Review

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Marine-Derived Bioactive Peptides as Immunomodulatory Agents: Organoid-Based Platforms and Translational Perspectives

Chae-Min Kim[1], Eon-Bee Lee[1,*]

[1]Laboratory of Aquatic Biopharmacology, Department of Aquatic Life Medicine, Pukyong National University, 48513 Busan, Republic of Korea
*Correspondence: eonbee@pknu.ac.kr (Eon-Bee Lee)
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Marine environments harbor immense biodiversity that yields peptides with potent immunomodulatory, antioxidant, and anti-inflammatory activities. These marine-derived immunomodulatory peptides act as molecular mediators of host defense and immune homeostasis across taxa ranging from fish to humans. However, current evaluation approaches, such as two-dimensional cell cultures and animal models, remain insufficient to capture the complex interactions underlying peptide-driven immune regulation. This review integrates recent advances in marine peptide discovery, structural–functional characterization, and elucidation of immunological mechanisms, while critically examining the translational limitations of conventional *in vitro* and *in vivo* systems. We highlight organoid technology as a next-generation platform that bridges these gaps through physiologically relevant three-dimensional microenvironments capable of reconstructing innate and adaptive immune responses. Intestinal, hepatic, pulmonary, and skin organoids provide unprecedented opportunities to assess peptide absorption, metabolism, and immunoregulation under near-physiological conditions. Furthermore, the development of fish-derived organoids offers a "fish-to-human" continuum, enabling cross-species validation of conserved pathways within a One Health framework. By integrating marine peptide research with organoid-based immune evaluation, this review proposes a translational roadmap that unites aquatic biomedicine, immunonutrition, and anti-inflammatory drug development. The convergence of organoid-on-a-chip systems, immune-cell co-cultures, and multi-omics analytics is expected to accelerate the preclinical-to-clinical transition of marine peptides, transforming them into functional foods, nutraceuticals, and immunotherapeutics. Collectively, this synthesis delineates how marine peptide–immune–organoid integration advances sustainable biotechnology and precision immunotherapy across species and disciplines.

Keywords: marine peptides; immunomodulation; organoid; fish models; translational biotechnology; immune evaluation; one health

Introduction

Marine environments, which cover more than 70% of the Earth's surface, represent an immense reservoir of biodiversity and a rich source of bioactive compounds. Among these, marine-derived peptides have attracted increasing attention due to their potent immunomodulatory, antioxidant, and anti-inflammatory properties across a wide range of species, including fish and mammals [1]. In aquatic organisms, these peptides function as key mediators of innate immunity, enabling adaptation to dynamic environmental stressors and pathogen exposure [2]. Despite growing interest in their potential applications as functional feed additives, nutraceuticals, and therapeutic agents, current research remains largely fragmented and predominantly confined to species-specific or model-limited observations. This limitation has hindered the establishment of an integrative framework linking peptide bioactivity in aquatic systems to human immunological outcomes, thereby constraining their translational potential.

Conventional approaches for evaluating the immunological activity of marine-derived peptides have largely relied on two-dimensional (2D) cell culture systems and animal models [3]. RAW 264.7 macrophage-based analyses of reactive oxygen species (ROS), nitric oxide (NO), and cytokines, as well as Caco-2 monolayer-based assessment of barrier function, are useful for initial screening but do not sufficiently recapitulate intercellular interactions or the complexity of the tissue microenvironment [4]. Fish and mammalian animal models have also made important contributions to developmental and immunological research. However, interspecies differences, translational limitations, and ethical constraints limit their ability to directly predict human applicability [5,6]. Indeed, many candidate compounds show promising immunomodulatory effects at the preclinical stage, but the same outcomes are often not reproduced in clinical settings, largely because conventional models do not sufficiently recapitulate the complexity of the human immune system [6]. Therefore, next-generation evaluation platforms with greater physiological relevance,

precision, and reproducibility are needed beyond conventional *in vitro* and *in vivo* approaches.

In this context, organoid technology has emerged as a promising next-generation platform for immunological evaluation. Organoids are three-dimensional structures generated through the self-organization capacity of stem cells and can relatively faithfully reproduce the cellular composition and functional architecture of native organs [7]. Organoids derived from various tissues, including the intestine, lung, liver, and skin, have been used to study immune-related pathologies, such as infection, inflammation, and cancer [8]. In addition, the integration of immune cells into organoid systems has enabled the development of immune organoid models to analyze both innate and adaptive immune responses [9]. In particular, applying marine-derived peptides to organoid systems enables more precise and physiologically relevant evaluation of epithelial barrier function, mucosal immunity, inflammation, and oxidative stress responses. This approach addresses the limitations of conventional cell and animal models and provides a novel translational evaluation framework for validating the efficacy and safety of marine peptides under clinically meaningful conditions.

Furthermore, the establishment of fish-derived organoid models may serve as an important bridge for advancing fish-to-human translational research. Although the fish immune system differs from that of humans, major signaling pathways, such as Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB), Mitogen-Activated Protein Kinase (MAPK), and Janus Kinase (JAK)/Signal Transducer and Activator of Transcription (STAT), are evolutionarily conserved, making them suitable for comparative immunological studies [10,11,12]. Therefore, by progressively extending data obtained from fish organoids to human organoid models, the immunological efficacy of marine peptides can be cross-validated between fish and human systems. This integrative framework can serve as a translational bridge beyond aquatic biomedicine toward human immune health, anti-inflammatory therapeutics, and functional food development, and may provide a strategic roadmap connecting marine peptides, immunity, organoid technology, and fish-to-human translation from a One Health perspective [13].

This review aims to summarize recent advances in marine-derived immunomodulatory peptide research and critically examine the limitations of conventional *in vitro* and *in vivo* models. In particular, this review discusses the potential of organoid-based immune evaluation as an alternative platform to address the limitations of conventional evaluation models and emphasizes the largely unexplored application of marine peptides in organoid systems. By proposing the concept of fish-to-human translation, this review also seeks to reappraise the immunological value of marine bioresources and present a differentiated framework that connects aquatic biomedicine with human health applications. Finally, future research directions and strategies are proposed to promote the academic investigation, industrial development, and clinical translation of marine-derived immunomodulatory peptides (Fig. 1).

Marine-Derived Immunomodulatory Peptides

Classification and Origins of Marine Immunomodulatory Peptides

Marine-derived immunomodulatory peptides originate from diverse marine organisms, including fish, mollusks, crustaceans, seaweeds, and microalgae (Fig. 2, Table 1, Ref. [14,15,16,17,18,19,20,21,22,23]). These origins do not merely represent taxonomic diversity, but reflect distinct evolutionary strategies acquired by each organism to maintain host defense, barrier protection, stress adaptation, and immune homeostasis [24,25]. Marine organisms have evolved under diverse ecological pressures, including microbial exposure, high salinity, temperature fluctuation, hypoxia, and tissue injury, and these environmental conditions have shaped the structural and immunological functions of their peptides. Therefore, the origin of a peptide provides important clues for understanding its functional properties, including antimicrobial defense, inflammatory regulation, oxidative stress control, tissue repair, and mucosal immune homeostasis [2,26]. Accordingly, an origin-based perspective can serve as an organizing framework for comparing marine immunomodulatory peptides from different source organisms and for interpreting their functional diversity.

Fish-derived peptides are among the most extensively studied groups of marine-derived immunomodulatory peptides. These peptides are mainly expressed in barrier tissues, such as the skin, mucus, gills, and intestine, as well as in systemic immune-related organs, including the liver and blood. Because fish are continuously exposed to aquatic pathogens, their peptides have evolved beyond simple antimicrobial effectors to function as regulators of mucosal immunity and inflammatory balance [24,25,26]. However, in addition to these endogenous host-defense peptides, many fish-derived bioactive peptides are generated by enzymatic hydrolysis of proteins obtained from edible muscle tissues or fish-processing by-products, such as skin, bones, heads, frames, and viscera, and have been investigated for food, nutraceutical, and industrial applications [27]. In this context, non-edible or discarded fish tissues may also serve as valuable substrates for the recovery of bioactive peptides and the high-value utilization of aquatic-processing by-products.

Piscidin is a representative amphipathic peptide with membrane-disruptive antimicrobial activity first identified in teleost fish, such as striped bass and tilapia. Beyond their direct antimicrobial activity, individual piscidins can exert immunomodulatory effects depending on their sequence and cellular context. For example, piscidin 1 and

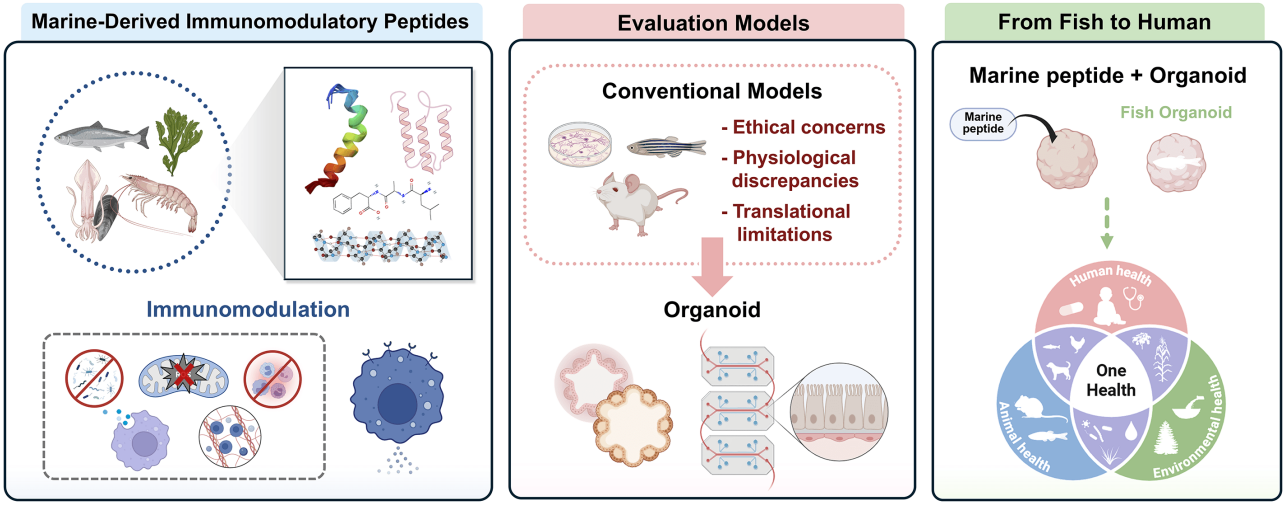

Fig. 1. Conceptual framework linking marine biodiversity, peptide immunomodulation, and organoid-based translational models. This figure outlines the overall conceptual flow of the review—from the vast marine biodiversity serving as a source of bioactive peptides, through their immunomodulatory mechanisms, to the introduction of next-generation organoid models as advanced evaluation platforms. The integration of marine peptide research with organoid technology establishes a "fish-to-human" translational continuum, bridging aquatic biomedicine and human immunology under the One Health paradigm. This figure was created using BioRender (BioRender Inc., Toronto, ON, Canada; https://www.biorender.com/).

