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REVIEW

Advances in Necrotizing Fasciitis Animal Models: Current Trends and Future Perspectives

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Abstract: Necrotizing fasciitis (NF) is a rapidly progressive soft tissue infection with high morbidity and mortality, yet its pathophysiology remains incompletely understood. Animal models are indispensable for dissecting disease mechanisms and testing therapeutic interventions, yet their translational relevance varies across species and experimental approaches. This review critically evaluates NF models in rodents, zebrafish, rabbits, pigs, and non-human primates, highlighting their relative strengths in replicating immunopathology, systemic dissemination, and biofilm-driven persistence. We examine methodological strategies including bacterial inoculation, trauma-induced infection, immunosuppression-enhanced infection, genetic engineering, and optical imaging, and discuss how these frameworks capture discrete aspects of NF pathogenesis. Emerging technologies, including CRISPR-mediated host factor engineering, multi-omics profiling, intravital and high-resolution imaging, organ-on-chip platforms, and artificial intelligence, are integrated into next-generation models to enhance predictive power, mechanistic insight, and translational fidelity. We also outline innovative therapeutic strategies validated in preclinical models, from precision-targeted monoclonal antibodies and immunotherapies to advanced drug delivery systems and AI-guided predictive modeling. Finally, we discuss practical and ethical considerations for NF modeling, emphasizing reproducibility, standardization, and the 3Rs principle, and propose a forward-looking framework for integrating in vivo, in vitro, and in silico platforms. Collectively, these insights provide a roadmap for refining NF animal models, accelerating mechanistic discovery, and informing clinically relevant interventions.

Keywords: fasciitis, necrotizing, disease models, animal, coinfection, precision therapeutics, translational medical research

Introduction

Necrotizing fasciitis (NF) is a life-threatening, rapidly progressive soft tissue infection characterized by extensive necrosis of the skin, subcutaneous tissue, and fascial layers.[1] The main pathogens, *Streptococcus pyogenes* and *Staphylococcus aureus*, exploit host weaknesses such as trauma or immunosuppression to trigger toxin-mediated tissue damage, sepsis, and multi-organ failure.[1,2] Clinical signs include severe pain, erythema progressing to violaceous skin discoloration, and serosanguinous discharge, which may quickly progress to hemorrhagic bullae, tissue necrosis, and hemodynamic instability.[3–6]

Although the global incidence is relatively low, estimated at 0.2 to 15.5 cases per 100,000 individuals annually,[1] the burden of NF is rising with an aging population, increasing diabetes rates, and wider use of immunosuppressive therapies.[6–11] Despite growing clinical awareness and recent consensus guidelines that emphasize early diagnosis, prompt surgical debridement, and empiric broad-spectrum antibiotics,[12–16] NF continues to be associated with high mortality (10–35%) and frequent complications. Delayed diagnosis, multidrug-resistant organisms, and the disease's fulminant course remain major contributors to poor outcomes.[1,3–5]

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In this context, antimicrobial resistance (AMR) poses an additional and increasingly recognized challenge. The inappropriate and extensive use of broad-spectrum antibiotics, coupled with the emergence of multidrug-resistant pathogens, complicates NF treatment and may undermine established therapeutic protocols. Addressing this threat requires integrative strategies such as the "One Health" approach,[17] which emphasizes the interconnectedness of human, animal, and environmental health in combating AMR. However, awareness of this framework among medical professionals remains limited, underscoring the need for greater dissemination and implementation. Incorporating "One Health" principles into NF management may not only help contain resistance but also strengthen preparedness for future infectious disease threats.

While early intervention is widely acknowledged as the most important prognostic factor,[18,19] individualized, evidence-based treatment pathways remain underdeveloped, particularly for complex cases or resource-limited settings. This gap partly reflects limitations in studying NF pathogenesis directly in humans. Preclinical animal models have therefore been critical not only for elucidating host–pathogen interactions but also for informing therapeutic strategies. Several adjunctive therapies, including hyperbaric oxygen therapy (HBOT), intravenous immunoglobulin (IVIG), and bacteriophage therapy, were first validated in animal studies before progressing to clinical evaluation, illustrating the translational impact of these models, even when efficacy remains debated.

However, existing reviews largely describe NF pathogenesis or individual models without providing a systematic, cross-species synthesis or integrating emerging translational platforms. This review addresses this unmet need by providing a structured, cross-species evaluation of NF animal models, from rodents and zebrafish to rabbits, pigs, and non-human primates, while assessing methodological approaches, pathophysiological fidelity, translational relevance, and ethical considerations. We further incorporate forward-looking perspectives on CRISPR-based immune humanization, multi-omics profiling, advanced imaging, organ-on-chip technologies, and AI-driven model optimization, thus offering a comprehensive and predictive framework that bridges preclinical modeling and clinical translation.

