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Zebrafish as a new approach methodology (NAM) in dermatology: current research and the regulatory landscape focusing on sustainable development goals

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Abstract

The conventional dependence on mammalian models in dermatological research is increasingly subject to ethical and sustainability-focused scrutiny, prompting a transition toward new approach methodologies (NAMs). Within this paradigm, zebrafish (*Danio rerio*) have surfaced as a persuasive alternative due to their genetic and physiological congruence with human skin, optical clarity during early developmental stages, accelerated life cycle, prolific reproductive capacity, and economically viable maintenance. This review describes recent research using zebrafish models in dermatology, including skin pigmentation disorders, barrier function, wound healing and regeneration, pharmacological discovery, genetic and inflammatory skin conditions, and melanoma. By examining the research, ethical, and logistical merits of zebrafish NAMs, this review emphasizes their contribution to fostering more humane and efficient research frameworks. Such integration also aligns with various United Nations Sustainable Development Goals (SDGs) 3, 9, 12, 13, 14, and 17, particularly regarding the accessible biomedical innovation in low- and middle-income countries (LMICs). The review also recognizes ongoing challenges, such as regulatory fragmentation and inconsistent validation of dermatological endpoints, which impede widespread translational acceptance. It concludes by presenting strategic recommendations for international collaboration, targeted financial support, cross-sector partnerships, and regulatory harmonization to realize the potential of zebrafish-based NAMs in dermatological research.

Keywords: zebrafish, dermatology, new approach methodologies, sustainable development goals, low- and middle-income countries

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Introduction

For centuries, animal models have been the foundation of biomedical research, dating back to ancient Greece (1). In dermatology, rodent models such as mice and guinea pigs, including their transgenic versions, have been used to mimic specific human skin diseases, study complex disease mechanisms, and evaluate the safety of chemicals and cosmetics, and also as an aid in discovering new therapeutic agents (2). This long-standing dependence is due to their physiological similarities to humans and the ability to modify their genetics to resemble human conditions.

However, the widespread and ongoing use of these traditional animal models is increasingly under intense scrutiny (3). This growing awareness arises from recognizing their inherent limitations and serious ethical concerns. From a scientific standpoint, a major challenge is the gap in translation between results from animal studies and human clinical outcomes. For example, comparative transcriptome analyses show that only about 30.2% of skin-related genes are the same between human and mouse skin, which may explain some difficulties in applying mouse study results to humans (4). In addition to biological differences, traditional mammalian models also involve high operational costs, long development times, and logistical challenges of maintaining large animal colonies (5). These factors hinder the efficiency and scalability of research.

The ethical dimension acts as a strong catalyst for change. Society's expectations and scientific principles support humane treatment of animals in research, summarized by the 3R framework: replacement, reduction, and refinement (6). The ongoing dependence on large numbers of mammals for preclinical testing, especially for conditions that may not fully apply, raises significant ethical concerns. This mix of scientific limitations, economic pressures, and ethical issues leads to a natural shift in perspective. This shift has become prominent lately: in July 2025, the U.S. National Institutes of Health (NIH) announced a complete departure from animal-exclusive projects (7). This move aligns with an NIH initiative, first unveiled in April, to prioritize human-based research technologies (8). Such a push for more effective, predictive, and ethically responsible research tools is not just about finding alternatives but about creating better methods that tackle systemic problems in research. This dual demand speeds up the adoption of new approach methodologies (NAMs), viewing them not only as replacements but as more advanced tools designed for specific research questions for which high throughput capacity and ethical concerns are key (9).

New approach methodologies

NAMs are a broad set of complementary alternatives to traditional animal-based research, with the potential to even replace the need for animal models in certain research contexts (10). Technically, they cover a diverse and evolving range of research approaches, broadly categorized to provide a deliberate tiered framework for toxicity and hazard assessment (Fig. 1). Simpler, faster, and more cost-effective methods (such as *in silico* and *in chemico*) can be used as initial high-throughput screens. Compounds that clear these

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early filters can then advance to more biologically relevant *in vitro* models. As such, zebrafish are increasingly seen as a bridge between *in vitro* assays and more complex mammalian *in vivo* studies, helping de-risk drug candidates and decrease the number of mammals needed in later stages (11). This tiered integrated approach optimizes resource use, accelerates decision-making, and steadily decreases dependence on higher-order animal models (12). This strategy enhances both scientific efficiency and ethical standards, supporting a more sustainable research approach. As such, NAMs are not meant to be isolated replacements but instead integrated components of a comprehensive testing pipeline (13).

In addition to supporting the 3R principles, the motivations behind adopting NAMs are diverse. These include the ability to uncover basic mechanisms in complex biological systems, improve scientific hypotheses with greater accuracy, and allow scalable testing across various input variables such as toxins or drugs (14). In addition to these advantages, NAMs play a key role in advancing scientific and technological frontiers, thereby speeding up the understanding of how toxic substances affect both human health and ecosystems (15).

