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Vitamin D-Induced Antimicrobial Peptides as Anti-Biofilm Agents: From Mechanisms to Therapeutic Opportunities

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Abstract

Biofilm-associated infections remain among the most persistent global health challenges because of their inherent resistance to antibiotics and ability to evade host immune responses. Antimicrobial peptides (AMPs), especially those induced by vitamin D, have emerged as promising innate immune effectors with significant anti-biofilm activity. Vitamin D regulates the expression of key AMPs, including cathelicidin (LL-37) and human β-defensins, through vitamin D receptor-mediated transcriptional activation, thereby strengthening epithelial and immune defenses against microbial biofilms. This review synthesizes current understanding of the vitamin D signaling pathway, vitamin D-responsive AMPs, and their mechanisms of action against biofilms produced by clinically significant pathogens. Evidence from *in vitro*, *in vivo* and clinical studies is critically assessed, and emerging therapeutic approaches, such as vitamin D supplementation, topical applications, AMP analogs, and synergistic antimicrobial combinations are examined. Despite notable progress, challenges remain regarding AMP stability, delivery, and safety. The review concludes by outlining future directions, including engineered vitamin D analogs, nanotechnology-based delivery systems, and AMP-centered precision therapies as next-generation strategies to combat biofilm-associated infections.

Keywords: Vitamin D; Antimicrobial peptides (AMPs); Cathelicidin LL-37; β-defensins; Biofilms; Innate immunity; Anti-biofilm therapy.
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1. Introduction

Biofilms are structured, surface-attached microbial communities embedded in a self-produced extracellular polymeric substance (EPS) composed of polysaccharides, proteins, lipids, and extracellular DNA (eDNA). Biofilm formation is now recognized as the dominant lifestyle for many bacteria and fungi in clinical environments. Estimates from the National Institutes of Health and subsequent analyses indicate that 60-80% of human microbial infections, as well as the majority of chronic infections, involve a biofilm component.[1-4] These infections include chronic wound infections, osteomyelitis, chronic rhinosinusitis, cystic fibrosis lung disease, recurrent urinary tract infections, endocarditis, periodontitis, as well as many infections associated with implanted medical devices such as catheters, prosthetic joints, and heart valves.[5,6]

The clinical significance of biofilms is primarily attributed to their high tolerance to antimicrobials and host immune responses. Within mature biofilms, steep gradients of nutrients, oxygen, and pH create heterogeneous microenvironments that support slow-growing or dormant microbial subpopulations, which are inherently less susceptible to antibiotics. The EPS matrix functions as a diffusion barrier, sequestering or inactivating antimicrobials, while elevated cell densities facilitate quorum sensing, horizontal gene transfer, and thus the development of multidrug resistance.[4,5] Furthermore, biofilm-embedded cells can differentiate into "persister" cells that survive otherwise lethal antibiotic exposures and subsequently repopulate the biofilm following treatment cessation. These characteristics account for the frequent failure of conventional antibiotic regimens to eradicate biofilm-associated infections, often resulting in relapse and necessitating surgical intervention.

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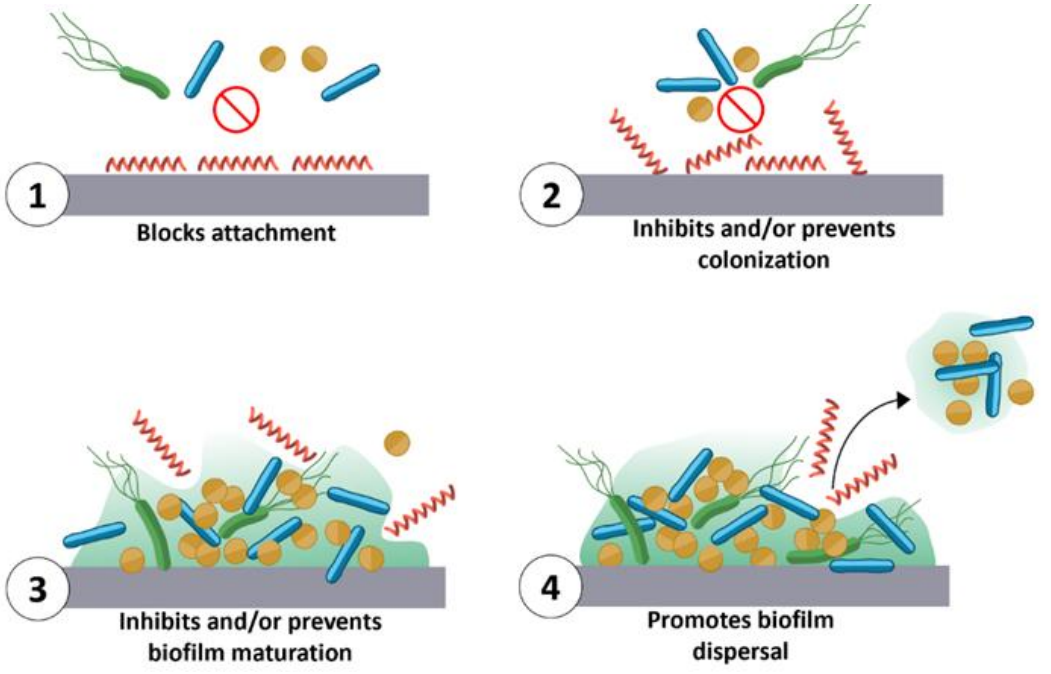