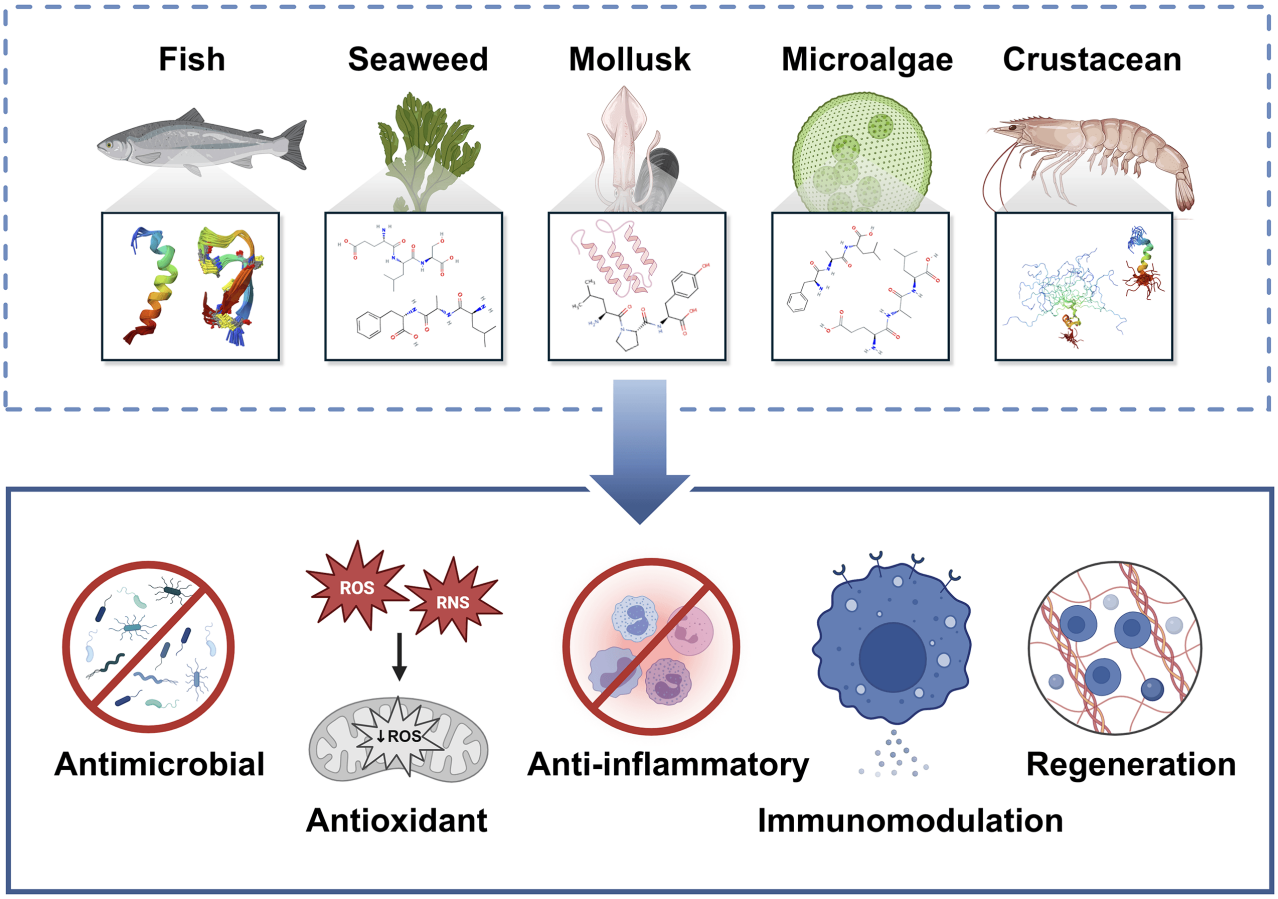

Fig. 2. Taxonomic origins and immunological functions of marine-derived immunomodulatory peptides. This figure was created using BioRender (BioRender Inc., Toronto, ON, Canada; https://www.biorender.com/).

Table 1. Representative marine-derived immunomodulatory peptides and hydrolysates.

Origin	Peptide/Hydrolysate	Function	Key immunological mechanisms	Reference
Ictalurus punctatus (Channel catfish)	β-defensin	Antimicrobial, immune-related	Immune-responsive expression after bacterial challenge; innate immune defense	[14]
Salmo salar (Atlantic salmon)	NK-lysin	Antimicrobial, immunomodulatory	↑ IL-1β, IL-8; MAPK (p38, ERK1/2, JNK) activation	[15]
Salmo salar (Atlantic salmon)	Cathelicidin (asCATH1, asCATH2)	Antimicrobial, immune-stimulatory	↑ IL-8 induction; leukocyte immune regulation	[16]
Mytilus spp. (Thick-shelled mussel)	Mussel peptide fraction (e.g., LVVLGH)	Immunomodulatory	↑ Phagocytosis; cytokine secretion via TLR4; activation of NF-κB and MAPK pathways	[17]
Patinopecten yessoensis (Scallop)	Scallop peptide hydrolysate (SCH)	Immunostimulatory	↑ Lymphocyte activation; ↑ IL-2, IL-6, TNF-α; Th1/Th2 modulation	[18]
Rapana venosa (Sea conch)	Peptide hydrolysate	Immunomodulatory, anti-inflammatory	NF-κB inhibition; ↓ TNF-α, IL-1β, IL-6; restoration of intestinal immune homeostasis	[19]
Litopenaeus vannamei (Shrimp)	SpPR-AMP1	Antimicrobial, immunoenhancing	↑ AMP genes in hemocytes → activation of innate immunity response	[20]
Scylla paramamosain (Mud crab)	Crustacean cardioactive peptide (CCAP)	Immunomodulatory neuropeptide	Regulation of hemocyte phagocytosis and apoptosis; modulation of immune-related signaling pathways	[21]
Arthrospira platensis (Spirulina)	Peptide-rich protein hydrolysate	Immunomodulatory	↑ Phagocytic activity; ↓ NO production; cytokine modulation in macrophages	[22]
Porphyra yezoensis (Seaweed, red)	Peptide-rich protein hydrolysate	Immunomodulatory	Macrophage activation → ↑ phagocytosis; ↓ NO; ↑ DTH response (*in vivo*)	[23]

IL, Interleukin; MAPK, Mitogen-Activated Protein Kinase; ERK, Extracellular Signal-Regulated Kinase; JNK, c-Jun N-terminal Kinase; TLR, Toll-Like Receptor; NF-κB, Nuclear Factor kappa-light-chain-enhancer of activated B cells; TNF, Tumor Necrosis Factor; Th1/Th2, T helper 1/T helper 2 cells; AMP, Antimicrobial Peptide; NO, Nitric Oxide; DTH, Delayed-Type Hypersensitivity; TNF-α, Tumor Necrosis Factor-alpha; ↑, increased or upregulated; ↓, decreased or downregulated; →, leads to or results in.

piscidin 2 from gilthead seabream differentially regulated inflammatory and Toll-like receptor-related genes in fish leukocytes [28]. Tilapia piscidin 4 also reprogrammed pro-inflammatory macrophages toward inflammation-resolving and tissue-repair-associated phenotypes [29]. These findings indicate that piscidins can contribute to host defense not only through direct antimicrobial activity but also by modulating inflammatory signaling and immune-cell function. Hepcidin is another important fish-derived peptide that links antimicrobial defense with iron metabolism. By regulating ferroportin and limiting iron availability, hepcidin suppresses microbial growth. In addition to its antimicrobial and iron-regulatory functions, teleost hepcidin can directly modulate inflammatory responses. A synthetic rainbow trout hepcidin was internalized by Rainbow Trout Spleen (RTS)-11 monocyte/macrophage cells and regulated Interleukin (IL)-10, IL-1β, and Tumor Necrosis Factor-alpha (TNF-α) expression in fish leukocytes and immune tissues [30]. In European sea bass, synthetic hepcidin also attenuated the pro-inflammatory response induced by betanodavirus infection while promoting immune-cell recruitment [31]. Collectively, these findings demonstrate that fish-derived immunomodulatory peptides can coordinate pathogen restriction with the regulation of inflammatory and immune-cell responses. Their conserved and species-specific activities therefore provide a comparative basis for investigating peptide-mediated immune regulation.

Mollusks represent another major source of marine peptides with high translational potential. Unlike vertebrates, mollusks lack an adaptive immune system and therefore rely on highly specialized innate immune molecules present in hemocytes and mucosal tissues. This immune architecture has led to the generation of peptides with broad antimicrobial, antiviral, wound healing, and immunomodulatory functions [2]. Myticin C derived from *Mytilus galloprovincialis* was initially characterized as an antimicrobial peptide but is now recognized as a multifunctional immunomodulatory peptide involved in antiviral defense, wound healing, tissue regeneration, and immune cell migration [32,33,34]. This functional diversity is particularly important for human application because chronic inflammation, impaired wound repair, and mucosal barrier dysfunction are key features of many human diseases. In addition, protein hydrolysates derived from oysters and mussels have been reported to inhibit nitric oxide production, reduce inflammatory cytokines such as TNF-α and IL-6, and enhance antioxidant enzyme activity in macrophage-based models [35,36]. These activities suggest that mollusk-derived pep-

tides may provide candidate bioactive fractions for further evaluation as anti-inflammatory nutraceuticals or functional materials targeting macrophage-mediated inflammation and tissue homeostasis.

Crustacean-derived peptides are closely associated with innate immune recognition and intercellular immune communication. Because crustaceans lack adaptive immunity, they rely on rapid and coordinated innate immune responses involving hemocytes, antimicrobial peptides, lectin-like proteins, and protease cascades for survival. Crustins and penaeidins are representative crustacean-derived peptides. Crustins were initially described as antimicrobial peptides, but recent studies have shown that they also participate in immune cell recruitment and inflammatory regulation. For example, LvCrustinIa-2 derived from *Litopenaeus vannamei* has been reported to mediate chemotactic recruitment of immune cells, indicating that crustins may contribute to immune response coordination beyond direct microbial killing. Penaeidins, which are primarily expressed in shrimp hemocytes, contain proline-rich and cysteine-rich domains and are involved in pathogen recognition, phagocyte activation, granulocyte migration, and cytokine-like immune responses [37]. In addition, several penaeidins have been shown to restrict white spot syndrome virus infection through direct interactions with viral structural proteins, such as the binding of PEN2 to p24 and the interference of BigPEN with VP28-mediated viral entry [38]. These findings indicate that crustacean-derived peptides participate in host defense through immune-cell-related functions and pathogen-specific molecular interactions, providing a useful biological basis for designing peptides capable of modulating innate immune-cell migration, inflammatory signaling, and host defense. However, because such mechanisms may depend on structure-specific interactions, further validation in mammalian cell or tissue models is required before their translational relevance can be established.

Seaweeds and microalgae are increasingly recognized as sustainable and scalable sources of immunomodulatory peptides. Compared with animal-derived peptides, algal peptides are particularly attractive for human application because they can be obtained from renewable biomass and incorporated into functional foods or nutraceutical formulations [39,40]. Peptides and protein hydrolysates derived from red algae such as *Porphyra* spp. have been reported to promote lymphocyte proliferation, regulate anti-inflammatory cytokine production, and suppress inflammatory mediators such as TNF-α and Interferon-gamma (IFN-γ) [41]. Green algal peptides from *Ulva* spp. have also been shown to exert immunomodulatory effects through Toll-Like Receptor (TLR)4-related pathways and downstream NF-κB and MAPK signaling [42]. These findings suggest that algal peptides may contribute to the regulation of oxidative stress and inflammatory signaling. Therefore, algal peptides may provide a useful source of candidates for marine biotechnology and immunonutrition, provided that their stability, bioavailability, and tissue-level effects are verified in appropriate human-relevant models.

Overall, marine-derived immunomodulatory peptides constitute a diverse class of bioactive molecules that reflect the ecological environments and immunological characteristics of their source organisms. Classification based on biological origin is useful for understanding the defense functions and potential immunological roles acquired by individual peptides. However, biological origin alone is insufficient to predict their bioactivity, stability, or translational potential. Peptides derived from taxonomically distinct organisms may exhibit similar immunomodulatory functions, whereas peptides originating from the same organism may display different activities depending on their amino acid sequences and physicochemical properties. A more comprehensive understanding of these peptides therefore requires an analysis of structural determinants, including peptide length, charge, hydrophobicity, amphipathicity, three-dimensional conformation, and disulfide bonding. These structural features govern peptide stability, target binding, membrane interactions, and immunomodulatory activity, thereby providing the basis for understanding their structure–function relationships.

Structure–Function Relationships

In addition to considering their biological origins, elucidating the structure–function relationships of marine-derived immunomodulatory peptides is essential for linking taxonomic diversity to biological activity. The amino acid sequence of each peptide determines its molecular weight and largely defines fundamental physicochemical properties, including charge distribution, hydrophobicity, and amphipathicity, while providing the basis for the formation of higher-order structures, such as three-dimensional conformation and disulfide bonding. These physicochemical and structural properties influence interactions with cellular membranes and receptors and the subsequent modulation of intracellular signaling pathways, thereby shaping the immunomodulatory functions of marine-derived peptides (Fig. 3). Understanding these structure–function relationships is therefore critical for predicting peptide stability, target specificity, bioavailability, and immunomodulatory activity and provides an important basis for selecting candidates for further functional or translational evaluation [43,44].