Current Status and Classification of NF Animal Models

Animal models remain indispensable for elucidating the pathophysiological mechanisms of NF, evaluating therapeutic strategies, and facilitating the translation of preclinical insights into clinical interventions. These models recapitulate key clinical features of NF, such as localized inflammation, bacterial dissemination, progressive soft tissue necrosis, and systemic inflammatory responses, thus enabling targeted investigation of antibiotics, immunomodulatory therapies, and surgical approaches.[20–22] However, substantial interspecies differences in anatomy, immunity, and microbiota composition limit the extrapolation of findings to human disease. Therefore, model selection should align with specific research objectives, balancing experimental accessibility against translational fidelity. Below, we classify and critically evaluate the major NF animal models in use today (Figure 1), highlighting their individual contributions and shared limitations.

Rodent Models

Rodents are the most frequently used species in NF research due to their low cost, rapid breeding, and genetic manipulability.[23–25] Murine models have provided critical insights into host-pathogen interactions, including inflammatory cascades, cytokine networks, and immune cell dynamics.[23,26] Despite their mechanistic utility, several limitations constrain their translational impact. The small size of rodents restricts serial sampling of blood and tissues, limiting dynamic monitoring of infection and treatment responses. In addition, differences such as thinner skin, simpler fascia, and a distinct microbiome limit how well these models replicate human tissue pathology.[27]

Of greater concern are the immunological disparities. Rodent immune systems differ in Toll-like receptor (TLR) expression, chemokine signaling, and leukocyte profiles compared to humans.[28] These differences can lead to overestimation of therapeutic efficacy in preclinical studies and subsequent failure in clinical translation. To enhance translational relevance, future directions include the incorporation of humanized immune systems and the integration of rodent studies with complementary platforms such as organoids or micro physiological systems.