Zebrafish as an animal alternative model

Among the various NAMs, the zebrafish (*Danio rerio*), a small freshwater vertebrate, has become a compelling and versatile alternative model for *in vivo* studies (16). Its growing prominence in biomedical research is due to a unique combination of biological features that overcome many limitations of traditional mammalian models. Zebrafish models can notably decrease the number of mammals needed in later stages of preclinical development, thus optimizing resource use and enhancing the overall success rate of translating new therapies. This strategic role is key to promoting sustainable drug discovery. The incorporation of zebrafish as NAMs into dermatological research significantly contributes to achieving various United Nations Sustainable Development Goals (SDGs). These benefits go beyond scientific progress, including ethical, economic, and environmental aspects, establishing zebrafish as a genuinely sustainable research model (Supplementary Appendix 1).

A key advantage of zebrafish is their remarkable genetic similarity to humans. About 70% of human genes have at least one ortholog in zebrafish, and this homology increases to nearly 87% for genes linked to human diseases (17). This high level of genetic conservation means that disease mechanisms and therapeutic targets found in zebrafish are often directly applicable to humans. In addition to genetic similarities, zebrafish offer unique experimental benefits. Their embryos and larvae are transparent, allowing real-time, non-invasive observation of cellular and molecular events within a living organism (18). This optical clarity makes it easier to observe dynamic processes such as tumor development, blood vessel formation, metastasis, and immune responses, which are difficult to study *in vivo* in opaque mammalian models without invasive procedures (19).

The rapid life cycle and high fecundity of zebrafish further improve their usefulness. They develop quickly, with major organ formation completed within 48 hours post-fertilization (hpf), and larvae become free-swimming by 5 to 6 days post-fertilization (dpf) (16). A single breeding pair can produce hundreds of eggs each week, providing large groups for statistically reliable experiments. These traits, along with their small size and low maintenance costs, make zebrafish much more economical than mammalian models, needing less space and resources.

Zebrafish research models offer significant social and economic benefits through their affordability and accessibility, especially for low- and middle-income countries (LMICs). The much lower costs for acquisition and upkeep, combined with their high fecundity and small size, create a strong opportunity for LMICs to develop and maintain high-quality biomedical research programs that would otherwise be too expensive with mammalian models. This economic benefit helps reduce disparities in research capabilities between high-income countries and LMICs, promoting equitability across the global scientific community (16).

Comparative analysis of zebrafish and human skin structures

A comparative analysis of zebrafish and human skin structure shows both important similarities and notable differences, guiding strategic use in dermatological research (20). Zebrafish skin is structurally divided into distinct epidermal and dermal layers, a key similarity with mammalian skin. The epidermis has multiple layers of keratin-producing cells (KCs), whereas the dermis con-

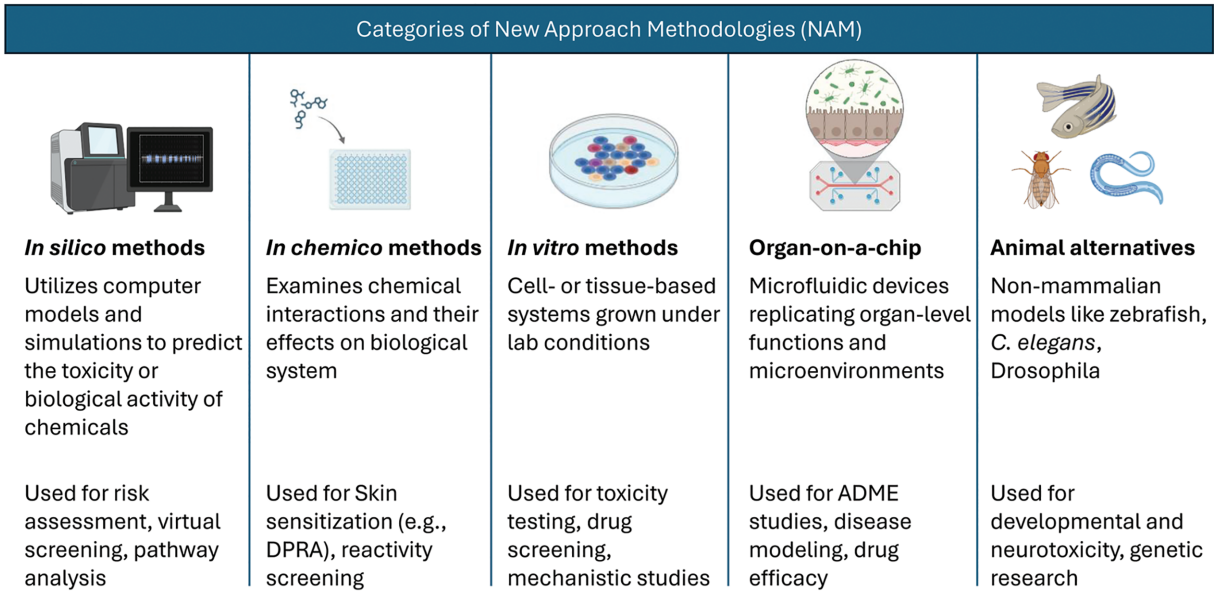

Figure 1 | Various categories of new approach methodologies (NAMs). The figure shows four types of NAMs with a brief description of each type and their use.

tains a varied mix of fibroblasts, KCs, pigment cells, and immune cells (21). Zebrafish skin also contains large amounts of keratin, a major structural protein in human skin (22). A clearly defined cutaneous basement membrane zone (BMZ) appears by 32 hpf and is fully formed by 48 hpf (23). This BMZ consists of collagens (types IV, VII, XII, and XIV) and other essential proteins such as laminin, α3-integrin, α6-integrin, and Fras1, all of which are vital for supporting and binding the dermal-epidermal junction. Moreover, gene expression patterns in zebrafish skin show a set of genes that are also found in developing human skin. Examples are keratin 1 (*KRT1*), keratin 5 (*KRT5*), 230-kDa bullous pemphigoid antigen (*BPAG1*), plectin (*PLEC*), type IV collagen subunits (*COL4A1–COL4A6*), collagen VII (*COL7A1*), and collagen XVII (*COL17A1*) (24, 25). This demonstrates the preservation of fundamental developmental pathways (22).