Fig. 1: Potential mechanisms by which antibiofilm peptides act on biofilms. Attachment of planktonic cells to surfaces may be prevented through inhibition of swimming or swarming motility and surface adhesion (1), and by disrupting cell aggregation (2). Biofilm formation and maturation can be hindered by destabilizing membrane potential, inhibiting quorum sensing, degrading EPS, or interfering with environmental stress responses (3). Additionally, biofilm dispersal may be promoted by stimulating twitching motility (4). Graphic credit: Nuraly S. Akimbekov, 2025.

Host innate immunity constitutes a parallel defense mechanism against biofilms. Antimicrobial peptides (AMPs) are essential effector molecules produced by epithelial cells and leukocytes, with the dual ability to directly damage microbes and to fine-tune inflammatory and repair responses. Human AMPs, including cathelicidin LL-37 and several β-defensins, are cationic and amphipathic, enabling them to bind to negatively charged bacterial membranes and matrix components, disrupt membrane integrity, and, in certain cases, modulate microbial gene expression.[7-10] In addition to direct antimicrobial activity, AMPs can disrupt biofilms by blocking microbial attachment, inhibiting microbial colonization, preventing biofilm maturation and promoting biofilm dispersal (Fig. 1). This multi-target functionality has generated significant interest in AMPs as natural anti-biofilm agents and as templates for novel therapeutics, with LL-37 receiving particular attention due to its broad antimicrobial, anti-biofilm, and wound-healing properties.[11-13]

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A significant advancement in the understanding of human innate immunity was the discovery that vitamin D directly regulates AMP gene expression.[7,14,15] In immune and epithelial cells, locally produced 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] after activation by enzyme CYP27B1 binds to the vitamin D receptor (VDR) and, in conjunction with the retinoid X receptor (RXR), promotes transcription of these AMP genes.[11,16] This mechanism has been demonstrated in numerous immune cells, indicating that the vitamin D-AMP axis functions at multiple barrier sites in the human body that are also frequent niches for biofilms.[5,17-19]

Vitamin D deficiency and insufficiency are widespread globally and have been associated with increased susceptibility to various infections.[20,21] Clinical and translational studies indicate that sufficient 25-hydroxyvitamin D [25(OH)D] levels enhance epithelial barrier integrity, increase AMP production, and modulate inflammatory responses, thereby contributing to protection against respiratory, gastrointestinal, urinary, and cutaneous infections.[15,16,22] Specifically, reduced serum vitamin D levels have been linked to increased severity of periodontal disease, elevated caries risk, and impaired wound healing: all conditions in which biofilms play a critical pathogenic role.[3,23] *In vitro* studies demonstrate that vitamin D metabolites enhance LL-37 induction in gingival epithelial

cells, keratinocytes, and macrophages, thereby improving their capacity to restrict bacterial growth.[24-26]

Although dedicated reviews have addressed vitamin D and innate immunity, the vitamin D-cathelicidin axis, and LL-37 as an antimicrobial agent,[11,12,15,23,27] a comprehensive synthesis specifically focused on vitamin D-induced AMPs as anti-biofilm effector molecules remains lacking. Key questions include how vitamin D signaling regulates AMP expression at biofilm-prone surfaces, how LL-37 and defensins interact with biofilm architecture and microbial communication, and how these mechanisms can be therapeutically exploited. This review examines the vitamin D-AMP-biofilm axis from mechanistic, experimental, and translational perspectives. The discussion first summarizes vitamin D signaling in antimicrobial immunity and the repertoire of vitamin D-responsive AMPs, then addresses biofilm biology and points of vulnerability to these peptides. Subsequently, *in vitro, in vivo* and clinical evidence for anti-biofilm effects of vitamin D and its induced AMPs are integrated, followed by an outline of emerging therapeutic strategies and future research directions.

2. Literature search strategy

This narrative review is based on a structured but non-systematic search of literature. We primarily searched PubMed/MEDLINE, Web of Science, and Scopus for English-language articles published between January 2000 and November 2025 using combinations of the following terms: “vitamin D”, “25-hydroxyvitamin D”, “1,25-dihydroxyvitamin D”, “calcitriol”, “antimicrobial peptide”, “host defense peptide”, “cathelicidin”, “LL-37”, “β-defensin”, “biofilm”, “quorum sensing”, “extracellular polymeric substance”, “device-associated infection”, “chronic wound”, and “periodontitis”. We also screened the reference lists of key original articles and recent reviews to identify additional relevant publications. Experimental in vitro and in vivo studies, clinical trials, and observational human studies that addressed vitamin D signaling, vitamin D-responsive AMPs, or their interaction with biofilm-forming pathogens were prioritized. Commentaries, editorials, and articles without primary data were used selectively to contextualize mechanistic or translational concepts.