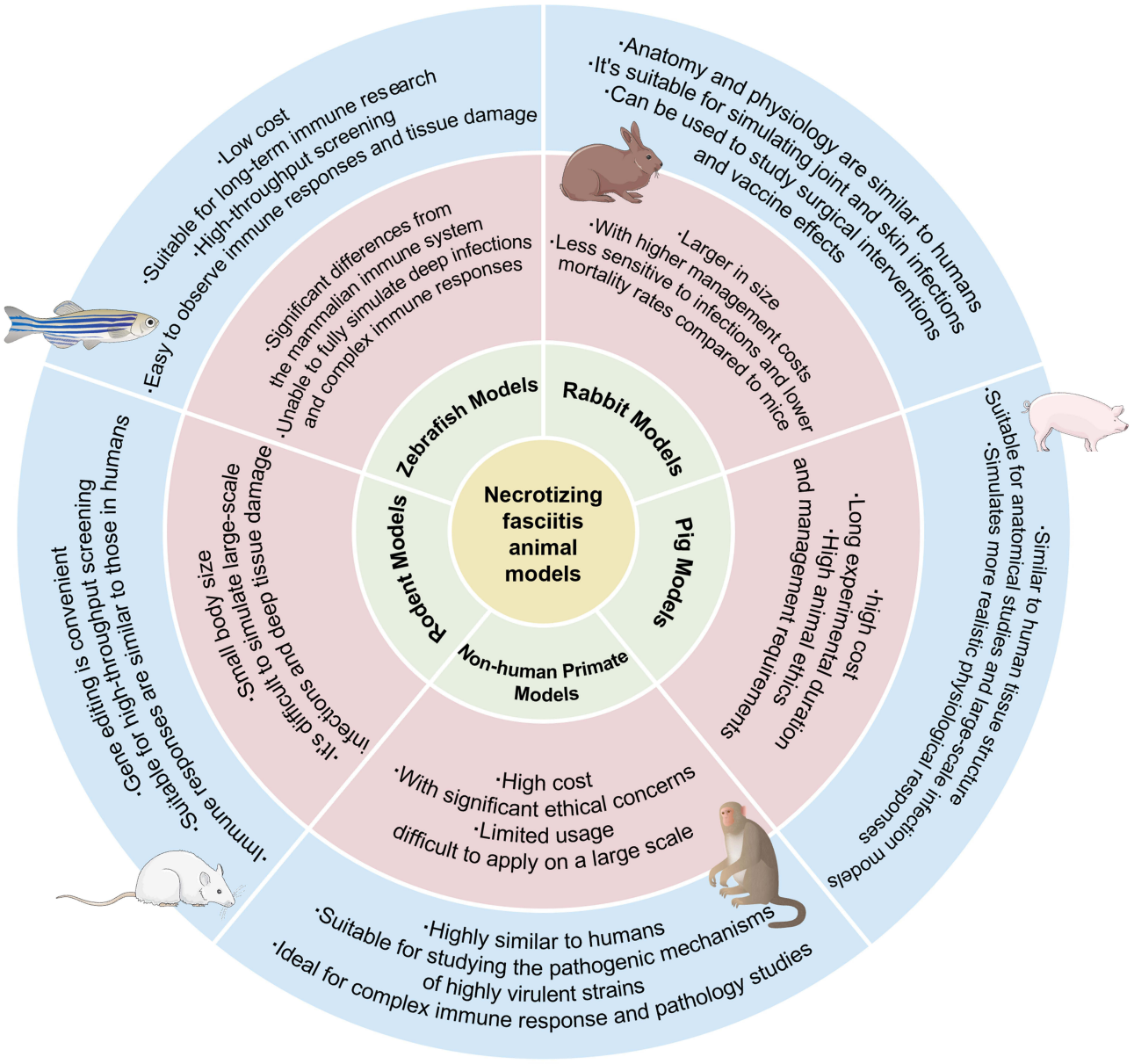

Figure 1 Overview of NF animal models (PowerPoint).

Zebrafish Models

The zebrafish (Danio rerio) has emerged as a unique model for visualizing host-pathogen interactions in NF due to its optical transparency and advanced imaging compatibility. Live imaging techniques, such as fluorescence microscopy and bioluminescence, enable real-time tracking of bacterial dissemination, immune cell recruitment, and tissue damage.[29–32] Additionally, the zebrafish possesses both innate and adaptive immune components, providing a conserved framework for exploring NF pathogenesis.[29]

Nonetheless, significant limitations curtail its translational utility. Zebrafish lack mammalian-specific immune mechanisms, such as TLR4 signaling and Fc-mediated phagocytosis, which are crucial in combating *Streptococcus pyogenes*. Moreover, the absence of fascia-like tissue architecture and temperature-dependent immune responses further limits its anatomical and physiological relevance. Thus, while zebrafish offer unparalleled advantages for high-throughput mechanistic studies, they are best suited as complementary tools, ideally integrated into broader pipelines that include mammalian and human-based systems.

Medium-Sized Mammalian Models

Rabbit models serve as a valuable intermediate between rodents and large animals, offering sufficient body size for serial tissue sampling, surgical intervention, and longitudinal lesion monitoring.[33–35] Compared to mice, rabbits demonstrate more human-like inflammatory profiles, including cytokine responses and leukocyte infiltration patterns, enhancing their utility for preclinical testing of topical and surgical treatments.

However, these models are not without limitations. *Streptococcus pyogenes* exhibits species-specific host tropism, and its pathogenesis in rabbits may not fully mimic human disease kinetics.[36] Furthermore, current rabbit models often overlook critical clinical variables such as polymicrobial infections, diabetes, or immunosuppression, thereby limiting ecological validity. Advancing these models will require the incorporation of clinically relevant comorbidities and pathogen combinations, as well as the use of multi-omics approaches to deepen mechanistic understanding.

Large Mammalian Models

Porcine models are widely regarded as the most physiologically relevant platform for studying NF due to their close anatomical, immunological, and dermatological resemblance to humans.[37,38] Pigs exhibit similar skin structure, including epidermal thickness, dermal collagen, and subcutaneous fat distribution, and their immune responses to bacterial infection align closely with those of humans.[39–41] As such, porcine models are particularly well-suited for studying polymicrobial infections, surgical management, and systemic sepsis in NF.

Despite their advantages, practical and ethical challenges limit broader adoption. High maintenance costs, specialized surgical infrastructure, and stringent ethical oversight reduce accessibility and scalability. Additionally, small sample sizes, driven by logistical constraints, undermine statistical power and generalizability. The lack of standardized protocols across institutions further hampers reproducibility. Future progress will depend on three key strategies: (1) development of non-invasive, longitudinal monitoring technologies; (2) harmonization of disease induction and assessment protocols; and (3) integration of porcine models with reduction-refinement strategies, such as hybrid in vitro–in vivo systems.

Non-Human Primate Models

Non-human primates (NHPs) provide unmatched immunological and physiological proximity to humans, particularly for modeling *S. pyogenes*-induced systemic infections and evaluating host-targeted therapies.[42–45] Their capacity to replicate human-like T-cell responses, cytokine kinetics, and immunopathological sequelae makes NHPs an invaluable model for late-stage vaccine and immunotherapy testing.

However, the ethical and logistical barriers are considerable. The high cost, limited availability, and public scrutiny surrounding NHP use restrict their utility to highly controlled, small-cohort studies, thereby limiting statistical robustness and experimental flexibility. Moreover, the absence of standardized NF induction protocols across primate studies reduces comparability. Moving forward, NHPs should be strategically reserved for critical validation steps within a tiered translational framework, supported by data from lower-order animals, human tissue models, and computational platforms. This approach balances ethical responsibility with the need for high-fidelity modeling.