Despite these similarities, important structural differences exist. Unlike human epidermis, zebrafish epidermis is non-keratinized and does not form a stratum corneum, the outermost protective layer with barrier functions similar to human skin (22). This is reflected in the absence of genes (encoding proteins) such as filaggrin (*FLG*), involucrin (*IVL*), and trichohyalin (*TCHH*) in the zebrafish genome, indicating a lack of terminal differentiation pathways found in mammals (26). In addition, zebrafish lack mammalian appendages such as hair follicles and sebaceous glands. Instead, they have specialized aquatic structures, including mucous-secreting cells and the lateral line system, which contains mechanosensory neuromast hair cells not present in human skin (22).

Practical applications of zebrafish in dermatological research and insights

Skin pigmentation disorders

Various approaches have been used to induce pigmentation disorders in zebrafish, including physical, chemical, and genetic methods (Supplementary Appendix 2). These different strategies provide not only experimental flexibility but also relevance to the human pigmentation spectrum and related dermatological conditions.

Physical induction methods, especially ultraviolet (UV) radiation, are widely used to model hyperpigmentation. Repeated ultraviolet B (UV-B) exposure, such as 300 mJ/cm² daily or 8,100 mJ/cm² intermittently, elicits notable melanogenic responses, including skin darkening, increased melanin production, and hormonal stimulation via alpha-melanocyte-stimulating hormone (α-MSH) and adrenocorticotropic hormone (ACTH) (27). These responses activate signaling pathways such as cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA)/cAMP response element-binding protein (CREB), which are well conserved in mammals (28). Although UV exposure effectively mimics environmental triggers of hyperpigmentation and allows real-time observation of response patterns, its reproducibility can be influenced by factors such as light source intensity and larvae positioning, requiring strict standardization. In addition, pulsed electromagnetic fields (PEMFs), delivered through Helmholtz coils (60 Hz, 2–20 G for 5 to 15 days), offer a non-invasive innovative method for inducing pigmentation (29). These fields enhance the expression of key melanogenic genes such as *TYRP1*, *DCT*, *MC1R*, and *MITF*, and affect extracellular signal-regulated kinase (ERK) and p38 mitogen-activated protein kinase (MAPK) pathways (29). However, their precise mechanism of action and long-term biological effects are not yet fully understood, necessitating further research before wider application.

Chemical induction methods have gained attention due to their ease of use and scalability. Small molecules such as serotonin (5-hydroxytryptamine), fisetin, and flumequine consistently increase melanin content through known melanogenic pathways (30). For example, fisetin activates microphthalmia-associated transcription factor (MITF) by inhibiting glycogen synthase kinase-3 beta (GSK-3β), and flumequine boosts mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinase (JNK) phosphorylation, mimicking overactivation of melanocytes seen in inflammatory or drug-induced pigmentation disorders (31, 32). These models offer the advantage of dose-dependent adjustable responses and are suitable for large-scale screening. However, variability in compound solubility, stability in aquatic systems, and potential systemic toxicity may affect results if not carefully managed. The use of multi-component plant extracts, such as Epimedii Folium extract (EFE) or rice bran ash mineral extract (RBM), introduces greater biological complexity (33). These substances have shown effectiveness in modulating tyrosinase activity and melanin deposition via MAPK/ERK mechanisms, but their undefined composition may limit mechanistic understanding and reproducibility between laboratories. Although they are promising for natural product discovery, such models require efforts in extract standardization and compound characterization to meet regulatory standards for reproducibility in NAMs.

Genetic approaches provide powerful tools for studying the molecular regulation of pigmentation and for modeling genetic pigmentation disorders and melanoma. Transgenic zebrafish expressing oncogenic *HRAS* (Harvey rat sarcoma viral oncogene homolog) in kita-GFP lines exhibit melanocyte hyperproliferation and disrupted pigment patterns by 3 dpf, with melanoma development under specific conditions (34). This model closely mimics human melanoma progression, especially when copper-dependent pathways and p53 activity are manipulated (35). Its strength lies in its genetic stability and usefulness in long-term tumor studies. However, it is less suitable for rapid compound screening due to the time needed for tumor development.

In another experiment, injection of human uveal melanoma cells with GNA11 or GNAQ mutations into Tg(fli1:EGFP) embryos created xenograft models of ocular and cutaneous melanoma, which are valuable for testing anti-tumor and anti-pigment therapies *in vivo* (36). Although they offer translational relevance, these models require specialized infrastructure and expertise in microinjection and imaging (37). More recently, clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9 (CRISPR-Cas9) mediated knockout of *bmp7b* has generated zebrafish with systemic hyperpigmentation and retinal melanin overproduction, uncovering new gene candidates (*wnt7ba*, *gna14*, and *erbb3b*) involved in pigment regulation (38). This method provides exceptional precision but demands careful validation of off-target effects and may cause developmental pleiotropy, complicating interpretations related to pigmentation.