3. Vitamin D signaling pathway in antimicrobial immunity

Vitamin D_3 (cholecalciferol) is synthesized in the skin from 7-dehydrocholesterol upon exposure to UV-B radiation or acquired through dietary intake. It is subsequently transported to the liver, where it undergoes hydroxylation to form 25-hydroxyvitamin D [25(OH)D], the principal circulating form and standard biomarker of vitamin D status. A second hydroxylation, primarily in the kidney by CYP27B1 (1α-hydroxylase), converts 25(OH)D to the biologically active hormone 1,25-dihydroxyvitamin D [$1,25(OH)_2D$].[28,29] Notably, CYP27B1 is also expressed in various extrarenal sites, such as epithelial cells of the skin, respiratory tract, and gut.[29-31] These cells are capable of locally converting 25(OH)D to $1,25(OH)_2D$ in an intracrine or paracrine manner in response to microbial or inflammatory stimuli, thereby directing vitamin D signaling to sites of infection rather than relying solely on endocrine mechanisms.

The active metabolite $1,25(OH)_2D$ binds to the VDR, a nuclear receptor expressed in monocytes/macrophages, dendritic cells, T and B lymphocytes, natural killer (NK) cells, and barrier epithelia.[32,33] Upon ligand binding, VDR forms a heterodimer with the RXR and interacts with specific VDREs in target genes, recruiting co-activators or co-repressors to regulate transcription. This mechanism enables vitamin D to modulate a broad array of innate immune genes, such as pattern-recognition receptors, cytokines, chemokines, autophagy-related proteins, and AMPs.[14,16,34] In macrophages, for instance, activation of toll-like receptors (TLRs) upregulates both VDR and CYP27B1, enhancing local production of $1,25(OH)_2D$ and VDR signaling in response to pathogens.[7,28] This process establishes a direct link between microbial recognition and vitamin D-dependent antimicrobial effector pathways.

A well-characterized outcome of VDR activation is the induction of AMP genes. Gombart and colleagues identified functional vitamin D response elements (VDREs) near the transcription start sites of the cathelicidin gene CAMP and DEFB4A, which encodes human β-defensin-2, thereby establishing the “vitamin D-AMP pathway” as a central axis in host defense.[7,23] Several β-defensins are upregulated by vitamin D in both epithelial and myeloid cells.[35,36] These observations support the existence of a vitamin D-cathelicidin/defensin axis, wherein sufficient 25(OH)D levels facilitate rapid transcriptional upregulation of AMPs.[23,27]

VDR and CYP27B1 are broadly expressed throughout both innate and adaptive immune compartments. Monocytes, macrophages, dendritic cells, T and B lymphocytes, and NK cells all express VDR, and many are capable of upregulating CYP27B1 upon activation, enabling local synthesis of $1,25(OH)_2D$.[32,37,38] Barrier epithelial cells in different organs also express VDR and CYP27B1 and strongly induce cathelicidin and β-defensins in response to vitamin D metabolites.[27,35,36] These sites represent key interfaces where biofilms frequently develop, including skin and wounds, airway mucosa, oral surfaces, and uroepithelium. This

distribution suggests that the vitamin D-AMP pathway is strategically positioned to modulate biofilm formation and persistence *in vivo*.

4. Vitamin D-induced antimicrobial peptides

Vitamin D signaling interacts with innate immunity primarily by inducing host defense peptides (HDPs), encompassing classical AMPs and related molecules that combine microbicidal activity with diverse immune-modulating roles. Human cathelicidin LL-37 and several β-defensins are the most extensively characterized vitamin D-responsive effectors; however, emerging evidence indicates that a wider range of HDPs is regulated by VDR.[39]

The human cathelicidin gene CAMP encodes a prepropeptide (hCAP18), which undergoes proteolytic processing in neutrophil granules and at epithelial surfaces to generate the mature 37-amino-acid peptide LL-37. LL-37 is a linear, cationic peptide (net charge approximately +6) that adopts an amphipathic α-helical structure in membrane-like environments. These properties facilitate electrostatic interactions with negatively charged bacterial surfaces and enable insertion into lipid bilayers.[40-43] LL-37 demonstrates broad-spectrum activity against Gram-positive and Gram-negative bacteria, fungi, and certain enveloped viruses, exerting its effects by forming membrane pores or inducing membrane perturbations that disrupt ion gradients.[11,42,44]

A pivotal discovery established that CAMP is a direct target of the VDR; specifically, $1,25(OH)_2D$ robustly induces CAMP/LL-37 expression in human myeloid and epithelial cells.[45-47] This regulatory mechanism is primate-specific and constitutes a central component of the "vitamin D-cathelicidin axis".[48,49] As discussed above, activation of TLRs upregulates CYP27B1 and VDR. This intracrine loop links microbial detection to LL-37 production.[45]

As was mentioned before, LL-37 exhibits pronounced anti-biofilm and immunomodulatory activities. It inhibits surface adhesion and early microcolony formation, penetrates and destabilizes the EPS, binds eDNA, and, at sublethal concentrations, modulates the expression of quorum sensing and virulence genes in various pathogens.[11,50,51] Furthermore, LL-37 functions as a chemoattractant, facilitates wound closure and angiogenesis, and regulates cytokine responses, establishing its role as a multifunctional effector at barrier sites prone to biofilm formation.[39,52]