Cationicity and hydrophobicity are major structural features commonly observed in many marine immunomodulatory peptides. Positively charged amino acids, such as lysine and arginine, enable electrostatic interactions with negatively charged bacterial membranes or immune cell surface molecules, whereas hydrophobic residues contribute to membrane insertion and the regulation of membrane permeability [45,46]. These properties may influence not only antimicrobial activity but also immune responses,

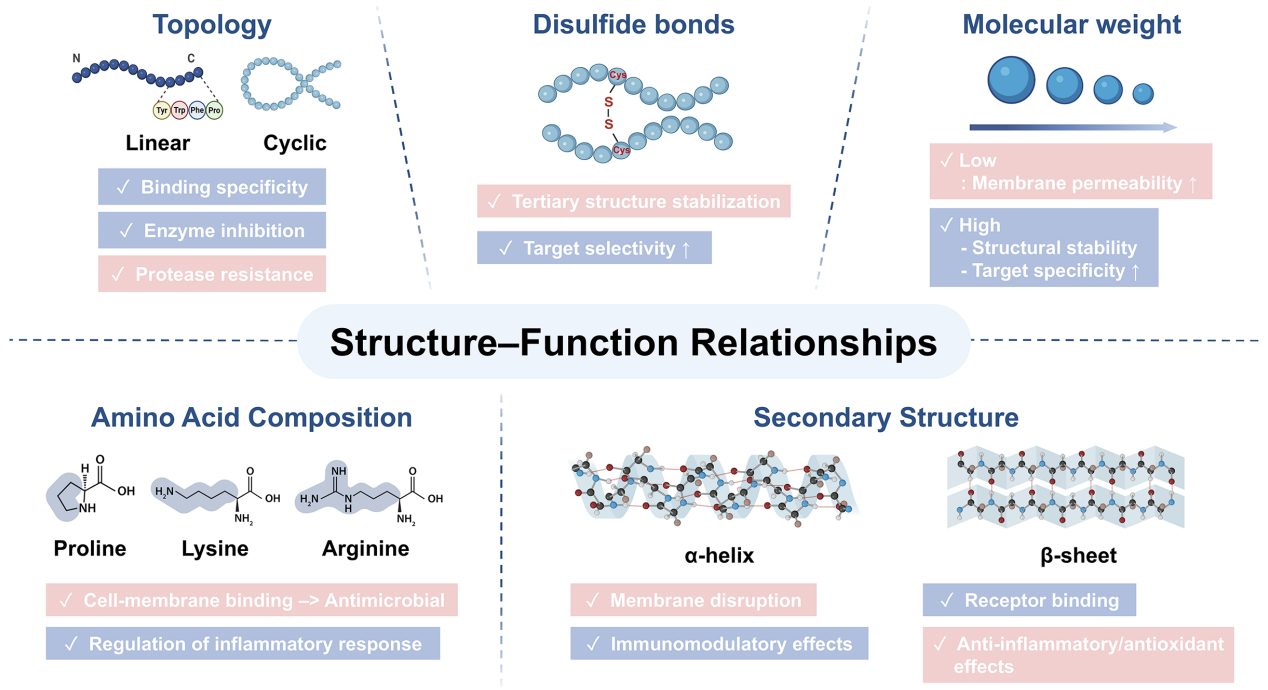

Fig. 3. Structure–function relationships of marine-derived immunomodulatory peptides. Marine peptides exhibit diverse structural determinants including topology, amino acid composition, disulfide bonds, molecular weight, and secondary structure that collectively define their bioactivity. Linear and cyclic topologies influence structural stability and target specificity, while residues such as Pro, Lys, and Arg modulate antimicrobial and immunomodulatory functions. Disulfide linkages and α-helix or β-sheet structures further stabilize peptides and govern membrane or receptor interactions. This figure was created using BioRender (BioRender Inc., Toronto, ON, Canada; https://www.biorender.com/).

including macrophage activation, regulation of inflammatory cytokines, and modulation of barrier function. In particular, peptides with amphipathic α-helical structures, such as piscidin, possess spatially separated hydrophilic and hydrophobic regions, allowing effective interactions with pathogen membranes while potentially regulating cellular signals involved in inflammation and tissue repair [47,48]. Thus, charge distribution and hydrophobic patterns mediate both antimicrobial activity and immune cell signaling, serving as structural determinants that explain the multifunctional properties of marine peptides [43,49].

Molecular weight and peptide length are also important factors that determine immunomodulatory activity. In general, low-molecular-weight peptides may exhibit relatively high tissue penetration, intestinal absorption, and intracellular accessibility, making them advantageous for modulating intracellular signaling pathways [50,51]. In contrast, relatively large peptides or those with complex structures are more likely to selectively bind to specific receptors, pathogen-associated components, or immune-related proteins. For example, some crustacean-derived peptides, such as anti-lipopolysaccharide (LPS) factor (ALF), are high-molecular-weight peptides stabilized by β-hairpin structures and disulfide bonds and can bind to LPS to modulate TLR4-mediated inflammatory signaling [52]. This indicates that structural stability and target-binding capacity can contribute to immunomodulatory function.

Structural stability is an essential requirement for developing marine peptides as practical functional materials or therapeutic candidates. Linear peptides are relatively easy to synthesize and modify, but they are susceptible to rapid degradation by endogenous enzymes. In contrast, cyclic peptides, disulfide bond-containing peptides, or peptides containing non-standard amino acids may exhibit enhanced resistance to proteolytic degradation, improved *in vivo* stability, and greater target selectivity [44,53,54,55]. Peptides such as Myticin C, paralithocins, and conotoxin families are representative examples showing that structural stability can be associated with antimicrobial and immunomodulatory activities [55,56,57]. These features must be considered for marine peptides to move beyond simple *in vitro* bioactivity and achieve sustained and reproducible effects *in vivo*.

Ultimately, understanding the structure–function relationships of marine-derived immunomodulatory peptides provides a basis for determining their stability under physiological conditions, their target immune cells and signal-

ing pathways, and their suitability for translational evaluation and potential human application. Therefore, future studies on marine peptides should move beyond separately reporting antioxidant, anti-inflammatory, or immune cell-modulatory activities and instead interpret their structural features and mechanisms of action in an integrated manner. This approach may help prioritize candidate peptides according to their functional relevance and provide a rational basis for their validation in physiologically relevant evaluation systems, including organoid-based platforms.

Immunomodulatory Mechanisms

Marine-derived immunomodulatory peptides contribute to the maintenance of host immune homeostasis through their antioxidant, anti-inflammatory, and immune-cell-modulatory activities [58]. However, these activities do not occur independently; rather, they operate within a complex regulatory network linking oxidative stress, inflammatory signaling, and immune-cell interactions. Excessive accumulation of ROS and reactive nitrogen species (RNS) can modulate redox-sensitive inflammatory signaling pathways, including NF-κB and MAPK, and promote NLR family pyrin domain-containing 3 (NLRP3) inflammasome activation, thereby enhancing the production of pro-inflammatory cytokines [59]. Conversely, activated macrophages and neutrophils generate additional ROS, RNS, and inflammatory mediators, further amplifying oxidative stress and inflammatory responses [60]. The resulting changes in redox status and the cytokine microenvironment can also affect macrophage activation states, lymphocyte differentiation, natural killer cell function, and intercellular immune interactions, ultimately contributing to the disruption of immune homeostasis. Therefore, the immunomodulatory effects of marine-derived peptides should be understood not merely as increases or decreases in individual biomarkers, but as an integrated capacity to simultaneously regulate oxidative stress, inflammatory responses, and immune-cell functions (Fig. 4).

Antioxidant Mechanisms in Cellular Stress Regulation

Oxidative stress is a common cellular stress response associated with infection, inflammation, metabolic disorders, and tissue injury. Physiological levels of ROS and RNS are required for normal cellular signaling. However, their excessive accumulation can damage lipids, proteins, and DNA, thereby aggravating inflammatory responses [59]. The antioxidant effects of marine-derived peptides should therefore be understood not merely as chemical reactions that eliminate reactive species, but as processes that restore redox homeostasis and balance immune responses.

Marine-derived peptides can attenuate such oxidative damage through radical scavenging, metal ion chelation, and regulation of antioxidant signaling pathways [36,61]. Beyond direct chemical antioxidant activity, selected marine-derived peptides have been reported to improve endogenous antioxidant defense under oxidative-stress conditions. For example, salmon leather collagen peptide alleviated Ultraviolet A (UVA)-induced oxidative injury by regulating the Nrf2/Keap1 pathway and enhancing antioxidant defense in a photoaging model [62]. Other marine-derived peptides have also been shown to reduce intracellular ROS accumulation and improve antioxidant status, although the specific enzymatic markers and upstream signaling mechanisms vary depending on peptide source, sequence, cell type, and experimental model [63]. These findings indicate that the antioxidant effects of marine-derived peptides should be understood as multi-mechanistic responses involving direct radical-scavenging activity, metal-ion chelation, and peptide- and model-dependent regulation of cellular redox homeostasis. Redox regulation also influences subsequent inflammatory responses. Excessive ROS and RNS can modulate NF-κB and MAPK signaling and promote NLRP3 inflammasome activation, thereby increasing the production of pro-inflammatory cytokines [59]. Antioxidant peptides should therefore be considered not merely as supplementary bioactive substances, but as candidate bioactive molecules capable of simultaneously modulating redox imbalance and inflammation-associated tissue injury.

Anti-Inflammatory Pathways and Cytokine Modulation

Inflammation is an essential defense response against pathogen invasion and tissue injury. However, excessive or prolonged inflammation can lead to chronic disease and tissue damage [64]. Marine-derived immunomodulatory peptides act by regulating excessive inflammatory signaling and restoring the balance between pro-inflammatory cytokines and anti-inflammatory mediators. The principal signaling pathways targeted by marine-derived immunomodulatory peptides include NF-κB, MAPK, and JAK/STAT signaling. When TLR4 is activated by LPS or inflammatory stimuli, the expression of inflammatory mediators such as TNF-α, IL-1β, IL-6, inducible nitric oxide synthase (iNOS), and Cyclooxygenase-2 (COX-2) is increased. Several marine peptides have been reported to modulate these signaling pathways, thereby reducing NO and prostaglandin E_2 (PGE_2) production and attenuating the expression of inflammatory cytokines [58]. For example, Glu-Gly-Leu-Leu-Gly-Asp-Val-Ph (EGLLGDVF), a peptide derived from the green mussel *Perna viridis*, suppresses pro-inflammatory cytokine production and the expression of iNOS and COX-2. Elastin peptides derived from skipjack tuna alleviated osteoarthritis-associated inflammation through the inhibition of JAK2/STAT3 signaling. An olive flounder head hydrolysate reduced NO, PGE_2, pro-inflammatory cytokines, iNOS, and COX-2 in LPS-stimulated RAW264.7 macrophages and attenuated inflammatory and oxidative responses in zebrafish [65,66,67].

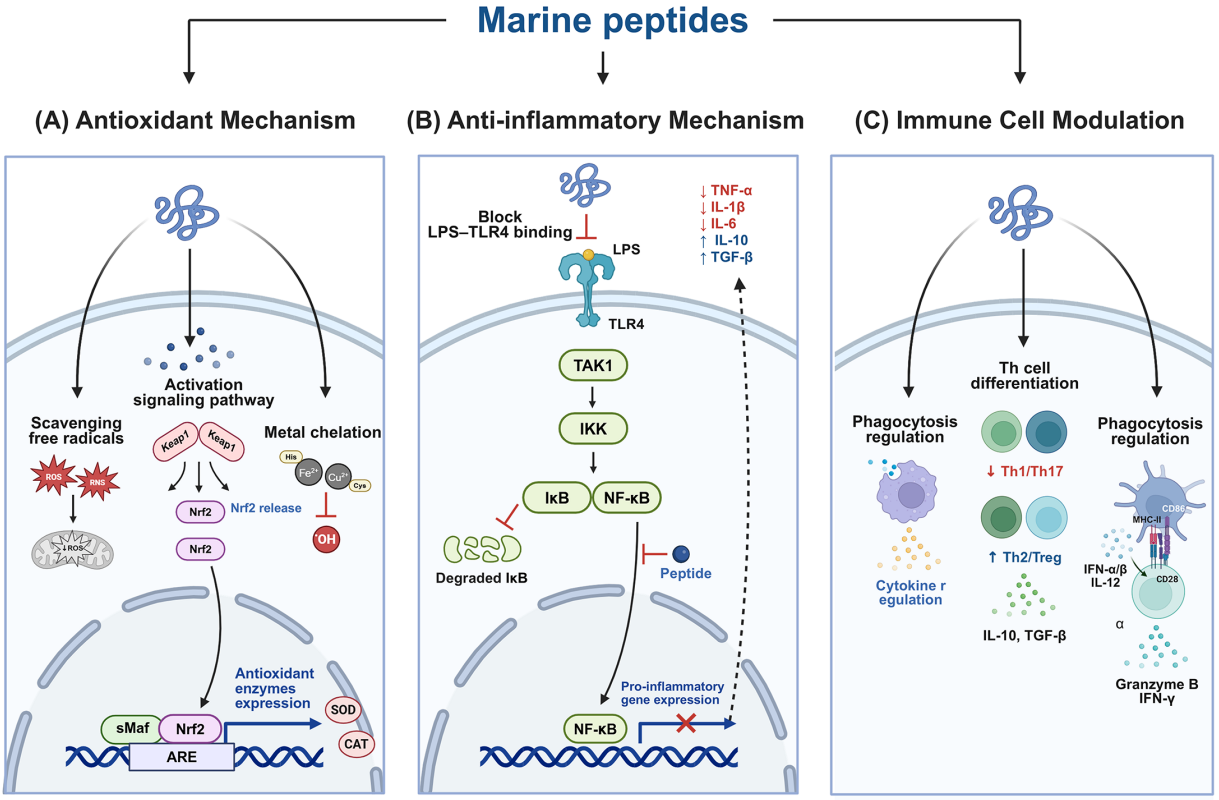

Fig. 4. Major immunomodulatory mechanisms of marine-derived immunomodulatory peptides. (A) Antioxidant mechanism: Marine peptides directly scavenge reactive oxygen and nitrogen species (ROS/RNS) and chelate transition metal ions (Fe^{2+}, Cu^{2+}) to inhibit hydroxyl radical formation via the Fenton reaction. They also activate the Nrf2/ARE signaling cascade by releasing Nrf2 from Keap1 inhibition, leading to the upregulated expression of antioxidant enzymes such as SOD and CAT, thereby restoring redox homeostasis and protecting cellular components from oxidative damage. (B) Anti-inflammatory mechanism: Certain peptides act as competitive inhibitors of LPS–TLR4 binding or suppress downstream inflammatory signaling pathways, including TAK1–IKK–NF-κB. This results in decreased transcription of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, COX-2) and enhanced expression of anti-inflammatory mediators (IL-10, TGF-β), collectively reducing tissue inflammation and injury. (C) Immune cell modulation: Marine peptides regulate immune balance by modulating Th cell differentiation (↓ Th1/Th17, ↑ Th2/Treg), enhancing macrophage and NK-cell activation, and promoting cytokine secretion (IL-10, IFN-γ, Granzyme B). Through these processes, they restore balanced immune activation and maintain overall immunological homeostasis. This figure was created using BioRender (BioRender Inc., Toronto, ON, Canada; https://www.biorender.com/). Nrf2, Nuclear Factor Erythroid 2-Related Factor 2; ARE, Antioxidant Response Element; Keap1, Kelch-Like ECH-Associated Protein 1; SOD, Superoxide Dismutase; CAT, Catalase; LPS, Lipopolysaccharide; TAK1, Transforming Growth Factor-Beta-Activated Kinase 1; IKK, IκB Kinase; COX-2, Cyclooxygenase-2; TGF-β, Transforming Growth Factor-Beta; Th1, T Helper 1 Cells; Treg, Regulatory T Cells; NK, Natural Killer Cells; IFN-γ, Interferon-Gamma.