Assessing pigmentation changes in zebrafish involves both imaging-based and biochemical methods, each with its own advantages and limitations (39). During early larval stages, the zebrafish's natural transparency allows non-invasive observation of melanin distribution with light or fluorescence microscopy. Image analysis tools such as ImageJ (NIH, public domain) make possible high-throughput, visually straightforward quantification. However, these methods can be affected by lighting conditions,

imaging resolution, and subjective thresholding (40). Biochemical methods supplement imaging by measuring melanin content and tyrosinase activity. The melanin production assay assesses absorbance at 405 nm after pigment extraction, and the tyrosinase activity assay measures absorbance at 475 nm from the supernatant (41). These are more objective and useful for confirming pigmentation patterns seen in imaging, but they require terminal sample processing, preventing longitudinal studies in the same fish.

Beyond methodological considerations, several studies have significantly improved the understanding of zebrafish pigmentation biology. The patterning of pigment cells such as melanophores (black), xanthophores (yellow), and iridophores (iridescent) follows principles consistent with Turing reaction–diffusion models, allowing real-time analysis of self-organizing systems (42). Importantly, the pigment pattern in zebrafish is not fixed; instead, it can regenerate remarkably after cellular ablation or genetic disruption. This regenerative ability allows the study of cellular interactions, lineage plasticity, and pigment pattern reestablishment, all highly relevant to conditions such as vitiligo, post-inflammatory hypopigmentation, and pigmentary mosaicism in humans (43). Comparative genetic analysis further shows that zebrafish *slc24a5*, a pigmentation gene controlling sodium–calcium exchange, corresponds to human alleles affecting ethnic skin tone variation, reinforcing the translational potential of zebrafish studies (44). In addition, UV-B–induced pigmentation responses are mechanically linked to oxidative stress and inflammation, with reactive oxygen species (ROS) serving as key mediators. Novel therapeutic agents, such as polyethylene glycol –modified nano-selenium (Nano-Se), have demonstrated anti-pigmentation effects in zebrafish by reducing ROS and modulating downstream pathways, highlighting the usefulness of the model in screening redox-based skin-lightening agents (45).

Skin barrier function studies

The transparent epidermis of developing zebrafish offers an accessible and genetically manageable model for studying epithelia *in vivo* (46). Advanced tools have been created to fluorescently label specific epithelial cell types, including periderm, basal cells, fibroblasts, and ionocytes, and to express genes in a mosaic pattern using Gal4 lines from enhancer trap screens (47). This ability allows for precise tracking, targeted cell removal, or real-time observation of single cells at subcellular resolution within the living organism (48). In addition, the use of photo-cleavable morpholino oligonucleotides targeting Gal4 enables researchers to control mosaic gene expression with precise timing during development (46). Gene function in the epidermis can be thoroughly examined using various methods, such as mutations, morpholinos, and chemical inhibitors (49). Although the zebrafish epidermis lacks a true stratum corneum, it contains specialized cell types that facilitate the exchange of oxygen, ions, and macromolecules. These exchanges function similar to exchanges performed by mammalian bilayered epithelia (26).

However, certain limitations exist. For instance, the absence of genes crucial for terminal differentiation and stratum corneum formation in zebrafish restricts the study of some human epidermal disorders that are primarily linked to these specific barrier functions. In addition, zebrafish skin lacks mammalian appendages such as hair follicles and sebaceous glands, further limiting the applicability of zebrafish for conditions related to these structures (25).

Cutaneous wound healing and regeneration

Experimental induction of skin wounds in zebrafish usually involves two main models: full-thickness flank wounds and tail fin amputation. Full-thickness wounds, about 2 mm across, are made using dermatology lasers such as the Erbium:YAG MCL29 Dermablate set at pulse energies of 500–600 mJ and 5 Hz frequency (50). These wounds remove both the outer and inner skin layers, including subcutaneous fat cells and scales, without harming the underlying muscle. This model simulates complex human skin wounds and allows detailed observation of skin healing and tissue reconstruction. However, it requires specialized tools and skills, which may limit its use in high-throughput or resource-limited settings. On the other hand, the tail fin amputation model is more common because it is simple and reproducible (51). It supports strong studies on white blood cell behavior, inflammation healing, and skin regrowth. The see-through nature of the zebrafish fin allows live imaging of cell actions after injury, but because it lacks layered skin and appendages it may be less similar to human skin.

One of the most remarkable features of zebrafish wound healing is the nearly complete absence of scarring, even after severe injury (50, 52). Re-epithelialization starts quickly and occurs independently of both inflammation and fibroblast growth factor (FGF) signaling (52, 53). Barrier function is restored by 12 hours post-wounding (hpw), and complete epidermal coverage is achieved by 24 hpw, as confirmed by methylene blue assays. By 28 days post-wounding (dpw), all elements of the original skin structure—epidermis, dermis, pigmentation, and scales—are fully restored (54, 55). This regenerative outcome starkly contrasts with the typical mammalian response, in which strong inflammation often results in fibrotic scar tissue. This difference challenges the common belief that inflammation naturally causes scarring. However, in zebrafish, inflammation is either tightly controlled, short-lived, or influenced by pro-regenerative signals that prevent fibrosis (56). Understanding this difference is essential for translational efforts to shift mammalian healing from mere repair toward genuine regeneration.