Methodological Approaches for NF Animal Model Development

The development of NF animal models primarily relies on five methodological strategies: bacterial inoculation, trauma-induced infection, immunosuppression-enhanced infection, genetic engineering, and optical imaging integration (Table 1). Each approach is designed to replicate specific aspects of NF pathogenesis and serves distinct experimental goals. Below, we critically evaluate these techniques and their translational limitations.

Bacterial Inoculation

Bacterial inoculation remains the most widely employed strategy for inducing NF in animal models. Pathogens such as *Streptococcus pyogenes* and *Staphylococcus aureus* are directly injected into subcutaneous or fascial layers to reproduce key clinical signs, including erythema, edema, tissue necrosis, and systemic sepsis.[20,31,41,46–49] Polymicrobial co-infection models better simulate clinical scenarios and often result in more severe disease phenotypes.

Table 1 Methods for Constructing an Animal Model of NF

Construction Method	Description	Advantages	Disadvantages
Bacterial Injection	The pathogen (eg, *Streptococcus pyogenes*) is injected into animals like mice, rabbits, or pigs to induce NF.	Simple, direct, easy to control bacterial dose.	Difficult to simulate the complexity of natural infections, and there is variability in bacterial strain virulence.
Trauma-Combined Infection	Physical injury (such as skin abrasions or cuts) is inflicted on animals, followed by infection with NF pathogens to simulate real-life clinical infections.	Closer to clinical reality, simulates the occurrence of trauma-induced infections.	The trauma treatment process may influence local immune responses.
Gene Editing	Gene-editing technologies are used to modify animal genes, simulating specific immune responses or disease susceptibility.	Precise control over the genetic background of animals, suitable for studying the role of specific genes in NF.	Long construction cycle, high cost, and potential ethical issues.
Polymicrobial Infection	Multiple pathogens are simultaneously introduced into animals to simulate the polymicrobial nature of NF.	More realistically simulates the complex pathogen interactions in clinical cases, reflecting the pathological process.	Complex to operate, difficult to control interactions between multiple pathogens.
Immunodeficient Model	Immunodeficient mice are used to study immune evasion and inflammation.	Simulates infection under immunosuppressive conditions, suitable for studying immune mechanisms.	Does not fully simulate normal immune responses, may lead to distorted pathological changes.

This method offers high reproducibility and technical simplicity, making it ideal for drug screening and mechanistic studies. However, the artificial use of high inoculum concentrations and the frequent exclusion of anaerobic bacteria or biofilm dynamics may oversimplify the multifactorial nature of human NF. Furthermore, most models capture only acute phases of infection, limiting their utility in investigating the full disease trajectory.

Trauma-Induced Infection

Trauma-based models mimic real-world NF triggers by combining mechanical injury, such as incisions, burns, or foreign body implantation, with bacterial contamination. In medium and large animals such as rabbits and pigs, full-thickness wounds and fascial incisions closely replicate human NF progression, including the interaction between tissue necrosis and host immune responses.[41,50] In mice, minor skin abrasions have been shown to significantly increase bacterial burden and mortality, underscoring the importance of trauma as a predisposing factor.[50]

These models are particularly useful for investigating wound microenvironments and infection dissemination. However, their technical complexity and sensitivity to surgical variation demand strict procedural standardization to ensure reproducibility and reliability.

Immunosuppression-Enhanced Infection

To model NF in high-risk populations, such as individuals with diabetes or undergoing chemotherapy, researchers have employed immunosuppressed animal models. Cyclophosphamide-induced neutropenia in mice enhances *S. aureus*-mediated soft tissue infections and highlights the intricate interplay between host immunity and microbial pathogenesis.[21] These models are valuable for dissecting immune evasion mechanisms.

Nevertheless, overly profound immunosuppression may exaggerate pathogen virulence, limiting the model's generalizability to immunocompetent hosts. Future models should consider incorporating mild or disease-relevant immunosuppressive conditions and coexisting host factors, such as metabolic dysfunction or trauma to more accurately reflect clinical NF risk profiles.

Genetically Engineered Models

Genome editing technologies such as CRISPR/Cas9, TALENs, and ZFNs have enabled the development of NF models with enhanced susceptibility to infection. For example, transgenic mice expressing human CD46, a complement regulatory protein, exhibit accelerated *S. pyogenes* dissemination and severe NF, implicating CD46 in host-pathogen interactions.[26]

These models provide valuable insight into genetic determinants of NF susceptibility and support personalized therapeutic development. However, the high cost, technical demands, and ethical considerations, especially when human genes increase disease severity, present significant barriers. Moreover, single-gene modifications may not capture the multifactorial nature of NF. Integration with humanized immune systems or multi-omics profiling may help bridge this gap and enhance translational relevance.