β-defensins are small, cationic peptides defined by a triple-disulfide-bonded β-sheet structure. Human β-defensin 2 (hBD-2, encoded by DEFB4A) and hBD-3 (DEFB103) are inducible defensins primarily expressed by epithelial cells. Both exhibit broad antimicrobial activity; hBD-2 is especially effective against Gram-negative bacteria, while hBD-3 demonstrates potent activity against *Staphylococcus aureus* and other Gram-positive species under physiological salt conditions.[39,53,54]

Vitamin D upregulates DEFB4A and other β-defensin-related genes, though generally to a lesser degree than CAMP. In human macrophages, $1,25(OH)_2D$ induces the expression of both CAMP and DEFB4A, and vitamin D-responsive regulatory elements have been identified in the DEFB4A promoter.[35,55] Similarly, keratinocytes and airway epithelial cells increase hBD-2 and hBD-3 expression in response to vitamin D metabolites and UVB exposure, which enhances cutaneous vitamin D_3 synthesis and links environmental UV exposure to epithelial defensin production.[39,52]

Defensins support anti-biofilm defense by inhibiting bacterial adhesion, permeabilizing the membranes of sessile cells, and acting synergistically with LL-37 and conventional antibiotics. Additionally, defensins play significant immunological roles, such as promoting chemotaxis of dendritic cells and T cells and modulating cytokine production, which may affect the inflammatory environment within biofilm-infected tissues.[39,54]

Although cathelicidin and β-defensins are the most extensively studied, vitamin D also regulates a wider array of host defense peptides. For example, RNase 7, a 14.5-kDa ribonuclease highly expressed by keratinocytes and urothelial cells, exhibits strong antimicrobial activity against uropathogenic *E. coli* and other pathogens.[55-57] Its expression is inducible by cytokines, growth factors, UVB, and microbial products. Several studies position RNase 7 within the broader vitamin D-HDP network, particularly in skin and urinary tract epithelia.[39,53,58]

Further vitamin D-regulated HDPs include S100-family proteins, such as psoriasin (S100A7), which contribute to nutritional immunity by chelating metal ions, as well as other short peptides identified through transcriptomic analyses of cells treated with $1,25(OH)_2D$.[39,55] While the specific anti-biofilm functions of these peptides are less well characterized than those of LL-37, together they constitute a multilayered antimicrobial barrier at epithelial surfaces. Elucidating the cooperative interactions among this broader HDP repertoire, cathelicidin, and defensins in biofilm control remains a key objective for future research.

5. Biofilm biology and targets for AMP intervention

Biofilms are currently recognized as spatially and functionally heterogeneous structures, rather than merely "slimy layers" on surfaces. Mature biofilms exhibit pronounced gradients of oxygen, nutrients, pH, and waste products, resulting in distinct

micro-niches. These include actively growing cells at the periphery, slow-growing or dormant cells deep within the matrix, and specialized persister subpopulations.[59,60] EPS embedding these communities comprises exopolysaccharides, proteins, lipids, and eDNA. EPS is not a passive adhesive; it organizes diffusion channels, provides mechanical stability, and functions as a biochemical filter for antimicrobials and host factors.[61,62]

Bacterial biofilm formation is a complex process that can be divided into four principal stages (Fig. 2). It is regulated by intracellular second messengers and quorum sensing networks. Increased concentrations of cyclic di-GMP promote EPS production and surface-associated lifestyles, while its degradation favors motility and dispersal. Quorum sensing systems, such as las/rhl and pqs in *Pseudomonas aeruginosa* or agr in *Staphylococcus aureus*, coordinate the expression of adhesins, matrix components, and virulence factors once a critical cell density is achieved.[59,63,64] These regulatory networks also control eDNA release, small regulatory RNAs, and multidrug efflux pumps that contribute to intrinsic tolerance.[62]

The structural and regulatory complexity of biofilms presents several strategic targets for vitamin D-induced AMPs beyond direct membrane disruption:

1. Initial adhesion and conditioning film. Initial bacterial attachment relies on surface structures such as pili, fimbriae, and flagella, as well as host-derived conditioning films composed of proteins and glycoproteins.[65,66] AMPs can modify bacterial surface charge and hydrophobicity, disrupt adhesin expression, or bind to host matrix proteins, thereby decreasing the likelihood of stable attachment. LL-37 has been demonstrated to reduce surface adhesion and promote twitching motility in *P. aeruginosa*, encouraging a transition away from a sessile state.[67]

2. EPS and eDNA as dual barrier and target. eDNA serves as a critical structural scaffold in many biofilms and can bind cationic AMPs, sometimes diminishing their antimicrobial activity. For instance, eDNA can sequester hBD-3 and reduce its efficacy against *Haemophilus influenzae*.[68] However, this electrostatic interaction can also be leveraged; high local concentrations of LL-37 or defensins may condense and destabilize the matrix, facilitating the penetration of other antimicrobials and immune factors.[69] Combination approaches that utilize AMPs alongside host DNases or DNase-loaded delivery systems may further disrupt eDNA-rich matrices.[61]