Mechanistically, zebrafish heal without scars through coordinated cell actions such as elongation and radial intercalation, which are controlled by Rho/ROCK and transforming growth factor (TGF) signaling pathways (57). Several conserved molecular cascades activate after injury. FGF signaling, although not essential for re-epithelialization in zebrafish, aids in angiogenesis, showing partial conservation with mammalian granulation tissue formation. H_2O_2 creates a tissue-scale gradient that facilitates rapid leukocyte recruitment to wound sites, peaking approximately 20 minutes post-injury (58). This ROS serves as a chemoattractant, enhancing the innate immune response and promoting the migration of immune cells to areas of injury (59). H_2O_2-mediated signaling especially involving epidermal growth factor (EGF), forkhead box protein O1 (FOXO1), and IκB kinase alpha (IKKα) has been linked to guiding immune cells to wound sites and supporting sensory axon repair, while also providing cytoprotection. Through the activation of extracellular signal-regulated kinases 1 and 2 (ERK1/2) and EGF receptor, the EGF pathway promotes DNA synthesis and keratinocyte proliferation, speeding up wound closure (60). In addition, the Wnt/β-catenin pathway is quickly upregulated, encouraging dermal cell growth and tissue remodeling by increasing nuclear β-catenin levels and activating cell cycle genes (57, 61).

The identification of these signaling axes highlights that zebrafish healing is not a passive or simple process, but a highly coordinated and regulated response. Importantly, many of these pathways, such as FGF, EGF, Wnt/β-catenin, and TGFβ, are conserved in mammals, yet their functional outcomes vary. This indicates that the "scar-free" phenotype is not caused by entirely new mechanisms but may instead result from differences in timing, strength, or interaction of conserved signaling networks (62). This raises important questions about how these pathways are controlled after injury in zebrafish compared to mammals, and whether their timing could be reprogrammed in mammals to promote a regenerative environment.

Drug discovery for cutaneous wound healing

A variety of pharmacologically active substances, including natural products, nanoparticles, and synthetic drugs, have been tested using zebrafish wound models. *Curcuma longa* extract (CLE) speeds up fin regeneration in adult zebrafish. This is due to CLE's broad-spectrum biological activities, including anti-inflammatory, antimicrobial, antioxidant, and anti-apoptotic effects (63). The CLE in nanomicelles forms improved tail regeneration within 3 days postamputation. Propolis, administered as ethanolic extract (EEP), demonstrates regenerative efficacy even under hyperglycemic conditions, increasing its relevance for diabetic wound models (64). EEP administration in zebrafish larvae exposed to lipopolysaccharide (LPS) resulted in reduced myeloid leukocyte migration and decreased pro-inflammatory cytokine expression like tumor necrosis factor (TNF)-α and interleukin (IL)-1β (65). The down-regulation of complement genes in LPS-challenged zebrafish further supports the anti-inflammatory potential of propolis (65). Similarly, *Clerodendrum cyrtophyllum* has been found to inhibit pro-inflammatory cytokines and eicosanoid signaling, making it a promising anti-inflammatory plant-based therapy. The extract significantly reduced the expression of inflammatory markers such as cyclooxygenase-2 (*COX-2*), phospholipase (*A2PLA2*), and pro-inflammatory cytokines (IL-1s, IL-8, TNF-α) in zebrafish exposed to copper sulfate–induced inflammation (66). These results highlight the usefulness of zebrafish in screening plant-derived compounds that influence key regenerative pathways. However, most studies remain preliminary, with limited dose-response analysis and a lack of standardization in extract compositions. This underscores the need for detailed phytochemical characterization and reproducibility testing before applying these findings.

Nanoparticle-based therapeutics present a new frontier for zebrafish wound healing research. Studies have shown that β-chitosan–derived zinc oxide nanoparticles significantly accelerate wound closure and improve tissue organization by modulating inflammatory responses and promoting collagen deposition (67). The synergistic effect of zinc oxide–cinnamic acid nanoparticles has been highlighted for their antioxidant and antimicrobial properties, leading to faster wound healing without toxicity (68). Silver nanoparticles (AgNPs) have attracted attention due to their antimicrobial properties and their ability to stimulate wound-related mediators such as TGF-β, matrix metalloproteinase (MMP)-13, IL-1β, and antioxidant enzymes (69). AgNPs improve wound closure when applied topically or through immersion. However, their use also highlights a key advantage of the zebrafish model; namely, the ability to detect subtle cytotoxic effects. Studies indicate that, despite initial benefits, AgNPs may impair granulation tissue function and slow fin regeneration, raising concerns about dose-dependent toxicity (70). *Spirulina maxima*–derived pectin nanoparticles (SmPNPs) offer a potentially safer alternative by effectively reducing ROS, enhancing immune modulation, and supporting pigment restoration and neoepidermis formation. Topical application of SmPNPs led to a higher percentage of wound closure (48.9%) compared to control treatments (38.8%) (71). Key wound healing markers, such as TGFβ1 and MMP-9, were upregulated, indicating active tissue regeneration and remodeling. This demonstrates how zebrafish models allow simultaneous assessment of regenerative effectiveness and oxidative stress, facilitating comprehensive therapeutic evaluation.