Optical Imaging-Integrated Models

Non-invasive imaging technologies such as bioluminescence imaging (BLI) and fluorescence reflectance imaging (FRI) allow real-time tracking of infection dynamics in vivo. For instance, *lux*-tagged *S. aureus* introduced into murine gluteal muscle permits longitudinal monitoring of bacterial proliferation and therapeutic response.[51] BLI also enables differentiation between acute and chronic infection states based on metabolic activity.

Despite these advantages, limitations in tissue penetration and spectral resolution restrict their accuracy in large animals or deep-seated infections. Additionally, the use of genetically modified pathogens may not fully recapitulate natural infection complexity. Future efforts should focus on integrating high-resolution imaging with more clinically relevant models to improve translational fidelity.

Comparative Translational Value of NF Animal Models

Rodents, zebrafish, rabbits, pigs, and non-human primates each offer distinct advantages for modeling NF, yet their translational relevance varies across different pathophysiological dimensions.

Small rodents, particularly humanized mice, excel in dissecting immune mechanisms, such as neutrophil extracellular trap (NET) formation, cytokine dynamics, and host-pathogen interactions, due to the availability of genetically engineered strains and CRISPR-mediated host factor modifications. Zebrafish models provide high-resolution visualization of bacterial dissemination and biofilm formation, enabling rapid, high-throughput screening of therapeutic interventions. Medium to large animals, including rabbits and pigs, better recapitulate systemic spread and organ-level pathophysiology, owing to their closer anatomical and hemodynamic similarity to humans. Non-human primates offer the highest translational fidelity for complex immune responses and multi-organ involvement, although ethical and logistical constraints limit their routine use.

By selecting models based on research goals such as immune mechanisms, systemic dissemination, or biofilm persistence, investigators can optimize translational value and guide clinical translation using CRISPR-engineered host factor studies, AI-driven therapeutic predictions, and organ-on-chip validation platforms.

Pathophysiological Mechanisms in NF Animal Models

Group A Streptococcus (GAS)-Mediated NF

GAS-induced NF is driven by a triad of bacterial virulence determinants, dysregulated host immunity, and microenvironmental remodeling (Figure 2 and Table 2).

Virulence Mechanisms

GAS employs M proteins (M1, M3, M6) to adhere to extracellular matrix (ECM) components and host integrins, facilitating epithelial invasion and biofilm formation.[52,53] M1 further enhances bacterial aggregation, amplifying tissue colonization.[54] Streptolysins O (SLO) and S (SLS) induce cytolytic pore formation, disrupting membrane integrity and triggering cytoplasmic leakage.[55] SLO synergizes with nicotinamide-adenine dinucleotidase (NADase) to deplete intracellular NAD+, impairing energy metabolism and inducing Golgi fragmentation, which exacerbates cell death.[56,57] NADase additionally subverts autophagy by inhibiting lysosome-autophagosome fusion, prolonging