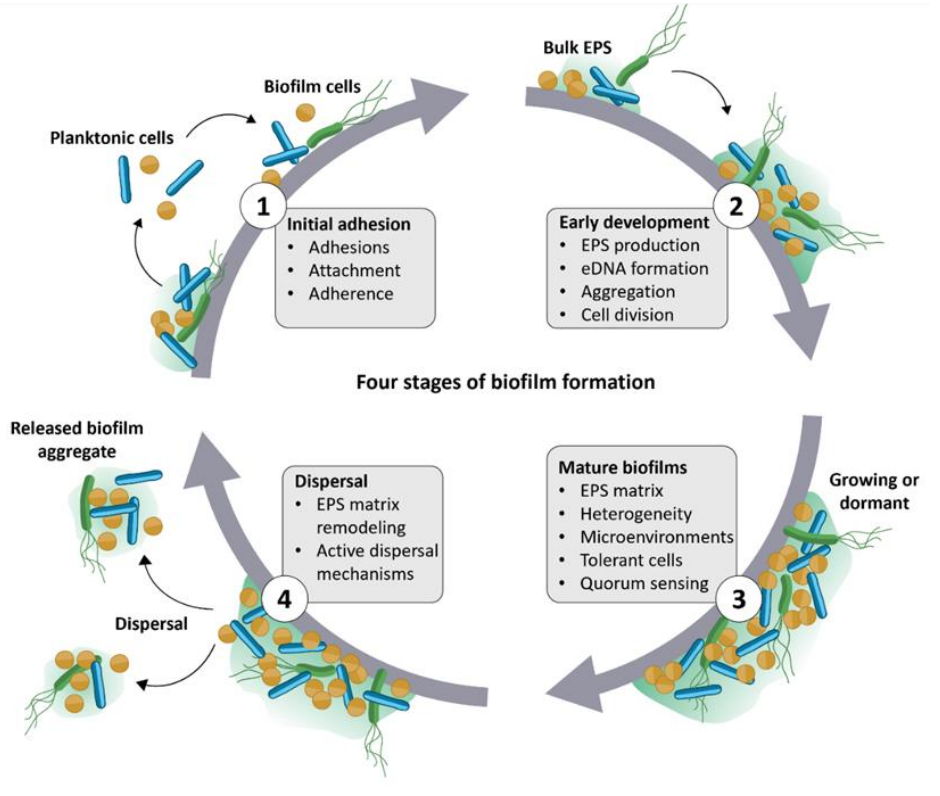

Fig. 2: The four stages of biofilm formation by planktonic bacteria are illustrated as follows: (1) initial adhesion to a surface; (2) early development involving aggregation, cell division, quorum sensing, and EPS production; (3) maturation into a structured, heterogeneous EPS-embedded biofilm, and (4) dispersal of cells or aggregates to colonize new sites. Graphic credit: Nuraly S. Akimbekov, 2025.

3. Quorum sensing and virulence circuitry. Subinhibitory concentrations of LL-37 modulate gene expression in *P. aeruginosa* by downregulating the las and rhl quorum sensing systems and genes essential for biofilm maturation, while simultaneously enhancing twitching motility.[67] Similarly, hBD-3 influences the transcription of the icaA and dltB loci, which regulate EPS production and cell surface charge in S. aureus during adhesion and early biofilm development.[70,71] By altering quorum sensing regulatory circuits rather than solely inducing cell death, AMPs can disrupt biofilms at the signaling level and attenuate virulence.

4. Dispersal-stage vulnerabilities. Dispersal is now recognized as an active and regulated phase of the biofilm life cycle.[64] Agents that induce dispersal transform resilient sessile bacteria into planktonic cells, which are significantly more susceptible to antibiotics and host AMPs. LL-37 and related peptides, as well as their murine analogs such as CRAMP, have been shown to reduce biofilm biomass and promote detachment in *P. aeruginosa*, indicating that targeted AMP exposure could be strategically employed to remove biofilms prior to or in conjunction with antibiotic therapy.[72,73]

In summary, contemporary biofilm biology demonstrates that vitamin D-induced AMPs function not only as microbicidal agents but also as modulators of adhesion, matrix integrity, and quorum sensing-driven community behavior. These multi-level targets establish a conceptual framework for the development of AMP-centered anti-biofilm strategies.

6. Evidence for Anti-Biofilm Activity of Vitamin D and Induced AMPs

6.1 *In vitro* evidence: cathelicidin and β-defensins

The most comprehensive *in vitro* data are derived from studies of LL-37 and its derivatives. Overhage *et al*. demonstrated that LL-37, at sub-MIC concentrations, effectively inhibits biofilm formation by *P. aeruginosa* by reducing initial attachment, stimulating twitching motility, and downregulating las/rhl quorum sensing regulons.[67] Subsequent research has confirmed that LL-37 can prevent or reduce biofilms formed by other pathogens, including *S. aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, and *Acinetobacter baumannii*.[11,66,74] In addition to blocking biofilm development, LL-37 at slightly higher concentrations can disperse pre-formed *P. aeruginosa* biofilms at levels well below the MIC for planktonic cells, indicating a specific anti-biofilm mechanism.[75]