Genetic skin disorders

The dermal–epidermal BMZ in zebrafish closely resembles that of mammals, and many skin-related genes show high orthology with human counterparts, providing a strong framework for modeling skin diseases at both the structural and molecular levels (Supplementary Appendix 3).

Among genetic skin disorders, epidermolysis bullosa (EB) is a key disease modeled using zebrafish (26). EB includes a group of rare but severe hereditary conditions characterized by mechanical fragility of the skin and mucous membranes, causing blistering after minor trauma (72). Genetic manipulation through morpholino-based knockdown has been used to impair homologous genes in zebrafish, mimicking phenotypes linked to impaired epidermal anchorage (73). Although specific EB models are still being refined, the overall approach highlights the usefulness of zebrafish in exploring genotype–phenotype relationships and discovering new regulators of skin integrity.

Ichthyosis, a diverse group of keratinization disorders characterized by excessive scaling and skin thickening, has also been modeled in zebrafish (74). Severe forms, such as harlequin ichthyosis, are caused by mutations in *ABCA12*, a gene that encodes a lipid transporter essential for stratum corneum formation. Another example is cerebral dysgenesis, neuropathy, ichthyosis, and keratoderma (CEDNIK) syndrome, which is linked to *SNAP29* mutations that interfere with vesicle trafficking. Zebrafish knockdowns of *abca12* and *snap29* using morpholino oligonucleotides cause epidermal defects that resemble ichthyotic skin, including compromised barrier function and abnormal lipid processing (75, 76). These models have shown that different mutations in genes controlling various epidermal pathways can lead to similar disease outcomes. It supports the use of zebrafish as a valuable system for studying the molecular basis of skin disorders with similar phenotypes.

A major advancement in this field is the development of the humanized zebrafish orthologous rescue (HuZOR) technique (77). This method involves creating zebrafish mutants with orthologous genes and testing whether introducing human mRNA variants can rescue the observed phenotype. It allows for *in vivo* functional validation of human polymorphisms or pathogenic mutations. HuZOR has been effectively used in pigmentation disorders (e.g., *slc24a5* and *slc45a2*) and is highly useful for skin disease research. This approach is especially valuable for distinguishing pathogenic from benign variants found through human genome sequencing, and it helps speed up the clinical application of genomic discoveries.

Inflammatory skin conditions

Zebrafish are increasingly recognized as a useful alternative model for studying human inflammatory skin diseases. In the case of psoriasis, zebrafish models have started to reveal the immune mechanisms involved and offer platforms for testing anti-inflammatory treatments. Psoriasis is a chronic immune-driven skin condition marked by keratinocyte hyperproliferation, acanthosis, damage to the epidermal barrier, and an overactive inflammatory response involving cytokines such as TNF-α, IFN-γ, and IL-17. Several zebrafish models aim to mimic aspects of this complex disease. Mutant zebrafish lines, such as those with *Clint1* mutations, exhibit psoriasis-like symptoms, including hyperproliferation and leukocyte infiltration, providing insights into inflammatory processes (78). Similarly, zebrafish models such as pen/lgl2 and Psoriasis/m14 exhibit psoriasis-like phenotypes through keratinocyte hyperproliferation and immune cell infiltration, allowing the study of psoriasis pathogenesis and potential therapeutic targets due to their genetic similarity to humans and versatile experimental applications (79). Chemically, imiquimod, a toll-like receptor (TLR)-7 agonist, has been shown to trigger sensory neuronal responses and itch-like behaviors in zebrafish, mediated by the TRPA1 ion channel (80). However, its ability to fully replicate the epidermal features of psoriasis in zebrafish is not yet well understood. This highlights a limitation in the model's accuracy because many key features of human psoriasis—such as parakeratosis and dermal infiltration—are not fully reproduced in zebrafish (81).

In contrast, copper sulfate ($CuSO_4$) exposure provides a well-established inflammatory zebrafish model. The transgenic Tg(mpx:EGFP) line, which expresses green fluorescent protein in neutrophils, allows for *in vivo* tracking of inflammatory responses (82). When exposed to $CuSO_4$, zebrafish larvae show strong neutrophil recruitment to damaged neuromasts, which can be measured quantitatively. This model has been effectively used to test the anti-inflammatory effects of phytocompounds such as astilbin and the empirical formula of Chinese medicine PSORI-CM02, demonstrating their ability to decrease neutrophil infiltration and promote macrophage-mediated resolution (83). The consistency and clarity of these responses make $CuSO_4$-based assays suitable as high-throughput platforms for screening agents that modulate inflammation (84, 85).

Skin cancer (melanoma)

Zebrafish have emerged as a robust and versatile model for studying melanoma, providing valuable insights into tumorigenesis, cell migration, invasion, and therapeutic responses (86). They allow real-time non-invasive visualization of tumor development and host–tumor interactions, positioning zebrafish as a NAMs-compliant alternative to mammalian models for oncological research, particularly in melanoma biology (87).