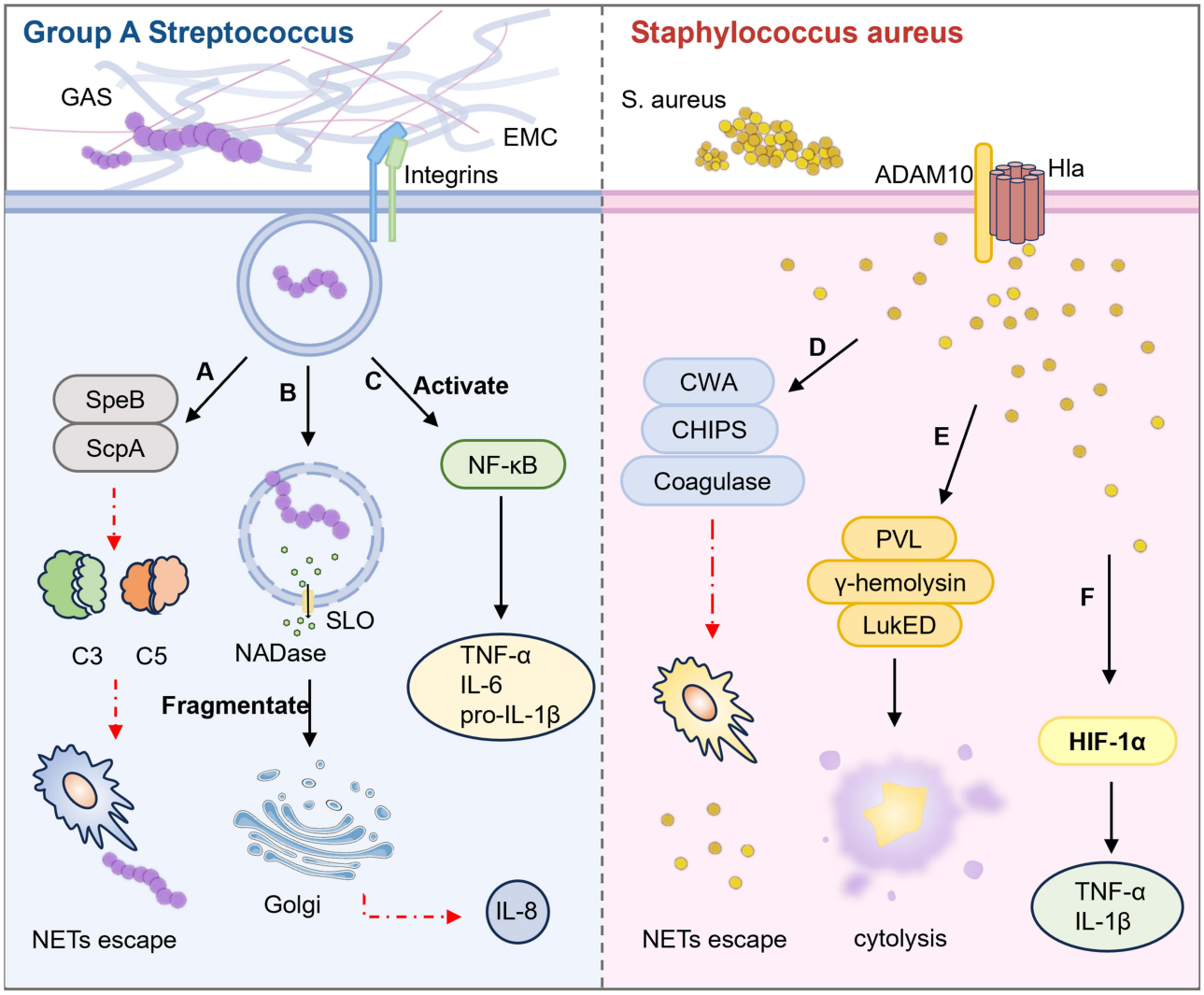

Figure 2 Pathophysiological Mechanisms in NF Animal Models. (A) GAS evades the immune system by degrading C3 and C5 through the action of proteases SpeB and ScpA. (B) SLO and NADase induce Golgi fragmentation and suppress IL-8 secretion. (C) GAS activates the NF-κB signaling pathway, leading to the upregulation of proinflammatory cytokines TNF-α, IL-6, and pro-IL-1β. (D) CWA proteins and coagulase help the bacteria evade host innate immune damage, while CHIPS blocks formyl peptide receptors on neutrophils, paralyzing chemotaxis and allowing immune evasion. (E) PVL, γ-hemolysin, and LukED lyse leukocytes and macrophages, paralyzing the phagocytic clearance function. (F) HIF-1α upregulates pro-inflammatory cytokines TNF-α and IL-1β. (PowerPoint).

intracellular GAS survival.[58,59] Concurrently, GAS metabolism consumes tissue oxygen and degrades H_2O_2, creating a hypoxic niche that promotes anaerobic co-infections and accelerates necrosis.[60]

Immune Evasion Strategies

GAS sabotages innate immunity through proteases SpeB and ScpA. SpeB inactivates complement C3b and cleaves chemotactic peptides (C3a, C5a), while ScpA degrades interleukin-8 (IL-8), collectively suppressing neutrophil recruitment.[61–65] This dual protease activity establishes an immune-privileged microenvironment conducive to bacterial proliferation.

Table 2 Comparative Pathophysiology: GAS vs *S. aureus*

Feature	GAS	*S. aureus*
Primary Toxins	SLO, SLS, NADase	Alpha-toxin, PVL, LukED
Immune Evasion	SpeB/ScpA-mediated complement inhibition	CHIPS-mediated chemotaxis suppression
Microenvironment	Hypoxia-driven polymicrobial synergy	Hypoxia-metabolic acidosis feedback loop
Therapeutic Target	NADase inhibitors	Alpha-toxin monoclonal antibodies

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Microenvironmental Dysregulation

GAS activates NF-κB signaling, upregulating pro-inflammatory cytokines (TNF-α, IL-6, pro-IL-1β) that drive vasodilation, vascular hyperpermeability, and plasma extravasation.[66–68] Concomitant activation of the coagulation cascade induces microthrombosis in dermal capillaries, exacerbating tissue ischemia.[69] Bacterial toxins and inflammatory mediators synergistically damage vascular endothelia, culminating in fibrinoid necrosis of arterioles and venules.[70] This hypoxic, nutrient-deprived milieu fosters bacterial overgrowth and cytotoxic metabolite accumulation, perpetuating a vicious cycle of necrosis and systemic inflammation.

Staphylococcus Aureus-Mediated NF

S. aureus NF is characterized by rapid tissue destruction and immune dysregulation, often progressing to toxic shock (Figure 2 and Table 2).

