino acids at the N-terminus of MSI-78 and substituting Gly^3 and Gly^{13} with Trp to enhance its activity and reduce its toxicity (Ma et al., 2020). Similarly, a cell-penetrating peptide was covalently coupled to tylotoin to improve its permeability (Mu et al., 2014), while lipidation and glycosylation have been shown to increase the bioavailability of two broad-spectrum antimicrobial peptides (Grimsey et al., 2020). These findings indicate that rational peptide engineering can help transform biologically active but pharmacologically fragile molecules into more viable therapeutic candidates.

In parallel, advances in delivery systems may help overcome pharmacological limitations by protecting peptides from degradation and enhancing local retention or tissue targeting. These materials, including hydrogels, nanofibers, scaffolds, nanoparticles, chitosan, and polymers, can be engineered into functionalized dressings and drug delivery systems to enhance peptide functionality. While traditional dressings serve as physical barriers to isolate the wound from the environment, active peptide-based dressings not only prevent infection but also actively promote tissue repair. Hydrogels, for instance, are 3D polymeric networks characterized by high water content. Integrating peptides into hydrogels combines the functional merits of the carrier (moist environment maintenance, adaptability to wound shape, elasticity, biocompatibility, and tissue adhesion) with the biological activity of the peptides, collaboratively accelerating healing. For example, incorporation of HPDAIR (hollow dopamine nanoparticles (HPDA) loaded with RL-QN15) (Sun et al., 2022) or HSN@RL-QN15 (hollow silica nanoparticles (HSNs) loaded with RL-QN15) into zinc alginate hydrogel (named HPDAIR&ZA and HSN@RL-QN15/ZA) (Qin et al., 2022), compared with RL-QN15 alone, HDPAIR, HPDAIR&ZA or HSN@RL-QN15/ZA is more effective.

Importantly, not all peptides should be considered equally promising for wound-healing applications. Robust evidence should extend beyond preliminary *in vitro* antimicrobial or pro-proliferative observations and ideally include mechanistic validation, efficacy in relevant animal wound models, and appropriate safety assessment. For example, some peptides may show strong antimicrobial activity or stimulate HaCaT and HSF proliferation in cell culture but remain of only preliminary interest if they lack mechanistic clarification, *in vivo* validation, or toxicity evaluation. By contrast, peptides such as FZ1, Ot-WHP, and *Buga*CATH are more compelling because available studies suggest that their wound-healing effects are not limited to simple antimicrobial action but also involve defined regulatory effects on inflammation, cell migration, and repair-related signaling pathways. At the same time, many otherwise interesting candidates still face major translation barriers, including peptide instability, cytotoxicity, insufficient selectivity, inconsistent activity across experimental settings, and the absence of suitable delivery strategies. Therefore, peptides with the greatest translational potential are likely to be those that combine multifunctional bioactivities with mechanistic clarity, reproducible *in vivo* efficacy, acceptable safety margins, and feasibility for formulation and targeted delivery.

CONCLUSION

Amphibian-derived peptides have emerged as a remarkably diverse and functionally versatile source of candidate therapeutics for skin wound repair. Beyond their classical antimicrobial activity, growing evidence indicates that these peptides act on multiple, interconnected stages of wound healing by directly modulating inflammation, enhancing keratinocyte and fibroblast migration and proliferation, promoting angiogenesis, stimulating extracellular matrix remodeling, and, in some cases, reducing oxidative stress to improve the quality of tissue regeneration (Brunetti et al., 2018; Fan et al., 2024; Xu & Lai, 2015). This multimodal mechanism is particularly attractive for chronic and infected wounds, where impaired healing is rarely driven by a single pathological process. Compared with conventional single-target drugs, amphibian peptides may therefore offer a systems-level therapeutic advantage by simultaneously addressing microbial burden, inflammatory imbalance, and regenerative insufficiency.

From a mechanistic perspective, a key concept emerging from current studies is that amphibian wound-healing peptides act not merely as endogenous antibiotics but serve as bio-inspired, immunoregenerative agents. Several peptides have been shown to activate signaling pathways pivotal to re-

epithelialization and healing, including EGFR, MAPK/ERK, PI3K/Akt, TGF-β/Smad, and focal adhesion-related pathways, while simultaneously modulating cytokine production and macrophage behavior in the wound microenvironment (Brunetti et al., 2018; Xu & Lai, 2015). Representative examples such as AW1, Ot-WHP, and *Buga*CATH support the view that amphibian peptides can promote healing not only by controlling infection but also by actively orchestrating tissue regeneration (He et al., 2019; Li et al., 2024; Zhou et al., 2024). These findings collectively suggest that the therapeutic value of amphibian peptides lies in their unique ability to couple anti-infective defense with innate repair-promoting bioactivity.

At the translational level, however, substantial barriers persist in bridging the gap between promising experimental observations and clinically viable therapeutics. As with many peptide-based agents, central challenges include rapid proteolytic degradation, short systemic half-lives, insufficient retention at wound sites, and the complexities of formulation stability and cost-effective manufacturing. Promisingly, advancements in peptide engineering and delivery science provide realistic paradigms to circumvent these hurdles. Sequence optimization, which involves residue substitution, terminal modification, cyclization, or hybrid design, can significantly enhance metabolic stability while preserving intrinsic bioactivity. Complementarily, biomaterial-assisted delivery systems, including hydrogels, nanoparticles, liposomes, and functionalized dressings, offer potent means to improve local retention, shield peptides from degradation, and reduce dosing frequency. Ultimately, the future success of amphibian-derived therapeutics will depend not only on the discovery of novel motifs but also on the synergistic integration of peptide engineering, advanced delivery platforms, and scalable biomanufacturing.

Despite rapid progress, several critical knowledge gaps persist. First, the structure–activity relationships governing the intricate balance among antimicrobial potency, immunomodulation, cytocompatibility, and regenerative efficacy remain to be fully elucidated. Second, given that most studies have relied on acute rodent models, the clinical relevance of current findings to human chronic wounds, such as diabetic ulcers, pressure injuries, and ischemic wounds, demands more rigorous validation. Third, the precise interactions between these peptides and the complex wound microenvironment, which is composed of biofilms, senescent cells, extracellular proteases, and immune-cell heterogeneity, are poorly understood. Fourth, issues of long-term safety, immunogenicity, repeated-dose tolerability, and batch-to-batch reproducibility must be systematically addressed to accelerate clinical translation. Finally, the field lacks standardized comparative frameworks to evaluate peptide candidates across the multidimensional axes of potency, stability, toxicity, and manufacturability.

Looking ahead, the field must transition from descriptive peptide discovery toward mechanism-guided and translation-oriented development. Harnessing high-throughput peptidomics, AI-assisted sequence design, structure-based optimization, and multi-omics analyses will facilitate the rational engineering of next-generation amphibian peptide analogues with superior stability, selectivity, and therapeutic indices. Concurrently, the establishment of standardized efficacy assessment across studies, including unified infection models, healing endpoints, and safety evaluation criteria, will be essential for improving cross-study comparability, facilitating meta-analysis, and accelerating the clinical translation of promising peptide-based therapeutics. Ultimately, multidisciplinary synergy among peptide chemists, biomaterial scientists, pharmacologists, and clinicians will be instrumental in transforming amphibian-derived peptides from biologically intriguing natural products into transformative therapies for recalcitrant wounds. Overall, the future of amphibian-derived wound-healing peptides will depend not only on the discovery of new molecules but also on the establishment of rigorous evidence standards, rational optimization strategies, and clinically relevant translational pipelines.

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

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