A major strength of the zebrafish model is its genetic tractability, which allows the development of transgenic lines that mimic key genetic mutations involved in human melanoma (88). Foundational models have been created for expression of the human *BRAFV600E* oncogene, often mutated in human cutaneous melanoma and *TP53* mutations (89). This results in aggressive pigmented tumors in zebrafish that mirror the histopathology and molecular features of human melanoma. Similarly, *NRAS* Q61K transgenic models have been created to explore the functional roles of *NRAS* mutations, another oncogenic driver in melanoma. Although the authors found the role of *NRAS* insufficient for tumorigenesis, the combined roles played by the loss of functional p53 was found responsible for melanoma (90). These models offer a valuable *in vivo* system for studying how different oncogenic pathways influence the initiation and progression of melanoma.

A notable innovation is the MiniCoopR system, which allows functional analysis of specific genes involved in melanoma development. By inserting a gene of interest under the mitfa promoter into *casper* zebrafish, an unpigmented double mutant, the system allows for both lineage tracing and functional assessment of melanocyte transformation (91). The reappearance of melanin expression upon successful transformation provides a visible indicator. This system has facilitated the identification of SETDB1, a histone methyltransferase, as a powerful promoter of melanoma progression. Likewise, the *crestin*–GFP model, in which the neural crest gene *crestin* is reactivated during the initiation of melanoma, offers a rare opportunity to observe the earliest stages of tumor formation, showcasing the zebrafish's unique capacity to track single-cell transformation *in vivo* (92). The *sox10* overexpression in melanocytes of the model resulted in accelerated melanoma formation.

However, despite their power, these models have limitations. The zebrafish melanocyte lineage differs somewhat from that of humans, and the fish's reliance on water-borne signaling molecules may affect tumor-immune interactions differently from terrestrial vertebrates (88). In addition, although zebrafish tumors mimic many aspects of human melanoma, including invasiveness and heterogeneity, they may not fully reproduce the complex stromal interactions and immune escape mechanisms seen in human skin cancers (93). These differences highlight the need for cautious extrapolation of zebrafish-derived findings to clinical settings.

Current regulatory acceptance of zebrafish NAMs

The transition of bench research to clinical applications through zebrafish-mediated preclinical studies requires regulatory approval. Although not yet universally mandated for all regulatory endpoints, the trend hints at wider global adoption. In this direction, the Organisation for Economic Co-operation and Development (OECD) plays a key role in fostering global harmonization and acceptance of alternative methods through its test guidelines. For example, the OECD's Test Guideline (TG) 236 guides the assessment of acute toxicity of chemicals using zebrafish embryos (94). This test provides valuable data for environmental risk assessment and can inform early human toxicity screening relevant to dermatology.

In the global north, the European Union (EU) has led efforts to promote NAMs (95). Directive 2010/63/EU, which requires the protection of animals used in scientific research, considers zebrafish larvae up to 120 hpf as *in vitro* models, offering a significant benefit for high-throughput screening and early toxicity testing without the full regulatory burden of *in vivo* animal studies (96). In addition, EU Cosmetics Regulation (EC) No. 1223/2009 has implemented a comprehensive ban on animal testing for cosmetics and their ingredients since 2013, encouraging the acceptance of non-animal models, including zebrafish, for general chemical safety and broader research uses that could influence dermatological science (97). Organizations such as the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) play

a key role in validating and supporting these new methods.

Similarly, in the U.S., the landmark U.S. Food and Drug Administration (FDA) Modernization Act 2.0, enacted in 2022, explicitly permits non-animal testing methods in drug approval processes (98). The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) plays a key role in validating and promoting these methods. The FDA is actively working to expand processes for qualifying alternative methods and to provide clear guidelines to stakeholders. It is also promoting the adoption of alternative methods for regulatory use through its New Alternative Methods Program, supported by new funding. This program aims to expand qualification processes and provide clear guidelines for external stakeholders developing alternative methods, including initiatives such as the Innovative Science and Technology Approaches for New Drugs (ISTAND) pilot program for novel nonclinical assays (99). The FDA also accepts alternative methods based on OECD guidelines for certain product types, such as reconstructed human cornea-like epithelium models for eye irritation and 3D reconstructed human epidermis models for primary dermal irritation.

In contrast, countries in Asia are rapidly expanding their use and development of alternative models. However, specific requirements for zebrafish in drug development and dermatological research are still evolving. For example, Japan, which has not yet issued specific regulations, widely accepts OECD-validated *in vitro* methods for cosmetics and chemical safety (100). This suggests the potential use of zebrafish in various applications, including pigmentation studies and basic skin biology. Similarly, India, under the New Drugs and Clinical Trial Rules (2023), specifically permits researchers to use non-animal and human-relevant methods, such as 3D organoids, organ-on-a-chip technologies, and computational models, for testing drug safety and efficacy (101). Although zebrafish are not explicitly listed in all these regulations, their general acceptance within the broader category of "non-animal and human-relevant methods" is implied, especially for early screening and mechanistic studies.

Challenges and recommendations

Although zebrafish models provide many scientific and logistical benefits for dermatological research, several limitations restrict their full translational use (Supplementary Appendix 4). A major challenge is the evolutionary gap between zebrafish and mammals, making it difficult to directly compare certain outcomes, especially those related to epidermal barrier function, sebum production, and skin immune responses (20). In addition, toxicological results from in-water dosing of zebrafish embryos often cannot be reliably applied to mammalian blood levels, making dose translation more complex in clinical toxicology (11).