Toxin-Driven Pathogenesis

Alpha-toxin (Hla) activates ADAM10 to cleave E-cadherin and vascular endothelial (VE)-cadherin, disrupting epithelial/endothelial barriers and promoting bacterial dissemination.[71–76] Pore-forming toxins (PVL, γ-hemolysin, LukED) lyse leukocytes and macrophages, crippling phagocytic clearance.[77–80] Coagulase and von Willebrand factor-binding protein (vWBP) induce microthrombi, facilitating ischemic necrosis.[81]

Immune Subversion

Cell wall-anchored (CWA) proteins (eg, clumping factor A) mediate host matrix adhesion while evading opsonization.[82,83] Chemotaxis inhibitory protein (CHIPS) blocks formyl peptide receptors on neutrophils, paralyzing chemotaxis.[81,84]

Host-Pathogen Feedback Loop

Toxin-induced vascular damage reduces perfusion, creating hypoxic foci where bacterial metabolic byproducts (eg, lactate) accumulate. Hypoxia-inducible factor 1α (HIF-1α) upregulates pro-inflammatory cytokines (TNF-α, IL-1β), amplifying tissue damage despite neutrophil infiltration.[85–87] In immunocompromised hosts (eg, diabetics, corticosteroid users), impaired neutrophil extracellular trap (NET) formation and macrophage dysfunction accelerate systemic spread.[88,89]

Future Directions and Innovations in NF Animal Models

To bridge the translational divide in NF research, the next generation of animal models must go beyond conventional frameworks. This review offers a forward-looking perspective by integrating emerging technologies and cross-disciplinary strategies that can reshape the field. Below, we outline key innovation pathways and propose directions that reflect a paradigm shift in NF modeling.

Enhanced Pathogen Complexity and Immune Humanization

Traditional NF models often rely on single-pathogen infections, underrepresenting the polymicrobial synergy characteristic of clinical NF. Future models should incorporate polymicrobial consortia, for instance, *Streptococcus pyogenes* with anaerobes such as *Clostridium perfringens*, to more accurately replicate the microbial dynamics driving extensive tissue damage.[49] At the same time, the introduction of humanized immune systems, such as transgenic mice expressing human cytokines (eg, IL-8/CXCL8) or specific HLA alleles, can help bridge species-specific immune differences. Models like NSG-SGM3 mice reconstituted with human hematopoietic stem cells enable functional interrogation of human-like immune responses, such as neutrophil extracellular trap (NET) formation during NF progression.[90,91] While these models offer substantial translational promise, they still fall short of fully capturing the complexity of human immunity and comorbid conditions.

Advanced Imaging and Multi-Omics Integration

The convergence of high-resolution imaging modalities and multi-omics profiling represents a transformative step in NF modeling. Technologies such as intravital microscopy and PET-MRI fusion allow real-time visualization of bacterial dissemination, immune cell trafficking, and treatment response in living hosts.[92,93] Meanwhile, single-cell RNA sequencing and spatial transcriptomics can unravel pathogen-specific transcriptional landscapes in infected tissues, providing unprecedented insight into host-pathogen interactions and potential therapeutic targets.[94] These tools not only enhance model granularity but also support data-rich, systems-level interpretations that were previously unattainable.

Precision Therapeutics and Resistance Mitigation

Addressing antibiotic resistance in NF demands models that support mechanistically driven drug development. Recent advances include monoclonal antibodies targeting virulence factors such as *S. aureus* α-toxin (Hla) or *GAS* NADase, both of which have demonstrated efficacy in preclinical settings.[95,96] Furthermore, immunotherapeutic approaches such as CAR-T cells and immune checkpoint inhibitors (eg, PD-1 blockade) may offer novel routes to restore immune competence in immunocompromised hosts.[97] Targeted drug delivery systems, like pH-responsive liposomes carrying DNase I, can disrupt biofilms and enhance antibiotic penetration, providing a strategic advantage in tackling persistent infections.[98]

AI-Driven Model Optimization

Artificial intelligence (AI) is emerging as a pivotal tool for refining NF research by integrating multi-omics datasets, CRISPR-engineered host models, and organ-on-chip platforms into a unified predictive framework. Machine learning algorithms can analyze large-scale transcriptomic, proteomic, and microbiome data derived from both CRISPR-modified animals and organ-on-chip systems to predict disease trajectories and therapeutic responses.[99,100] Deep learning frameworks are also being applied to automate quantification of necrotic areas in histological or imaging datasets, thereby reducing observer bias and improving standardization.[101,102] Additionally, generative AI is now capable of designing candidate small molecules that target conserved bacterial structures, such as streptolysin O pore domains.[103] By linking mechanistic insights from genetically precise animal models with high-throughput, human-relevant organ-on-chip data, AI provides a data-driven, iterative platform that accelerates therapeutic discovery, optimizes experimental design, and enhances the translational fidelity of NF models.