Recent studies indicate that these effects may not be restricted to a single signaling pathway. For example, a jellyfish toxin-derived peptide simultaneously reduced ROS production and suppressed NF-κB/NLRP3 signaling, thereby modulating the connection between oxidative stress and inflammasome activation [68]. Peptides derived from abalone viscera reduced the expression of IL-1β, IL-6, and TNF-α while inhibiting the phosphorylation of ERK, JNK, and p38 [69]. These findings indicate that the anti-inflammatory effects of marine-derived peptides may involve multi-target responses encompassing redox regulation and the suppression of inflammatory signaling.

Changes in inflammatory signaling and the cytokine microenvironment can also affect immune-cell activation and function. Reductions in pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-6, together with increases in regulatory cytokines such as IL-10, may alter macrophage activation states, lymphocyte differentiation, and natural killer cell function [70]. Restoration of cytokine balance therefore represents an important intermediate step linking the suppression of inflammatory signaling to the reprogramming of immune-cell functions and provides a mechanistic basis for immune-cell regulation.

These anti-inflammatory and immunomodulatory activities may be associated with human diseases involving macrophage hyperactivation, intestinal mucosal inflammation, skin inflammation, and metabolic inflammation. Therefore, marine peptides can be regarded not merely as anti-inflammatory agents but as candidates capable of reducing tissue damage and restoring immune balance by regulating inflammatory signaling networks. However, many current studies remain focused on 2D cell models, and whether the same regulatory effects can be reproduced in actual tissue environments has not yet been sufficiently validated. Future studies should evaluate tissue-level indicators, such as epithelial barrier integrity, immune cell recruitment, and tissue repair, and further demonstrate therapeutic potential through pharmacokinetic and drug delivery studies, as well as validation in complex disease models.

Immune Cell Modulation

Immune cells connect innate and adaptive immunity and serve as key mediators that translate oxidative stress and inflammatory signals into tissue-level responses. Marine-derived peptides contribute to immune homeostasis by modulating the activation, differentiation, migration, and interactions of these cells. A previous study has reported that marine-derived peptides can regulate macrophage phenotypes and cytokine production [29]. Beyond these macrophage-mediated effects, protein hydrolysates derived from *Nibea japonica* skin enhanced cellular and humoral immunity by increasing splenocyte proliferation and immunoglobulin levels, whereas peptides derived from *Colochirus robustus* increased lymphocyte proliferation and NK-cell activity [71,72]. These findings suggest that marine-derived peptides may not act exclusively on a single immune-cell population but may regulate tissue-level immune responses through interactions among epithelial cells, macrophages, lymphocytes, and the microbiota.

Immune-cell regulation is both a consequence of antioxidant and anti-inflammatory activity and a feedback mechanism that further modulates redox and inflammatory states. Marine-derived peptides may first attenuate oxidative stress, thereby reducing redox-sensitive inflammatory signaling. The resulting cytokine changes can then reshape macrophage, lymphocyte, and NK-cell functions, these immune cells can in turn further influence ROS production, cytokine secretion, phagocytosis, and inflammatory resolution. This reciprocal relationship explains why antioxidant, anti-inflammatory, and immune-cell-modulatory activities should be interpreted as an interconnected regulatory axis rather than as three independent mechanisms. This integrated perspective indicates that individual biomarkers alone are insufficient for evaluating the immunomodulatory efficacy of marine-derived peptides. Accurate characterization of their immunomodulatory effects requires the simultaneous assessment of redox responses, inflammatory signaling, cytokine networks, immune-cell states, barrier function, and tissue repair. This requirement is directly related to the limitations of conventional evaluation models, which cannot adequately recapitulate complex intercellular interactions and tissue microenvironments, and supports the need for multicellular and organoid-based assessment platforms.

Clinical Development Status of Marine-Derived Peptides

Marine-derived peptides have attracted attention as therapeutic candidates because of their structural diversity, target specificity, and broad biological activities. To date, the most successful or clinically advanced marine-derived peptides and peptide-based therapeutics have been developed primarily for pain management and oncology [73]. These clinical examples demonstrate that marine-derived peptide scaffolds can achieve clinical translation when their mechanism of action, delivery route, tissue exposure, therapeutic window, and target indication are appropriately aligned. In contrast, marine-derived peptides intended to directly regulate inflammation, immune homeostasis, or barrier immunity remain largely at the preclinical or early translational stage [74,75]. This contrast provides important context for evaluating marine-derived immunomodulatory peptides, because antioxidant, anti-inflammatory, or immune-cell-modulatory activity observed *in vitro* does not by itself ensure clinical applicability.

Ziconotide represents a prominent example of the successful translation of a marine-derived peptide into an approved pharmaceutical product. It is a synthetic analogue of ω-conotoxin MVIIA, originally identified in the venom of the marine cone snail *Conus magus*. Ziconotide selectively blocks N-type voltage-gated calcium channels in the spinal cord, thereby inhibiting the release of neurotransmitters involved in pain transmission. It has been approved in the United States and the European Union as an intrathecally administered treatment for severe chronic pain refractory to conventional therapies [76]. Compared with opioid analgesics, ziconotide has several advantages, including the absence of tolerance development, respiratory depression, and withdrawal symptoms. However, its clinical application is limited by the requirement for intrathecal administration, a narrow therapeutic window, and dose-dependent central nervous system and neuropsychiatric adverse effects. This case demonstrates that the high target specificity of a marine peptide can translate into clinical efficacy, while also illustrating that administration route and safety management remain major translational barriers.

Plitidepsin is a cyclic depsipeptide derived from the marine tunicate *Aplidium albicans* and represents another clinically advanced marine peptide-based anticancer agent. Plitidepsin targets eukaryotic elongation factor 1A2, thereby disrupting proteostasis and survival signaling in cancer cells and ultimately inducing apoptosis. In Australia, plitidepsin was approved in combination with dex-

amethasone for patients with relapsed or refractory multiple myeloma who had failed or become resistant to previous treatments [77,78]. Beyond multiple myeloma, plitidepsin has been evaluated for other malignancies and antiviral indications, including a Phase III clinical trial in hospitalized adults with moderate COVID-19 [79]. Nevertheless, its clinical utility depends on indication-specific efficacy, toxicity profiles, and appropriate patient selection. The clinical development of plitidepsin therefore demonstrates that marine-derived peptides and depsipeptides can provide novel molecular targets, while emphasizing that successful translation requires an appropriate alignment among mechanism of action, disease biology, therapeutic window, and target patient population.

Dolastatin 10 illustrates the importance of delivery strategies in the clinical translation of marine peptide-derived compounds. It is a potent cytotoxic pentapeptide originally isolated from the marine mollusk *Dolabella auricularia*. Although dolastatin 10 exhibits strong anticancer activity by inhibiting tubulin polymerization, its clinical development as a free drug was limited by the inability to adequately separate systemic toxicity from therapeutic efficacy [80]. Subsequently, the dolastatin-derived auristatin analogues monomethyl auristatin E and monomethyl auristatin F were incorporated as cytotoxic payloads into antibody–drug conjugates, enabling more successful clinical translation [81]. This example indicates that strong cytotoxic activity must be coupled with an appropriate delivery strategy to overcome systemic toxicity and unfavorable tissue distribution. It also highlights the importance of targeted delivery systems for improving the therapeutic window of marine peptide-derived compounds.

Together, these clinical examples highlight both the therapeutic potential and the translational constraints of marine-derived peptide-based compounds. This issue is particularly relevant to marine-derived immunomodulatory peptides, for which clinical evidence remains limited despite extensive reports of antioxidant, anti-inflammatory, barrier-protective, and immune-cell-modulatory activities in cellular and animal models. In many cases, these bioactivities have not yet been sufficiently linked to key translational parameters, including gastrointestinal stability, bioavailability, tissue-specific exposure, long-term safety, reproducibility, delivery feasibility, and therapeutic window [82]. Therefore, the clinical development of marine-derived immunomodulatory peptides requires evaluation strategies that move beyond simple activity-based screening and more accurately predict efficacy and safety in physiologically relevant tissue environments. This translational gap underscores the limitations of conventional evaluation models and supports the use of more clinically predictive platforms, including organoid systems.

Conventional Evaluation Models and Their Limitations

In Vitro Models (2D Cell Systems)

Two-dimensional cell-based models are the most fundamental *in vitro* systems for evaluating the immunomodulatory activity of marine-derived peptides. Cell lines such as RAW 264.7 macrophages, THP-1 human monocytic leukemia cells, and Caco-2 intestinal epithelial cells have been widely used to evaluate antioxidant activity, NO production, cytokine expression, NF-κB, MAPK, and JAK/STAT signaling, and epithelial barrier function [83,84,85,86]. These models offer high reproducibility through the use of established cell lines and are useful for initial screening and mechanistic analysis because of their experimental accessibility. However, because 2D cell models are cultured as monolayers, they do not sufficiently reflect the structural complexity of native tissues. Intercellular interactions, extracellular matrix (ECM)-dependent signaling, oxygen and nutrient gradients, and crosstalk between immune and epithelial cells are limited in these systems, making it challenging to conclude that anti-inflammatory or antioxidant activities observed at the cellular level will be reproduced in native tissues [87,88]. For example, macrophage monocultures are useful for evaluating cytokine changes and phagocytic activity, but they cannot reflect interactions with intestinal epithelial cells, dendritic cells, or lymphocytes. Caco-2 monolayers are used to assess intestinal absorption and barrier function, but they do not sufficiently recapitulate the mucus layer, immune microenvironment, or microbiota interactions of the native intestinal mucosa [85,86]. Therefore, although 2D *in vitro* models are suitable for initial functional validation of marine peptides, they have structural limitations in extending these findings to complex immune responses at the tissue and organismal levels (Table 2, Ref. [84,87,89,90,91,92,93,94,95,96,97,98,99]). These limitations indicate that marine peptide research should move beyond simple cell-based marker analysis and expand toward physiologically relevant multilayered three-dimensional (3D) or multicellular evaluation systems to assess immunological activity more precisely.

In Vivo Models (Fish and Mammalian)

In vivo models have played an important role in evaluating the efficacy, toxicity, and immune responses of marine-derived peptides. Among these models, fish, particularly zebrafish and other teleost species, have been widely used to investigate development, inflammation, infection, and toxicity. Because they possess both innate and adaptive immune components, fish models are useful for evaluating the *in vivo* activity of immunomodulatory candidates [100,101]. In particular, zebrafish have advantages in studies of inflammation, infection, and tissue regeneration because of their transparent embryos, rapid development, genetic manipulability, and availability of reporter lines [102].

Table 2. Representative 2D cell-based models relevant to immune and tissue-level evaluation and their limitations.