LL-37-derived peptides, such as KE-18, KR-12, and RP557, retain or enhance antibiofilm activity while exhibiting improved stability and reduced cytotoxicity. These analogs inhibit biofilms formed by multidrug-resistant Gram-negative isolates and can eradicate biofilm-embedded cells *in vitro*, supporting the concept that the LL-37 scaffold represents a promising template for anti-biofilm drug development.[76-78]

β-defensins offer additional evidence. Parducho *et al*. showed that hBD-2 inhibits *P. aeruginosa* biofilm production at low micromolar concentrations without significantly affecting metabolic activity, indicating that hBD-2 primarily influences biofilm regulation rather than direct bactericidal activity.[79] In contrast, hBD-3 exhibits both potent bactericidal and antibiofilm effects: it reduces biofilm biomass, interferes with the expression of ica-dependent matrix genes in *S. aureus*, and outperforms chlorhexidine against mature multispecies oral biofilms *in vitro*.[69,70,80-82]

6.2 *In vitro* effects of vitamin D on biofilms

While many experiments utilize purified peptides, several recent studies have directly evaluated the effects of vitamin D. In Gram-negative pathogens such as *A. baumannii* and *K. pneumoniae*, cholecalciferol or vitamin D_3 significantly reduces initial attachment and eradicates mature biofilms, accompanied by downregulation of key biofilm genes (*e.g.* cusD, bssS, pelA).[83,84] Because these experiments were performed in the absence of host cells, their antibiofilm effects cannot be attributed to vitamin D-induced LL-37 or β-defensins; instead, they likely reflect direct physicochemical actions of vitamin D on bacterial membranes, metabolism, or regulatory networks. Narrative reviews by Benson and colleagues synthesize these data and propose that, in vivo, vitamin D supplementation may attenuate biofilm-associated infections through a combination of such direct effects and indirect, AMP-mediated mechanisms.[85]

6.3 *Ex vivo* and *in vivo* models

Ex vivo and animal studies serve as an intermediary step toward clinical application. In the urinary tract, Hertting *et al*. demonstrated that vitamin D supplementation in postmenopausal women increased bladder epithelial cathelicidin responses; infected biopsies from supplemented individuals produced more LL-37 and exhibited enhanced ex vivo killing of uropathogenic *E. coli*.[86] In mouse wound models, topical or local LL-37 treatment accelerates closure of *S. aureus*-infected wounds, reduces bacterial counts, and diminishes histological features associated with biofilm persistence.[27,87,88] Murine cathelicidin (CRAMP) also reduces *P. aeruginosa* biofilm biomass both *in vitro* and *in vivo*, with omics analyses indicating that biofilm dispersion and quorum sensing modulation are central to this effect.[60,72]

Although these models do not always explicitly quantify EPS structure, they consistently demonstrate that cathelicidin-

driven responses enhance clearance of infections that are typically biofilm-prone, including chronic wounds and device-associated infections.[66,89]

6.4 Clinical and translational correlations

Direct clinical trials with explicit biofilm endpoints are limited; however, multiple lines of evidence support similar conclusions. Observational studies associate higher serum 25(OH)D concentrations with lower prevalence and severity of periodontitis, reduced gingival bleeding, and decreased attachment loss, all of which are closely linked to plaque biofilm burden.[90,91] Short-term vitamin D_3 supplementation following non-surgical periodontal therapy improves clinical attachment levels, indicating that correcting deficiency may enhance host control of oral biofilms.[92,93] In the oral cavity, low salivary LL-37 levels are correlated with increased caries activity, and vitamin D has been shown to induce cathelicidin in oral epithelial cells, supporting a functional relationship between vitamin D status, AMP expression, and dental biofilm behavior.[27,94,95]

Beyond the oral cavity, vitamin D-cathelicidin activity has been implicated in the protection of the urinary tract and respiratory mucosa, where biofilm formation by uropathogens and *P. aeruginosa* is a significant contributor to chronic infection.[23,86,96] Although these studies seldom directly visualize biofilms, they support the broader concept that vitamin D-induced AMPs enhance host control of biofilm-prone infections, thereby justifying the need for targeted clinical trials that assess biofilm structure or biomarkers as explicit outcomes.

Direct *in vivo* evidence for the antibiofilm activity of vitamin D or LL-37 in humans remains limited. Most clinical and translational studies evaluate outcomes such as infection incidence, wound area reduction, or surrogate inflammatory markers, rather than directly assessing biofilm structure, biomass, or composition. Consequently, antibiofilm effects are primarily inferred from mechanistic and *ex vivo* data. There is a need for dedicated clinical studies that incorporate biofilm-specific endpoints, such as imaging, histology, or molecular markers of extracellular polymeric substances and quorum-sensing activity.

7. Mechanistic integration

The anti-biofilm effects of vitamin D-induced AMPs arise from multiple organizational levels, including intracellular host defense, local tissue microenvironments, and cooperative interactions with other antimicrobials, rather than relying on a single direct killing mechanism. Mechanistic actions of vitamin D-induced AMPs on biofilms are summarized schematically in Fig. 3.