Translational Pipeline Integration

Animal models remain indispensable to the NF research pipeline, as they effectively recapitulate key clinical features, such as neutrophilic infiltration, cytokine surges (eg, IL-6, TNF-α), and extensive necrosis, that are consistent with patient tissue samples and systemic biomarker profiles.[22,23,26,33,42] New platforms like organ-on-a-chip systems using patient-derived tissues are helping to bridge preclinical and clinical domains by simulating human-like tissue environments under controlled conditions.[104] International initiatives, such as the FNIH Biomarkers Consortium, are further accelerating progress by promoting standardization, open-access data sharing, and cross-institutional reproducibility.[98]

Beyond scientific validity, the feasibility of NF models is strongly influenced by resource demands and laboratory infrastructure. Rodent models are generally cost-effective and can be implemented in standard biosafety level 2 (BSL-2) facilities, making them accessible to a wide range of laboratories. In contrast, large-animal models, such as pigs or rabbits, require advanced surgical capacity, anesthesia monitoring, and specialized housing conditions, thus limiting their availability to highly equipped centers. Next-generation approaches, including humanized systems, intravital imaging, and microfluidic organ-on-chip platforms, demand substantial initial infrastructure and technical expertise, but hold the promise of improving reproducibility and reducing dependence on live animals over time.

Several therapeutic strategies validated in animal models have already informed clinical practice. For instance, HBOT demonstrated tissue-protective effects in preclinical models, with meta-analyses suggesting potential benefit in selected NF subgroups.[105,106] Similarly, IVIG shown in murine models to neutralize streptococcal superantigens,[107] has progressed into clinical evaluation, though retrospective studies suggest that its therapeutic impact in NF may be limited.[108] More recently, bacteriophage therapy, initially explored in zebrafish and murine models against multidrug-resistant *Staphylococcus aureus*, has

advanced into early-phase clinical applications, though its long-term safety and efficacy require further validation.[109,110] These examples illustrate both the translational promise and the ongoing controversies surrounding NF adjunctive therapies, highlighting the critical role of animal models in guiding innovation from bench to bedside while underscoring the need for rigorous clinical trials.

Ethical Considerations in NF Animal Research

Animal models have been instrumental in advancing our understanding of NF pathophysiology and in evaluating therapeutic candidates. However, the use of models that induce rapidly progressing and highly painful infections brings substantial ethical challenges. Ensuring humane treatment and minimizing suffering is essential not only from an ethical standpoint but also for maintaining scientific rigor and validity.

All animal experiments cited in this review were conducted under protocols approved by Institutional Animal Care and Use Committees (IACUC) or equivalent ethical oversight bodies, and in accordance with relevant regulations such as the ARRIVE and NIH guidelines. Moving forward, NF research should rigorously adhere to the 3Rs principle, Replacement, Reduction, and Refinement, to ensure responsible and ethical use of animal models.

Innovative alternatives such as organoids, organ-on-a-chip systems, and computational modeling are being increasingly explored to reduce reliance on live animals.[111–114] These platforms offer unique advantages for high-throughput screening and mechanistic investigations, especially in the early stages of research. However, current limitations, such as the inability to simulate systemic immune responses, polymicrobial infection dynamics, and multi-organ involvement, restrict their capacity to fully replace in vivo models. Additionally, these systems have not yet achieved widespread validation for NF-specific applications.

Thus, while these emerging tools provide valuable supplementary strategies, they cannot yet supplant animal models in NF research. A hybrid approach that integrates refined animal experiments with advanced in vitro and computational models may provide the most ethical and scientifically robust strategy.

Conclusions

Although current animal models cannot fully replicate the complexity of human NF, they remain indispensable for elucidating disease mechanisms and guiding therapeutic innovation. Comparative analyses show that rodents excel in dissecting immune mechanisms and host-pathogen interactions, zebrafish enable high-throughput visualization of bacterial dissemination and biofilms, medium-to-large animals recapitulate systemic spread and organ-level pathology, and non-human primates provide the highest fidelity for complex immune responses and multi-organ involvement.

The integration of CRISPR-engineered host factors, multi-omics profiling, organ-on-chip platforms, intravital imaging, and AI-driven analytics is poised to transform NF modeling across multiple dimensions: (1) development of clinically relevant polymicrobial and humanized models that faithfully mirror infection dynamics and host responses; (2) real-time monitoring of disease progression and therapeutic efficacy through imaging and microfluidic platforms; and (3) mechanistically guided interventions, including targeted antibodies, immunotherapies, and biofilm-disrupting delivery systems, validated in preclinical studies to improve translational outcomes.

To translate these advances into clinical benefit, future efforts must emphasize standardized model validation, transparent data sharing, and ethical refinement of experimental design. Collectively, these innovations elevate NF animal models from descriptive tools to predictive, mechanistically informed platforms, enabling precision therapies and offering a tangible path toward reducing NF-associated morbidity and mortality.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically

reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests.

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