Organ	Cell	Features	Application	Limitation	Ref
Intestine	HT-29 (human colon epithelium)	Mucus-secreting epithelial model	Gut mucosal immunity, cytokine release, drug transport, pathogen adhesion	Low digestive enzyme expression; weak barrier (low TEER); slow differentiation	[87]
Liver	Huh7 (human hepatocyte-derived)	Human hepatoma line retaining inflammatory and metabolic responsiveness	Drug metabolism and hepatotoxicity testing; immunomodulatory and inflammatory studies	Cancer-derived; partial CYP450 activity; lacks multicellular hepatic structure	[89,90]
Liver	Hep3B (human hepatoma)	Cancer-derived hepatocyte line with albumin secretion and HBV genome integration; retains partial hepatic metabolic and immune functions	Anti-inflammatory and antioxidant peptide screening; immune-related hepatotoxicity evaluation	Low CYP450 activity; limited secretory function; lacks complex hepatic architecture	[91,92]
Skin	HaCaT (human keratinocyte)	Epidermal keratinocyte line with innate immune function	Skin barrier and wound-healing studies, irritation and inflammation testing	Lacks immune cell crosstalk and 3D architecture	[93,94]
Lung	A549 (human alveolar epithelial)	Airway epithelial cell with inflammatory responsiveness	Respiratory inflammation, toxicity assessment	Limited immune receptor diversity	[95]
Brain	BV2 (mouse microglia)	Microglial immune cell analog	Neuroinflammation and neuroimmune signaling studies	Murine origin; differs from human microglia phenotype	[96]
Kidney	HK-2 (human proximal tubular epithelial)	Normal renal epithelial line; sensitive to oxidative/inflammatory stress	Nephrotoxicity, renal inflammation evaluation	Low immune gene expression; lacks *in vivo* nephron complexity	[97,98]
Endothelium	HUVEC (human umbilical vein endothelial cell)	Primary vascular endothelial model expressing adhesion molecules and eNOS	Cardiovascular, oxidative stress, and inflammation studies	Limited long-term stability; differs from microvascular phenotype	[99]
Immune	THP-1 (human monocyte/macrophage)	Human monocyte precursor; differentiates into macrophage-like cells	Cytokine release, phagocytosis, inflammation and immunomodulation assays	Requires differentiation; differs from primary macrophages	[84]

TEER, Transepithelial Electrical Resistance; CYP450, Cytochrome P450 enzyme family; HBV, Hepatitis B virus; eNOS, Endothelial nitric oxide synthase.

Although fish models are valuable as translational bridges based on certain immunological similarities with humans, they have limitations in directly predicting human applicability. Fish immune responses can vary considerably depending on environmental factors such as water temperature, salinity, and water quality. Moreover, fish possess physiological characteristics distinct from those of mammals, including skin and gill exposure, temperature-dependent metabolism, and fish-specific antibody systems [103,104]. Therefore, fish models are useful for understanding the biological functions of marine peptides and evaluating their initial *in vivo* efficacy, but they cannot directly substitute for human immune responses or pharmacokinetic profiles.

Mammalian models are the most widely used *in vivo* systems in human disease and drug development research. Mice and rats are genetically tractable, and their immune and metabolic systems are relatively well characterized. Therefore, they can be used to evaluate the efficacy and safety of marine peptides in models of inflammatory diseases, infection, cancer, and metabolic disorders [105,106]. However, mammalian models also fail to fully recapitulate the human immune system. Differences in immune cell composition, cytokine responses, microbiota, metabolism, drug absorption, and clearance across species can lead to the failure of preclinical findings to be reproduced in clinical settings [107,108]. In addition, high costs, ethical regulations, and the complexity of long-term experiments limit

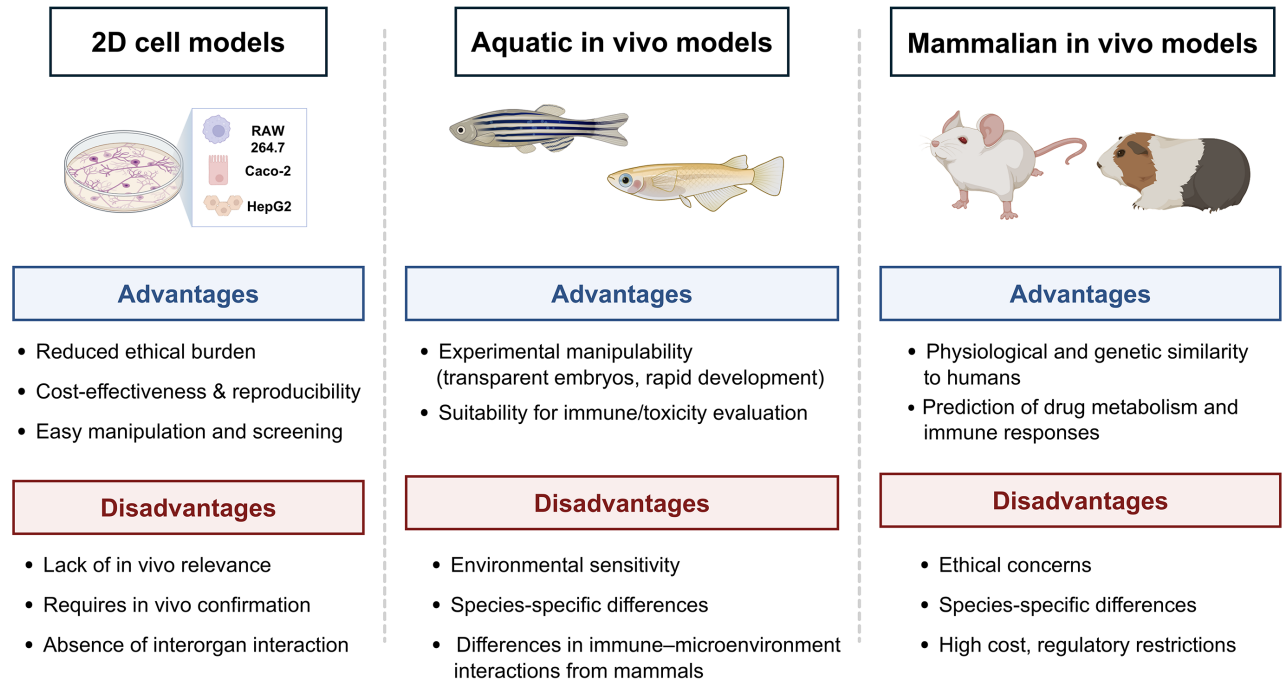

Fig. 5. Comparative overview of conventional immunological evaluation models, summarizing their relative strengths and limitations across experimental complexity, physiological relevance, and ethical feasibility. This figure was created using BioRender (BioRender Inc., Toronto, ON, Canada; https://www.biorender.com/).

the practical use of mammalian models. Therefore, fish and mammalian *in vivo* models are necessary for evaluating the organism-level effects of marine peptides, but human-relevant platforms that can address the limitations of conventional models are also required to predict their applicability to humans (Fig. 5).

Translational Limitations

Conventional *in vitro* and *in vivo* models each provide useful information, but they remain limited in directly linking the biological activity of marine peptides to human tissue-level responses. Two-dimensional cell models are useful for mechanistic screening, but their planar architecture cannot reproduce intercellular interactions, ECM-dependent signaling, or oxygen and nutrient gradients, and long-term culture may alter gene expression and cellular morphology [88,109]. Fish models are valuable for evaluating organism-level immune and toxicity responses, but extrapolation to humans is limited by temperature-dependent immune regulation, fish-specific antibody systems, and distinct exposure routes through the skin and gills [103,104,110]. Mammalian models are essential for preclinical evaluation, but species differences in immune-cell composition, cytokine responses, microbiota, metabolism, and drug absorption, distribution, metabolism, and excretion (ADME) pathways limit clinical predictability [107,111].

These limitations are particularly relevant to marine peptide research because peptide efficacy depends on multiple interconnected factors, including structural stability, protease resistance, intestinal absorption, tissue distribution, immune-cell targeting, and cytokine network modulation. Conventional models are often unable to evaluate these parameters in an integrated human tissue context, limiting their ability to predict whether *in vitro* or animal-based bioactivity will translate into human-relevant effects. Therefore, the translational development of marine-derived peptides requires evaluation platforms that better reproduce tissue architecture, epithelial barrier function, immune-cell interactions, and inflammatory microenvironments. Organoid-based systems can address part of this gap by recapitulating selected aspects of tissue-specific differentiation, epithelial organization, immune-cell co-culture, and disease-associated inflammatory responses, thereby enabling more tissue-relevant assessment of peptide transport, toxicity, anti-inflammatory activity, and immune-cell-modulatory functions [4,112]. In this context, 3D culture systems, organoids, and cross-validation strategies are increasingly emphasized as complementary tools for improving translational predictability [6,113].

Organoid-Based Models for Immunological Evaluation

Overview of Organoid Systems

Organoids are three-dimensional cellular structures generated through the self-organization of stem cells or progenitor cells that recapitulate the structural and functional

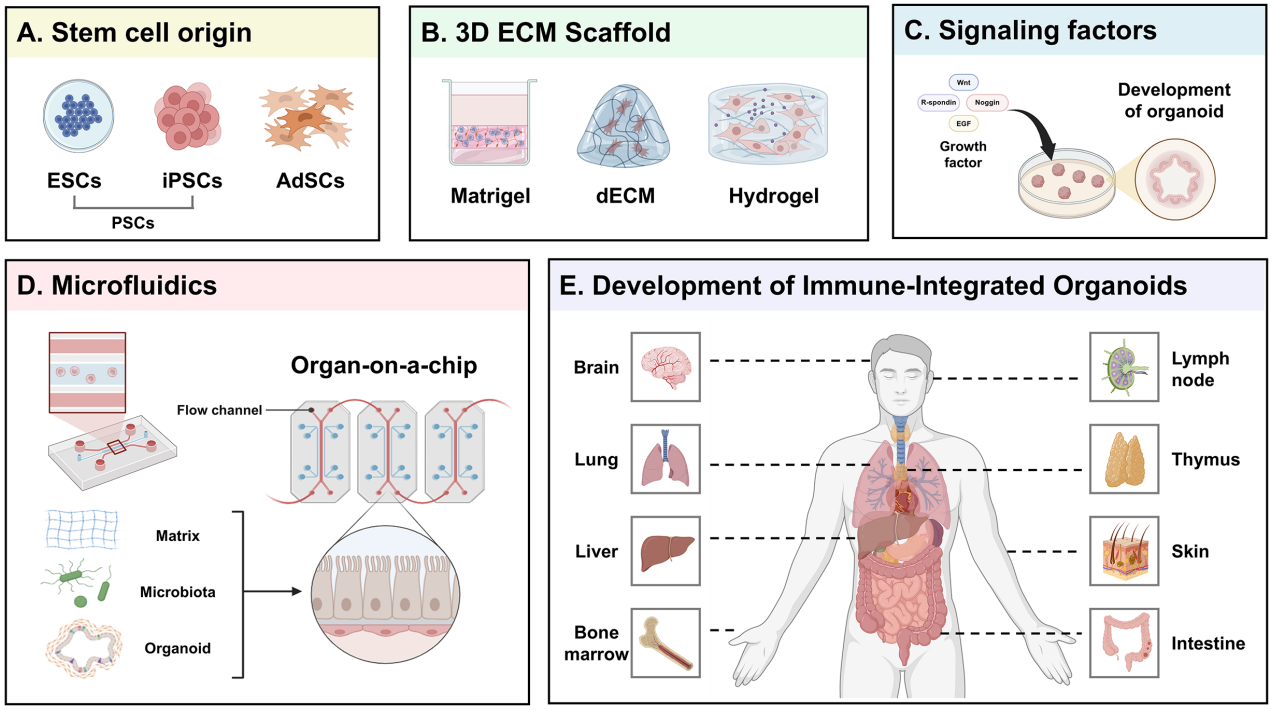

Fig. 6. Organoid technology as a next-generation platform for immunological evaluation. (A) Stem cell origin: Organoids are derived from pluripotent stem cells (PSCs), including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) or from adult stem cells (AdSCs), each providing distinct advantages for modeling organ development, tissue regeneration, and immune response. (B) 3D ECM scaffold: The extracellular matrix (ECM) scaffold, composed of natural (Matrigel, decellularized ECM) or synthetic (hydrogel) materials, provides structural and biochemical cues essential for self-organization and lineage differentiation. (C) Signaling factors: Growth factors and morphogens such as Wnt, R-spondin, and Noggin activate developmental signaling pathways that guide organoid formation and maturation, enabling tissue-specific structural and functional organization. (D) Microfluidics: Integration of organoids into microfluidic "organ-on-a-chip" systems allows dynamic control of oxygen, nutrient, and mechanical gradients, as well as co-culture with microbiota and immune cells, thereby enhancing physiological relevance and reproducibility. (E) Development of immune-integrated organoids: Immune-integrated organoid models have been reported or are being developed across multiple tissues, including the brain, lung, liver, bone marrow, intestine, skin, thymus, and lymph nodes, enabling comprehensive reconstruction of innate and adaptive immune interactions for disease modeling and immunotherapeutic evaluation. This figure was created using BioRender (BioRender Inc., Toronto, ON, Canada; https://www.biorender.com/).

complexity of native tissues *in vitro* [7]. They possess self-renewal and differentiation capacities, and their spatially organized cells form tissue-like microenvironments, including selected immunological features that resemble those of native organs. Accordingly, organoids are recognized as next-generation platforms capable of integratively evaluating tissue-specific immune responses, inflammatory signaling, infection dynamics, and drug responsiveness, which are difficult to recapitulate using conventional 2D cell lines or single-tissue models. They have been widely applied in developmental research, disease modeling, and the evaluation of drug efficacy and toxicity [4,112] (Fig. 6, Table 3).