The dosing methodology also has limitations. Relying on aqueous exposure in zebrafish larvae confines the model to water-soluble compounds, excluding many hydrophobic or poorly soluble drugs commonly used in dermatology (11). Although injection-based methods exist, they are labor intensive and not suitable for high-throughput screening, making them less practical for large-scale drug discovery (102). In addition, the regenerative capacity of zebrafish, although beneficial for tissue repair studies, may complicate the interpretation of long-term toxicological or wound healing results. Their rapid regenerative responses might hide chronic or cumulative effects that are more relevant to humans (11).

A lack of standardization in husbandry, environmental controls, and genetic strain maintenance remains a major obstacle (103). Variability in experimental conditions across laboratories reduces reproducibility and makes it harder to compare results in collaborative or multi-center studies. Unlike mammalian models, which benefit from well-established protocols and extensive control datasets, zebrafish research often lacks detailed reporting of environmental and procedural factors (11). This limits its usefulness in regulatory or clinical settings. Specifically in cancer research, challenges include variability in xenograft engraftment, temperature incompatibilities with human tumor cells, and differences between the zebrafish yolk sac microenvironment and the human tumor niche (104). These influence cell behavior, immune responses, and drug effectiveness.

Importantly, the regulatory landscape for zebrafish-based models remains fragmented. Although international regulatory agencies—such as the OECD, the European Union, and the U.S. FDA—are increasingly accepting zebrafish NAMs, specific guidance on dermatological applications is still limited. The lack of harmonized protocols for key endpoints related to skin diseases, such as inflammatory markers, pigmentation changes, or wound healing metrics, hinders broader regulatory adoption. This fragmentation not only delays validation efforts but also discourages investment in zebrafish-focused dermatology pipelines.

To fully harness the sustainable and equitable benefits provided by zebrafish NAMs in dermatology, a series of coordinated efforts are necessary (Supplementary Appendix 4). First, targeted investment in research confirming the relevance of zebrafish for dermatological endpoints is crucial. This includes creating and sharing standardized protocols for evaluating pigmentation, inflammatory responses, wound healing, and genodermatoses. Second, international regulatory harmonization must be enhanced by expanding OECD Test Guidelines to incorporate zebrafish-based models for dermatology. Such harmonization would promote mutual acceptance of data across jurisdictions, streamline drug development processes, and decrease dependence on traditional animal testing.

Third, building capacity in LMICs must be prioritized to ensure fair participation in this evolving field of research. Technical training, infrastructure support, and knowledge transfer programs would empower researchers in these regions to use zebrafish models for local public health challenges, promoting both scientific innovation and international collaboration. Finally, ongoing multi-stakeholder dialogue is essential. Dermatologists, toxicologists, academic researchers, industry stakeholders, and regulatory bodies must work together to define research priorities and create a clear roadmap for zebrafish integration in dermatology. Such dialogue will also encourage data sharing, reduce duplication of effort, and speed up the validation of zebrafish NAMs.

Conclusions

Zebrafish offer a mechanistically conserved, ethically viable, and cost-effective vertebrate model that enhances molecular discovery to preclinical dermatology. Although they cannot fully replicate all architectural or immunological complexities of human skin, their high genetic and signaling concordance provides a powerful platform for elucidating fundamental cutaneous processes and advancing translational dermatological innovation. The model has become a cornerstone in dermatological research by bridging mechanistic insights with translational potential across pigmentation, barrier function, wound healing, genetic skin dis-

orders, inflammation, and melanoma. Nonetheless, translational interpretation requires careful context. Zebrafish are less suitable for modeling late-stage keratinization, sebaceous gland–related disorders, or stratum corneum–dependent pathologies, for which human epidermal models or mammalian systems remain essential. Furthermore, their robust regenerative capacity may mask chronic or fibrotic processes that are clinically relevant to humans.

Despite these differences, zebrafish provide an unparalleled platform for real-time visualization of dermatological processes, such as pigment migration, immune cell trafficking, angiogenesis, and wound re-epithelialization, offering insights that are challenging to capture in mammalian models. These conserved mechanisms demonstrate the successful use of zebrafish in modeling human pigmentation disorders, inflammatory dermatoses, and genetic skin diseases, and in the preclinical screening of nanoparticles, phytocompounds, and regenerative agents.

As such, to realize the complete potential of zebrafish as NAMs in dermatology, their usefulness must be strategically combined with complementary mammalian models to ensure translational accuracy. A multi-model approach is therefore essential. It must use the high throughput and genetic accessibility of zebrafish while taking advantage of the anatomical relevance and clinical history of rodent systems. This dual strategy allows researchers to benefit from the strengths of each model while addressing their individual weaknesses, ultimately enhancing the process from research to clinical application.

In the long run, a coordinated cross-sector strategy that aligns scientific innovation with regulatory clarity and global equity will be critical. If properly addressed, the challenges outlined above can become opportunities. Such a strategy can promote dermatological research that is both ethically responsible and scientifically sound, while also making meaningful contributions to achievement of the SDGs.

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