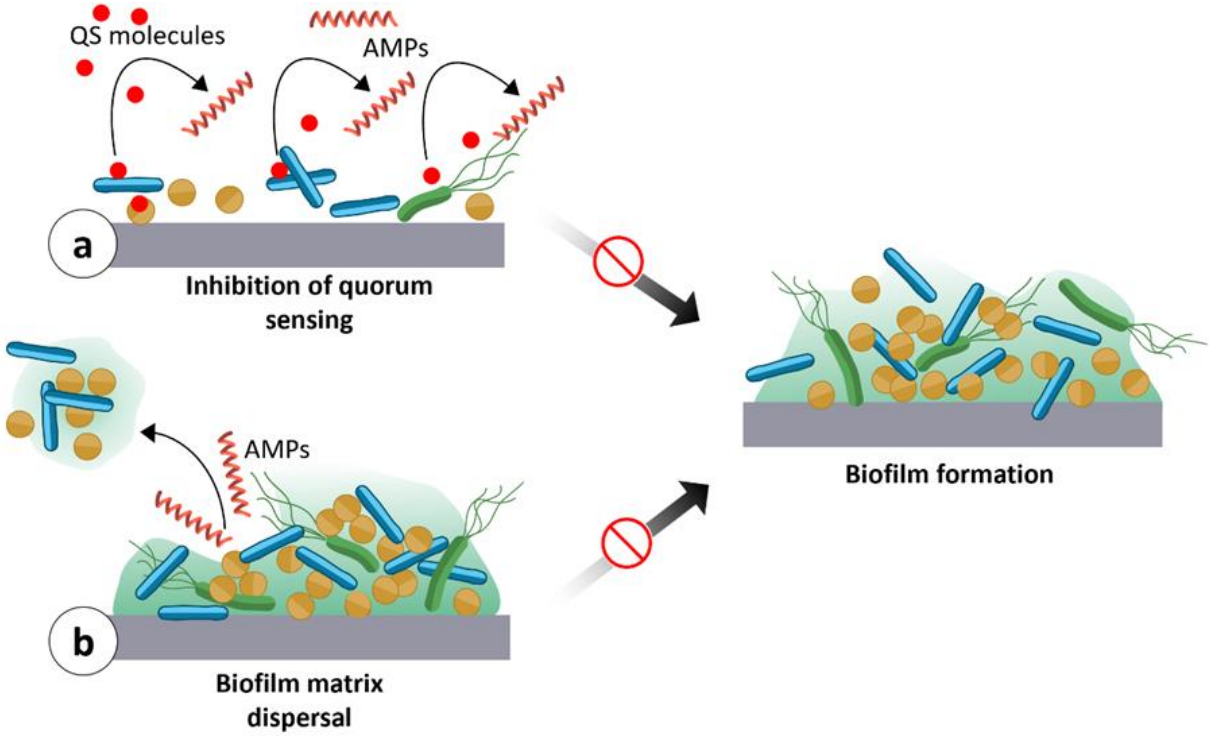

Fig. 3: Mechanisms by which vitamin D-induced antimicrobial peptides affect biofilms. (a) interfere with quorum-sensing regulons and stress-response pathways, thereby reducing expression of adhesins, exopolysaccharides, and virulence factors; (b) promote biofilm dispersal by enhancing twitching or swimming motility and converting sessile cells into more antibiotic-sensitive planktonic forms. Graphic credit: Nuraly S. Akimbekov, 2025.

7.1 Intracellular control: autophagy and phagolysosomal maturation

Randomized controlled trials investigating vitamin D supplementation for the prevention of acute respiratory infections (ARIs) have generally demonstrated modest or context-dependent benefits. Recent trials and meta-analyses report small protective effects, primarily among individuals with significant baseline deficiency, while other studies find no significant impact on overall ARI incidence or severity in populations that are largely vitamin D replete.[97,98] These trials did not include biofilm-specific endpoints, and AMP induction was rarely assessed, which limits the ability to directly associate clinical outcomes with the vitamin D-AMP axis. Therefore, systemic vitamin D repletion should currently be considered a rational and cost-effective adjunct to optimize host defense, rather than a primary antibiofilm therapy.[99,100]

Mechanistically, LL-37 facilitates the co-localization of microbes and autophagic compartments, serving as a "molecular bridge" that directs bacteria into degradative organelles.[98,100] Although these studies did not specifically address biofilms, enhanced intracellular killing reduces the population of planktonic and cell-associated bacteria that may contribute to recurrent biofilm formation on mucosal surfaces or medical devices. Therefore, the vitamin D-cathelicidin-autophagy axis represents an upstream regulatory mechanism that restricts biofilm initiation.

7.2 Microenvironmental reprogramming at barrier surfaces

At epithelial surfaces, vitamin D-induced AMPs reprogram the local microenvironment rather than solely targeting biofilms externally. LL-37 and related peptides promote epithelial proliferation, migration, and barrier restoration, partly by inducing growth factors such as EGF and VEGF and modulating transcription factors like TFEB, which regulate autophagy and lysosomal biogenesis.[51,101,102] These wound-healing and barrier-strengthening effects decrease the availability of exposed surfaces for biofilm establishment and foster a dynamic tissue environment capable of continuously shedding colonized cells.