Organoids can be classified according to their cellular origin into pluripotent stem cell (PSC)-derived organoids, adult stem cell (AdSC)-derived organoids, and patient-derived organoids (Table 4). PSC-derived organoids are useful for modeling developmental processes and early tissue differentiation, whereas AdSC-derived organoids relatively well preserve the characteristics and functions of mature tissues, such as the intestine, liver, lung, and skin [114,115]. Patient-derived organoids can retain donor-specific genetic and phenotypic characteristics and are therefore widely used in disease modeling, drug response prediction, and precision medicine research [116]. These organoid systems can be combined with extracellular matrix-based scaffolds, such as Matrigel or hydrogels, growth factors such as Wnt, R-spondin, and Noggin, and microfluidic platforms to more precisely reproduce tissue-specific structures and functions [117,118,119].

Therefore, organoids are important models for evaluating the functions of marine-derived immunomodulatory peptides at the tissue level rather than merely through cell-level markers. In particular, intestinal, pulmonary, and

Table 3. Comparative overview of 2D and 3D cell culture systems.

Category	2D cell culture	3D cell culture
Cell morphology	Flat monolayer growth on planar surfaces	Cells maintain natural morphology and grow in three-dimensional spheroids or aggregates
Cell junctions	Limited and non-physiological cell–cell contacts	Active cell–cell and cell–ECM interactions resembling *in vivo* tissues
Extracellular matrix (ECM)	Single-layer coatings such as collagen; limited reconstruction	Combination of natural and synthetic ECM components; tunable stiffness and tissue-specific architecture
Microenvironment	Homogeneous and static	Spatial gradients of oxygen, nutrients, and mechanical cues; dynamic microenvironment
Differentiation and heterogeneity	Predominantly single cell types with low differentiation	Multilineage differentiation and cellular heterogeneity reflecting native tissue organization
Drug/metabolic response	High drug sensitivity; limited metabolic activity	Drug diffusion and metabolic gradients mimic *in vivo* pharmacological behavior
Gene/protein expression	Expression profiles differ substantially from *in vivo* tissues	Transcriptomic and proteomic patterns closely resemble *in vivo* levels
Immunological relevance	Simplified innate immune responses; absence of complex immune networks	Representation of both innate and adaptive immune components and signaling pathways
Reproducibility & cost	Highly reproducible and cost-effective	Higher cost and technical complexity; standardization required

Table 4. Comparison of organoids derived from different stem cell origins.

Category	PSC-derived organoids	AdSC-derived organoids	Patient-derived organoids
Cell origin & differentiation potential	ESC/iPSC-based; pluripotent to all three germ layers; suitable for complex organ modeling	Tissue-specific adult stem cells; retain native architecture and function	Derived from patient tumors or tissues; preserve genetic and phenotypic heterogeneity
Immune microenvironment	Adaptive immunity via co-culture with immune cells	Innate immune axis (epithelial–macrophage) for inflammation studies	Patient-specific immune response and drug sensitivity via autologous co-culture
Applications	Developmental biology, disease modeling, immune cell differentiation, vaccine testing	Infection, inflammation, and regeneration studies in barrier organs	Precision medicine, drug response prediction, toxicity testing, personalized therapy
Advantages	High pluripotency, cellular diversity, organ-level function	Easy culture, high tissue fidelity, physiological relevance	Clinical translatability, personalized modeling accuracy

skin organoids are useful for evaluating barrier-associated responses, whereas hepatic organoids can support the assessment of metabolism and hepatotoxicity. These systems may therefore contribute to the tissue-relevant evaluation of peptide transport, toxicity, anti-inflammatory activity, and barrier-function modulation in human-relevant translational research.

Applications in Immune Evaluation

Organoid-based models have expanded from three-dimensional tissue reconstruction to tissue-relevant platforms for investigating immune responses and epithelial-immune interactions. Many immune responses are initiated at barrier tissues, including the intestine, lung, and skin, which are continuously exposed to antigens, pathogens, and inflammatory stimuli. Because these organs possess tissue-specific epithelial and immune compartments, immune-integrated organoids and organoid–immune-cell co-cultures can provide physiologically relevant platforms for examining epithelial-immune crosstalk and inflammatory responses [120]. For example, macrophage–intestinal-organoid co-culture models have enabled the simultaneous assessment of macrophage polarization, inflammatory signaling, epithelial injury, and barrier restoration

[121]. Moreover, combining organoids with immune cells, pathogen exposure, or inflammatory stimulation allows barrier integrity, cytokine responses, immune-cell behavior, and tissue recovery to be evaluated within a multicellular context that is difficult to reproduce using conventional 2D cultures [122,123]. Accordingly, these platforms may facilitate the mechanistic evaluation of marine-derived peptides with antioxidant, anti-inflammatory, and immune-cell-modulatory activities under tissue-relevant conditions.

Intestinal Organoids

The intestine is one of the most important barrier organs in the human immune system, and the intestinal mucosa contains spatially organized epithelial, stromal, and immune-cell populations that collectively regulate barrier defense, immune tolerance, and responses to dietary and microbial antigens [124].

The intestinal epithelium is composed of enterocytes, goblet cells, Paneth cells, and other specialized cell types, and it participates in innate immune responses not only by forming a physical barrier but also through antimicrobial substance secretion, mucus layer formation, and cytokine signaling. Intestinal organoids can reproduce these intestinal epithelial structures and functions *in vitro*, making them suitable models for evaluating mucosal immunity and barrier function [125]. Adult intestinal stem cells can generate self-organizing crypt–villus-like epithelial structures containing differentiated intestinal cell lineages, and human adult stem-cell-derived epithelial organoids can be maintained and expanded over prolonged culture periods [126]. In parallel, pluripotent stem cells can be directed to form intestinal organoids containing epithelial and mesenchymal components, providing a model of human intestinal development [127]. More recently, the incorporation of macrophages into intestinal organoids has enabled direct investigation of epithelial-immune crosstalk, inflammatory signaling, epithelial injury, and barrier recovery. For example, macrophage-augmented intestinal organoid models have been shown to reproduce NF-κB activation and increased expression of TNF-α, IL-1β, and IL-6 following infection, suggesting their applicability for evaluating inflammatory bowel disease and candidate anti-inflammatory compounds [123].

These features make intestinal organoids particularly relevant for evaluating marine-derived immunomodulatory peptides. Because many marine peptides are being developed for oral, functional food, or nutraceutical applications, their intestinal absorption, epithelial barrier protection, cytokine regulation, and contribution to mucosal immune homeostasis should be evaluated together. Therefore, intestinal organoids can serve as key platforms for determining whether marine peptides exert anti-inflammatory and barrier-restorative effects in an intestinal mucosa-like environment, beyond simply demonstrating anti-inflammatory activity in macrophage-based 2D models.

Lung Organoids

The lung is a barrier tissue that is in direct contact with the inhaled environment and is continuously exposed to pathogens, pollutants, and oxidative stress. The pulmonary epithelium interacts with various immune cells, including macrophages, dendritic cells, and T cells, and participates in host defense against infection, inflammatory regulation, and tissue repair [128,129]. Lung organoids can recapitulate this epithelial-immune microenvironment and are used as important platforms for studying respiratory infections and inflammatory lung diseases [129].

Lung organoids can be generated from adult stem cells or pluripotent stem cells and may contain lung tissue-specific cell types, such as ciliated cells, mucus-producing cells, and alveolar epithelial cells. AdSC-derived lung organoids are useful for evaluating inflammatory cytokine responses in bronchial or nasal epithelium, whereas PSC-derived lung organoids contain both epithelial and mesenchymal cells and are suitable for analyzing IFN responses, NF-κB signaling, antiviral defense, and tissue regeneration [129,130]. In particular, studies using induced pluripotent stem cells (iPSC)-derived alveolar organoids have identified alveolar type II cells as key cells that induce type I IFN responses following viral infection, demonstrating that lung organoids can be used to evaluate not only infection responses but also the dynamics of antiviral immune responses [131,132]. They have also been applied to models of immune-mediated lung diseases, such as chronic obstructive pulmonary disease (COPD) and other inflammatory or fibrotic lung diseases, for evaluating anti-inflammatory candidates and immunotherapeutic responses [133,134,135]. In this regard, lung organoids may serve as useful models for evaluating whether the antioxidant and anti-inflammatory effects of marine-derived peptides can regulate respiratory epithelial injury, cytokine imbalance, and oxidative stress.

Skin Organoids

The skin is a primary defensive barrier against the external environment and maintains immune homeostasis and tissue repair through interactions among keratinocytes, fibroblasts, Langerhans cells, macrophages, and T cells. Skin organoids can reproduce the multicellular structure of skin tissue and are therefore used in studies of skin inflammation, infection, wound repair, and immune-mediated diseases. Recent advances in iPSC- and AdSC-based skin organoid technologies have enabled the development of complex models that include not only epidermal and dermal architecture but also vascular and immune components. For example, vascularized skin organoids have been proposed as platforms for studying cutaneous inflammation, infection, and graft-associated immune responses by integrating CD45-positive hematopoietic cells, CD68-positive macrophages, and CD207-positive Langerhans-like cells into skin structures [136]. In addition, psoriasis-like skin

models reproduced a Th17-dependent cytokine network, IL-6, IL-8, IL-23A, and antimicrobial peptide expression following stimulation with IL-17A and TNF-α, and treatment with bimekizumab or adalimumab normalized inflammatory cytokine and AMP expression [137]. These findings demonstrate that skin organoids can be used to evaluate cytokine networks and immunotherapeutic responses in immune-mediated skin diseases.

Skin organoids are also relevant for evaluating marine-derived peptides. Because some marine peptides exhibit antioxidant, anti-inflammatory, tissue-regenerative, and wound-healing activities, skin organoids can be used to evaluate oxidative stress suppression, inflammatory cytokine reduction, epithelial repair, and barrier restoration at the tissue level. Therefore, skin organoids can serve as translational models for evaluating whether marine-derived peptides can attenuate skin barrier dysfunction and inflammatory responses, beyond simple assessment of anti-inflammatory or antioxidant activity.

Application to Marine Peptides

Organoid technology is increasingly used to evaluate the tissue-level actions of immunomodulatory compounds. Compared with conventional 2D cell cultures and animal models, organoids can model selected aspects of tissue architecture, epithelial barrier organization, cytokine responses, metabolic activity, and epithelial-immune interactions. These features make organoid systems useful for determining whether candidate compounds exert biological effects at the tissue level, rather than only producing changes in isolated cellular markers. In particular, under specific culture conditions, patient-derived organoid systems can retain selected tumor-associated stromal or tissue-resident immune compartments and support the evaluation of patient-specific therapeutic responses [138].

In a model in which patient-derived lung cancer organoids were co-cultured with peripheral blood mononuclear cells using a gel–liquid interface method, treatment with an anti-PD-1 antibody induced T-cell infiltration and tumor-cell apoptosis, which showed a strong association with actual patient responses [139]. In addition, treatment of pancreatic cancer organoids with the STING agonist D166 activated STING-associated signaling in a time- and dose-dependent manner and increased IL-6 and CXCL10 expression [140]. These studies demonstrate that organoids can be used not only as models for evaluating drug efficacy but also as platforms for analyzing complex immune mechanisms involving immune cell responses and cytokine regulation.

Although direct studies on marine-derived immunomodulatory peptides remain limited, organoid studies using bioactive peptides and marine natural products provide useful precedents for evaluating diverse bioactive compounds at the tissue level (Table 5, Ref. [140,141,142,143,144,145,146]). The phycocyanin-derived bioactive peptide PCP3 (MFDAFTK) attenuated lipopolysaccharide-induced inflammation in murine intestinal organoids by reducing inflammatory mediators and oxidative stress while restoring the expression of tight-junction and intestinal epithelial-cell markers [147]. In addition, trabectedin, a non-peptidic marine-derived compound, has been evaluated in organoid-based cancer models, further demonstrating the feasibility of using organoids to assess tissue-level responses to marine bioactive compounds [73,148].

This organoid-based evaluation framework is particularly relevant to marine-derived immunomodulatory peptides because their intended applications often involve tissue-level processes that cannot be fully captured by macrophage-based 2D assays. For orally administered functional foods or nutraceuticals, intestinal models should evaluate peptide stability, epithelial transport, tight-junction integrity, mucosal cytokine responses, barrier recovery, and tissue-level inflammatory resolution. For skin, lung, or liver applications, organ-specific models can be used to assess wound repair, respiratory inflammation, oxidative injury, peptide metabolism, and toxicity. Therefore, organoids can help determine whether marine peptides retain functional activity within tissue-relevant microenvironments and whether their effects involve coordinated regulation of barrier function, cytokine networks, immune-cell interactions, and tissue repair.

Despite this potential, studies directly applying marine-derived immunomodulatory peptides to organoid-based immune evaluation remain limited. This gap is important because peptide activity can be strongly affected by protease stability, tissue exposure, epithelial transport, local metabolism, and immune-cell crosstalk. Future studies should therefore combine organoid systems with immune-cell co-culture, apically accessible intestinal formats, and organoid-on-a-chip technologies to evaluate peptide delivery, barrier responses, cytokine regulation, tissue repair, and safety under controlled tissue-relevant conditions. Microfluidic organoid-on-a-chip systems can provide controlled luminal flow and mechanically active culture conditions while supporting tissue-specific epithelial differentiation and quantitative assessment of barrier function [149]. Thus, organoid-based evaluation can provide a more coherent translational strategy for determining whether marine-derived peptides can progress from simple bioactive candidates to functional foods, nutraceuticals, or immunomodulatory therapeutic candidates.

Translational Integration

Marine-derived peptides are bioactive molecules originating from the distinct physiological and immunological environments of aquatic organisms. However, biological activities observed in fish and other aquatic species cannot be assumed to occur identically in humans because of interspecies differences in immune architecture, metabolism,

Table 5. Representative studies employing organoid-based models for immunological evaluation of drugs and natural products.

Compound	Organoid model	Key immunological mechanism/marker	Outcomes	Ref
STING agonist D166	Human pancreatic tumor organoids and organoid-immune-cell co-culture	cGAS-STING activation, T-cell activation, macrophage polarization, immune-cell infiltration	Activated STING signaling and reshaped the pancreatic tumor immune microenvironment	[140]
Epigenetic inhibitors	Mouse- or patient-derived breast tumor organoids + tumor-specific cytotoxic T cells	MHC-I upregulation, antigen presentation, T-cell-mediated cytotoxicity	Enhanced antigen presentation and potentiated T-cell-mediated tumor-cell killing	[141]
Tofacitinib (JAK inhibitor)	Human colonoid + TNF-α/IFN-γ stimulation	Inhibition of p-STAT1/3; reduced IL-6, IL-8, CXCL10; restored ZO-1 expression	Prevented cytokine-induced epithelial barrier damage and normalized JAK–STAT signaling	[142]
Moringa oleifera leaf extract	Human intestinal organoid + TNF-α stimulation	Suppression of IL-6, IL-1β, TNF-α expression	Reduced TNF-α–induced inflammation in intestinal organoids; demonstrated anti-inflammatory activity consistent with *in vivo* colitis model	[143]
Aloe emodin (*Aloe vera anthraquinone*)	Human intestinal organoid	Regulation of FFAR1 signaling; modulation of enteroendocrine cell differentiation	Preserved $SOX9^{+}$ label-retaining cells; promoted epithelial regeneration and mucosal healing	[144]
Betulinic acid (birch triterpenoid)	Mouse intestinal organoid–macrophage co-culture	Activation of PPAR-γ; inhibition of NF-κB; upregulation of tight-junction proteins; modulation of inflammatory cytokines	Restored intestinal barrier integrity and alleviated inflammation via PPAR-γ/NF-κB pathway	[145]
STW 5-II (multi-herbal preparation)	Mouse intestinal organoid (cytokine + LPS/flagellin-induced inflammation)	Inhibition of pNF-κB, pSTAT1, and iNOS; restoration of ZO-1 expression; reduced FITC permeability	Attenuated cytokine-induced inflammation and preserved epithelial barrier integrity through multi-target NF-κB/STAT1/iNOS modulation	[146]

STING, Stimulator of Interferon Genes; MHC-I, Major Histocompatibility Complex Class I; JAK, Janus Kinase; IFN, Interferon; p-STAT, Phosphorylated Signal Transducer and Activator of Transcription; CXCL, C-X-C motif chemokine ligand; ZO, Zonula Occludens; FFAR, Free Fatty Acid Receptor; SOX, SRY-box transcription factor; PPAR, Peroxisome Proliferator-Activated Receptor; iNOS, Inducible Nitric Oxide Synthase; FITC, Fluorescein Isothiocyanate.

microbiota, tissue organization, and environmental adaptation [150,151]. Therefore, evaluating the potential human applications of marine-derived peptides requires a stepwise fish-to-human validation strategy capable of distinguishing evolutionarily conserved peptide responses from species-specific responses, rather than relying on direct interspecies extrapolation.

Although organoids have been proposed as next-generation platforms for immunological and toxicological assessment, fish organoid research remains less developed than mammalian organoid research (Fig. 7) [152]. Many currently available fish-derived 3D models consist of spheroids, epithelial cell aggregates, cell-line-derived cultures, or tissue-explant-like systems originating from the gill, intestine, or liver. These models should therefore be distinguished from stem- or progenitor-cell-derived organoids in which self-renewal, multilineage differentiation, and tissue-level self-organization have been experimentally validated. Nevertheless, these models represent an important advance over conventional monolayer cultures. For example, Holgersson et al. [153] established three-dimensional spheroids using the rainbow trout liver cell line RTL-W1 and demonstrated that these cultures maintained viability and metabolic activity during extended culture, developed tissue-like ultrastructural features, and exhibited increased expression of genes associated with xenobiotic and lipid metabolism. In particular, *cyp1a* expression and CYP1A-dependent ethoxyresorufin-O-deethylase activity were higher in spheroids than in monolayer cultures, and the two culture systems showed distinct responses to selected toxicants [153]. These findings demonstrate that three-dimensional organization can substantially influence the metabolic and toxicological properties of fish-derived *in vitro* models.

Recent advances in comparative thymus biology provide an important perspective for future fish immune-organoid research. Nusser et al. [154] investigated the developmental trajectories and evolutionary origins of thymic

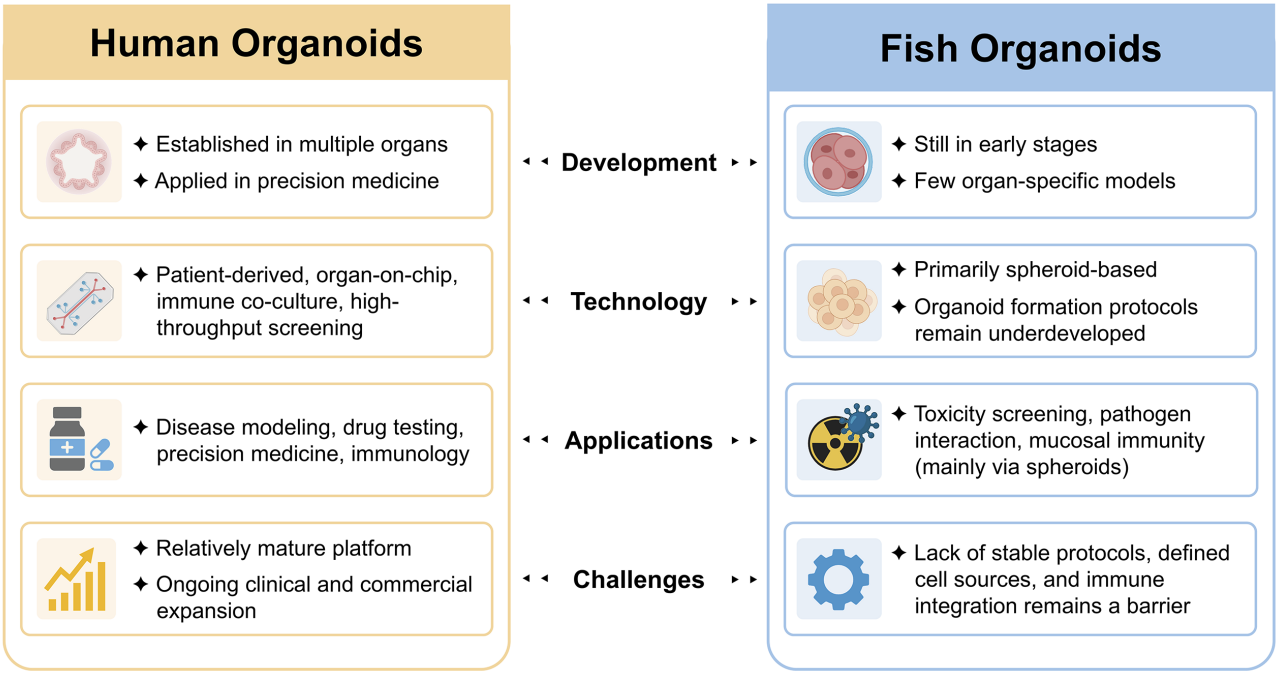

Fig. 7. Comparative overview of human and fish organoid systems. This figure was created using BioRender (BioRender Inc., Toronto, ON, Canada; https://www.biorender.com/).

mimetic cells and demonstrated that these cells emerge through two successive developmental waves within the mouse thymic microenvironment. The authors also reconstructed thymic microenvironments using evolutionarily ancient *FOXN1/4*-family genes, including *FOXN* genes derived from cartilaginous fish, and showed that thymic epithelial programs exhibit both conserved and divergent features across vertebrate evolution. Furthermore, cells expressing peripheral-tissue-associated genes were identified in the thymus of cartilaginous fish and the thymoid of lampreys, suggesting that mechanisms associated with central immune tolerance emerged early in vertebrate evolution [154]. Although this study did not establish a fish organoid in the strict sense, it demonstrates that cross-species microenvironmental reconstruction and developmental-trajectory analysis can reveal conserved and species-specific programs of immune-organ development. It also provides a conceptual and methodological basis for designing fish thymic organoid systems aimed at modeling epithelial differentiation, mimetic-cell formation, antigen-presentation programs, and lymphocyte developmental trajectories.

Therefore, next-generation fish organoid models should extend beyond barrier-function or toxicological applications toward the reconstruction of immune-related tissues, such as the thymus, head kidney, spleen, gill, and intestine. To validate these models, it will be necessary to establish high-resolution reference atlases of native fish immune tissues using single-cell RNA sequencing, single-nucleus RNA sequencing, spatial transcriptomics, lineage tracing, and proteomics. Such reference maps would allow the cellular composition, differentiation states, epithelial-immune niches, cytokine networks, and immune activation programs of fish organoids to be compared with those of native tissues [155,156]. Thymic models should focus particularly on FOXN-dependent epithelial differentiation, mimetic-cell populations, antigen-presentation pathways, and T-cell developmental programs. In head kidney, gill, and intestinal models, interactions among hematopoietic and lymphoid cells, epithelial barrier integrity, immunoglobulin T (IgT)-associated mucosal immunity, cytokine responses, oxidative-stress regulation, and interactions with microorganisms or immune cells could be incorporated as major functional readouts.

From this perspective, fish-derived three-dimensional models and fish organoids could serve as early-stage platforms for evaluating the dose–response relationships, environmental stability, barrier-protective activity, pathogen defense, oxidative-stress regulation, and evolutionarily conserved signaling pathways of marine peptides, including NF-κB, MAPK, JAK/STAT, and Nrf2. Candidate peptides exhibiting reproducible and non-cytotoxic activities in fish models should subsequently be evaluated in corresponding human intestinal, hepatic, pulmonary, skin, or immune-organoid systems.

This fish-to-human validation framework could be further strengthened through integration with organoid-on-a-chip systems, immune-cell co-culture, and microfluidic environmental-control technologies [157]. These systems would enable dynamic assessment of peptide delivery,

metabolism, cytokine secretion, immune-cell migration, and tissue repair under controlled oxygen, nutrient, temperature, and flow conditions. Fish-derived models could therefore be used to evaluate the biological context and initial efficacy of marine peptides, whereas human organoids could validate their functionality, safety, and translational relevance. Ultimately, the integration of fish and human organoid platforms may provide a mechanism-based translational pathway for advancing marine-derived peptides across applications ranging from aquaculture-oriented bioactive materials to functional foods, nutraceuticals, and immunomodulatory therapeutic candidates.

Bridging Fish Models and Human Applications

Marine-derived immunomodulatory peptides exhibit antioxidant, anti-inflammatory, barrier-protective, and immune-cell-modulatory activities, but their relevance to human health cannot be inferred solely from activity observed in fish or other marine organisms. A translational framework is therefore needed to connect source-organism biology, peptide structure, conserved immune mechanisms, and human tissue-level validation using organoids, multi-omics approaches, and organoid-on-a-chip systems.

Key Insights

A key insight of this review is that marine-derived peptides should not be regarded merely as antimicrobial or antioxidant substances but rather interpreted as immunomodulatory candidates capable of regulating immune homeostasis at the tissue level. Peptides derived from fish, mollusks, crustaceans, seaweeds, and microalgae reflect distinct ecological pressures and immune adaptation strategies, and their functions are associated with antimicrobial defense, oxidative stress regulation, inflammatory suppression, immune cell activation, and barrier tissue repair. In particular, examples such as piscidin, hepcidin, Myticin C, crustins, penaeidins, and algal peptide hydrolysates demonstrate that marine peptides are not merely bioactive components but can act as molecular regulators of host defense and immune balance.

In addition, the translational potential of marine peptides is determined not only by their origin or biological activity itself but also by the clarity of their structure–function relationships and mechanisms of action. Structural characteristics such as charge distribution, hydrophobicity, amphipathicity, molecular weight, disulfide bonds, and cyclic structures determine peptide stability, target specificity, protease resistance, and immune cell interactions. These structural features can be linked to conserved immune and stress signaling pathways, such as NF-κB, MAPK, JAK/STAT, and Nrf2, enabling comparative immunological interpretation between fish and humans.

Conventional 2D cell models and animal models are useful for the initial functional evaluation of marine peptides, but they do not sufficiently recapitulate the complex immune microenvironment of human tissues. In contrast, organoids can integratively analyze epithelial barriers, tissue-specific differentiation, cytokine responses, and immune-cell co-culture, making them suitable for validating the tissue-level functions of marine peptides. Under specific culture conditions, human organoids containing autologous tissue-resident immune populations can enable analysis of tissue-specific epithelial-immune interactions. Macrophage–intestinal-organoid co-cultures can further support assessment of inflammatory crosstalk and barrier recovery [120,121].

Together, these insights indicate that the translational value of marine-derived immunomodulatory peptides should be defined not simply by source organism or isolated bioactivity, but by reproducible tissue-level immune regulation in human-relevant systems. Organoid-based platforms can bridge simplified cell assays, animal models, and human applications by integrating barrier function, cytokine networks, immune-cell interactions, and tissue repair. Thus, marine peptide research should move toward a unified evaluation framework linking marine biodiversity, peptide structure, immunological mechanism, and human tissue-level validation.

Translational Challenges

A major challenge in fish-to-human translation of marine peptides is interspecies physiological differences. Fish have developed unique immune and metabolic systems through adaptation to aquatic environments characterized by water temperature, salinity, oxygen concentration, and pathogen pressure. For example, fish possess an IgT-centered mucosal immune system and gut microbiota composition distinct from those of humans, and these differences may alter immune responses to peptides [151,158,159]. In addition, fish differ from mammals in amino acid and lipid metabolic pathways, oxygen consumption rates, and responses to environmental stress [160]. Therefore, anti-inflammatory or antioxidant effects observed in fish should not be interpreted as directly reproducible in humans. Instead, conserved responses should be distinguished from species-specific responses.

Differences in the models themselves also represent an important limitation. Fish organoids can be used as early-stage platforms in marine peptide research to evaluate dose-response relationships, stability under environmental stress conditions, and activation of conserved signaling pathways. However, fish-derived models may differ from human tissues in structure, cellular composition, and physiological function, and these differences can vary across organs. For example, some cellular components important in human intestinal organoid research, such as Paneth cells, are absent in the fish intestine [161].

Technical standardization also represents an important challenge. Currently, aquatic cell models are generally

maintained at relatively lower culture temperatures and under variable oxygen and salinity levels, and these factors can influence peptide stability, cellular uptake, metabolic rate, and immune signaling [162,163]. In addition, Dolgos et al. [164] demonstrated that extracellular matrix composition and culture format can influence cell lineage representation and transcriptional programs in patient-derived organoids. These findings highlight the need to document and standardize matrix conditions when conducting cross-species comparisons of fish and human three-dimensional models. In human organoid research, high-resolution analytical technologies such as transepithelial electrical resistance (TEER), single-cell transcriptomics, proteomics, high-throughput screening, and high-content analysis are widely used, whereas studies using aquatic systems often remain limited to morphological evaluation [152]. Therefore, standardized analytical methods and automated technologies are warranted to improve the reproducibility and quantitative rigor of fish organoid-based evaluation.

Finally, the human application of marine peptides requires consideration of not only efficacy but also safety, bioavailability, metabolic stability, delivery routes, and regulatory criteria. For marine peptides to be developed into functional foods, nutraceuticals, or anti-inflammatory therapeutic candidates, their evaluation must extend beyond demonstrating cytokine reduction in macrophage-based 2D assays and instead integrate validation of intestinal absorption, hepatic metabolism, barrier recovery, immune-cell crosstalk, and tissue repair. For this reason, a cross-validation framework based on common readouts is needed, rather than simple transfer of data from fish models to human organoids. Such a framework would help distinguish conserved peptide responses from species-specific effects while improving the reliability of human translational prediction.

Future Directions

Future research on marine-derived immunomodulatory peptides should move beyond natural-product discovery toward a precision biomedical framework integrating molecular, cellular, tissue-level, and environmental contexts. Four technical priorities should be pursued within an overarching One Health framework.

First, precise cellular maps of fish immune tissues should be established and subsequently used to guide the development and validation of fish-derived three-dimensional models. Single-nucleus transcriptomic profiling of the Atlantic salmon gill has provided an important foundation for identifying the cellular composition and immune-signaling pathways involved in mucosal immunity [155]. In addition, the fish-derived gill epithelial cell line ASG-10 retains xenobiotic biotransformation capacity associated with cytochrome P450 (CYP) and UDP-glucuronosyltransferase (UGT) activity and can therefore support mechanistic evaluation of metabolism and toxicity [165,166]. Future research should extend single-cell and spatial-omics approaches to the thymus, head kidney, spleen, gill, and intestine to establish reference maps of tissue-specific immune-cell populations, epithelial-immune niches, cytokine networks, and activation states. Metabolic and toxicological responses observed in fish-derived cell lines and spheroid models should also be compared with those of fish organoids to determine the extent to which emerging three-dimensional models reproduce the cellular composition, differentiation states, and immune functions of native tissues. These reference maps and validation criteria would provide a foundation for interpreting tissue-specific immunological and metabolic responses to marine-derived peptides.

Second, the dynamic mechanisms through which marine-derived peptides act within the organoid microenvironment should be resolved at single-cell spatial and temporal resolution. Integrating single-cell RNA sequencing and spatial transcriptomics with time-course peptide exposure, lineage tracing, and proteomics would allow early- and late-responding cell populations to be distinguished and signaling interactions among epithelial cells, macrophages, dendritic cells, and lymphocytes to be traced. A key question is whether a peptide directly modulates NF-κB, MAPK, JAK/STAT, or Nrf2 signaling within specific cell populations or acts indirectly through cytokine secretion and intercellular communication. The spatial distribution of these responses across regions of barrier disruption, inflammation, and tissue regeneration should also be examined. The recent application of spatial transcriptomics across multiple organoid types to generate high-resolution spatial whole-transcriptome reference data demonstrates the technical feasibility of this approach [167]. Such analyses would shift peptide evaluation from single time-point average responses toward dynamic, cell-state-specific and spatially resolved mechanisms of action. Where appropriate, these omics-guided analyses may be combined with immune-cell co-culture or organoid-on-a-chip platforms to evaluate cytokine secretion, immune-cell migration, barrier integrity, and tissue-level responses under more physiologically relevant conditions.

Third, a parallel and application-specific comparative framework should be established to connect fish and human organoid systems. Because marine-derived immunomodulatory peptides are closely associated with the physiological and immunological environments of aquatic organisms, fish-derived organoids provide important value for analyzing aquatic-species-specific responses that are difficult to assess using human-derived organoids alone. In particular, fish organoids can be used to evaluate how peptides act under conditions related to water temperature, salinity, aquatic pathogens, environmental contaminants, epithelial barrier injury, oxidative stress, and fish-specific immune signaling. Therefore, for candidate peptides intended for aquaculture and aquatic-animal health applications, fish organoids may

provide directly relevant information on species-specific efficacy, toxicity, barrier protection, pathogen defense, and environmental stress responses.

For candidates intended for human application, absorption, metabolism, toxicity, anti-inflammatory activity, barrier responses, and immune-cell interactions require additional independent validation using corresponding human intestinal, hepatic, pulmonary, skin, or immune-organoid systems. In this context, fish organoids can be used not simply as screening tools for predicting human efficacy but as reference platforms for interpreting early biological responses in the aquatic biological context from which marine peptides originate and for comparing these responses with those observed in human-derived models. For example, when common quantitative readouts, such as barrier integrity, cytokine profiles, oxidative-stress markers, immune-cell states, metabolic stability, and tissue repair, are applied to both fish and human organoids, it may be possible to distinguish whether specific peptide responses reflect conserved mechanisms across species or responses specific to aquatic organisms or human tissues. In addition, integrative comparison of single-cell and spatial-omics data can help analyze how peptide-induced changes in cell states, signaling pathways, and tissue-microenvironment remodeling differ among species. Thus, the use of fish organoids before or in parallel with human organoid-based evaluation is meaningful for assessing aquatic-species-relevant efficacy, distinguishing conserved immunomodulatory mechanisms from species-specific responses, and selecting appropriate downstream evaluation models according to the intended application of each candidate peptide.

Fourth, artificial intelligence (AI)-based peptide discovery, big-data analytics, and peptidomics approaches should be incorporated into candidate screening, de novo design, and sequence optimization. Although conventional marine peptide research has largely relied on empirical bioactivity screening, machine-learning and deep-learning approaches can enable the prediction of immunomodulatory activity, receptor binding, protease stability, tissue permeability, toxicity, and potential immunogenicity from peptide sequences, physicochemical descriptors, and predicted structural features [168]. These computational strategies may also facilitate the prioritization of candidates from marine peptidomic datasets and guide rational sequence modification to improve stability, selectivity, and bioavailability. AI-selected or AI-optimized candidates should subsequently be evaluated in fish and human organoid systems to assess dose–response relationships, barrier protection, immune signaling, metabolic stability, tissue-specific activity, and toxicity. The resulting experimental data can then be incorporated back into predictive models to establish an iterative design–validation–learning workflow. Particular attention should be paid to training-data quality, species-related bias, model interpretability, and whether computationally predicted activities are reproduced in tissue-level experimental systems.

Finally, these technical advances should be integrated within a One Health framework encompassing aquatic-animal health, human health, and marine-ecosystem health. From an aquatic-animal health perspective, research should determine whether marine-derived peptides can improve barrier immunity, infection resistance, oxidative-stress control, and tissue repair in farmed fish while reducing dependence on conventional antibiotics [169,170]. From a human-health perspective, activities identified in fish models should be cross-validated in human organoids to determine their efficacy, safety, and bioavailability as functional foods, nutraceuticals, or immunomodulatory therapeutic candidates. Fish-derived models can be used in parallel to clarify aquatic-species-specific responses and to identify mechanisms that may be compared across vertebrate systems. From an ecosystem-health perspective, evaluation should encompass the sustainable sourcing of marine resources and valorization of fishery by-products, as well as the environmental persistence of peptides and their delivery systems, toxicity toward non-target organisms, and potential effects on aquatic microbiota and antimicrobial-resistance selection pressure. Within this framework, shared efficacy, safety, and environmental-impact endpoints across fish-derived models and human organoids could provide a practical strategy for connecting aquaculture disease management, human immune and nutritional health, and marine-ecosystem sustainability.

Conclusion

Marine-derived immunomodulatory peptides represent a promising class of bioactive molecules with the potential to regulate host defense, inflammatory responses, barrier function, and immune homeostasis owing to their structural diversity and multifunctional activities. However, evidence for their immunological efficacy and safety remains largely limited to conventional cell cultures and animal models, necessitating rigorous evaluation of their bioavailability, tissue-level biological relevance, cross-species reproducibility, and relevance to human physiology beyond source-based classification and simplified bioactivity assays. Organoid-based platforms can recapitulate key aspects of tissue-specific epithelial, metabolic, and immune microenvironments, enabling the activity, bioavailability, and safety of marine peptides to be evaluated under physiologically relevant conditions. In particular, the complementary application of fish-derived and human organoids may help distinguish evolutionarily conserved immunomodulatory responses from species-specific effects and improve the reliability of fish-to-human translational assessment. This integrated validation framework can determine whether bioactivities observed in conventional models are reproduced in physiologically relevant tissue envi-

ronments and provide evidence to support the development of marine-derived peptides as functional foods, nutraceuticals, and immunomodulatory therapeutic candidates. Ultimately, integrating marine peptide research with organoid-based validation can advance fish-to-human translational research by cross-validating immunomodulatory activities identified in fish-derived models using human organoids, thereby supporting a One Health framework that bridges aquatic biomedicine and human health.

Availability of Data and Materials

Not applicable.

Author Contributions

CMK: Writing – original draft, Conceptualization, Data curation. EBL: Writing – original draft, Writing – review & editing, Formal analysis, Funding acquisition, Supervision. Both authors gave final approval of the version to be published. Both authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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