Concurrently, LL-37 and defensins function as immunomodulators. They act as chemoattractants and activators for neutrophils, monocytes, and specific T-cell subsets, and they modulate cytokine and chemokine profiles to either enhance or resolve inflammation, depending on the context.[103-106] In biofilm infections, vitamin D-induced AMPs not only exert direct effects on the biofilm but also coordinate the recruitment and functional modulation of phagocytes capable of clearing detached bacterial aggregates and debris.

7.3 Regulatory rewiring: quorum sensing, stress responses and dispersal

At the bacterial level, cathelicidin and other AMPs modulate regulatory networks that control biofilm behavior. LL-37 has been shown to disrupt *P. aeruginosa* quorum-sensing systems and virulence factor expression, in some cases downregulating las/rhl-controlled exoproducts and promoting twitching motility and biofilm dispersal.[74] Similarly, murine cathelicidin (CRAMP) decreases c-di-GMP levels and exopolysaccharide production while increasing motility and rhamnolipid synthesis, collectively promoting biofilm dispersion.[72]

However, these effects are dependent on both dose and context. Other studies have demonstrated that sublethal exposure to LL-37 can upregulate quorum sensing and virulence genes and stimulate efflux pump expression in *P. aeruginosa*, potentially facilitating adaptive resistance if peptide concentrations are inadequate for bactericidal activity.[107] This duality highlights the necessity of achieving optimal local peptide concentrations, whether through endogenous vitamin D-mediated induction or exogenous peptide administration, to favor quorum sensing disruption and biofilm dispersal rather than enhancement of virulence.

7.4 Cooperative interactions with conventional antibiotics

Vitamin D-induced AMPs also engage in pharmacological synergy with conventional antibiotics. By disrupting cellular membranes and partially degrading EPS, LL-37 enhances antibiotic penetration into otherwise protected cells and reduces the minimal biofilm eradication concentration. Recent studies have demonstrated strong synergy between LL-37 and polymyxin B or colistin against multidrug-resistant *E. coli* and *P. aeruginosa*, with these combinations preventing biofilm formation and eradicating established biofilms at significantly lower antibiotic doses.[108,109] Comparable synergistic effects have been observed for LL-37 derivatives combined with β-lactams or glycopeptides against Gram-positive biofilms.[50,110,111]

In vivo, vitamin D supplementation functions as an indirect combination therapy by increasing endogenous LL-37 and defensin levels, thereby enabling standard antibiotics to act within an AMP-primed tissue environment. This integrative mechanism may account for the observed improvements in antibiotic efficacy against biofilm-associated infections following vitamin D repletion or topical AMP administration, even when vitamin D or the peptide alone exhibits limited direct bactericidal activity.

7.5 A multi-scale model of the vitamin D-AMP-biofilm axis

Collectively, these findings support a multi-scale mechanistic model in which systemic vitamin D status determines the ability of immune and epithelial cells to produce cathelicidin and defensins. These peptides subsequently (i) enhance intracellular pathogen clearance through autophagy, (ii) remodel epithelial barriers and immune microenvironments, (iii) disrupt biofilm structure and signaling networks, and (iv) synergize with antibiotics to eliminate residual microbial communities.[23,50,97,104] Within this framework, vitamin D-induced AMPs act not as isolated "biofilm drugs" but as central integrators that coordinate host and pharmacological responses against biofilm-associated pathogens. Fig. 4 presents an overview of the multi-scale concept, illustrating the connections between systemic vitamin D status, VDR signaling, AMP induction at epithelial and immune interfaces, subsequent effects on biofilm structure and quorum sensing, and the potential for adjunctive and topical therapeutic strategies.

7.6 Separating direct vitamin D actions from AMP-mediated effects

To enhance clarity, three mechanistic layers can be distinguished within the vitamin D-AMP-biofilm axis. The first layer involves direct actions of vitamin D on host cells, including modulation of autophagy, phagolysosomal maturation, tight junctions, barrier integrity, and broad immunoregulatory effects on myeloid and lymphoid cells.[14,27,31] These effects have been demonstrated in systems where $1,25(OH)_2D$ acts on host cells, even when AMP induction is modest or not measured.

The second layer comprises AMP-mediated actions downstream of vitamin D, as defined by experiments demonstrating that vitamin D-VDR signaling induces LL-37 or β-defensins. Blockade of these peptides, through genetic or pharmacological approaches, abrogates the antimicrobial or antibiofilm phenotype. This mechanism is most clearly documented for cathelicidin-dependent autophagy in macrophages and for epithelial LL-37/β-defensin induction at barrier sites.[7,23,35,45,85]

The third layer involves studies utilizing purified LL-37 or β-defensins, or their synthetic analogues, in the absence of vitamin D. In these instances, antibiofilm effects are attributable to the intrinsic properties of the peptides, although in vivo these peptides may be upregulated by vitamin D.

Accordingly, this review refers to "vitamin D" when discussing outcomes demonstrated for $1,25(OH)_2D$ or 25(OH)D at the level of host cells or whole organisms, and to "vitamin D-induced AMPs" when effects are demonstrated for LL-37/β-defensins themselves or for responses that are lost upon inhibition of these peptides.

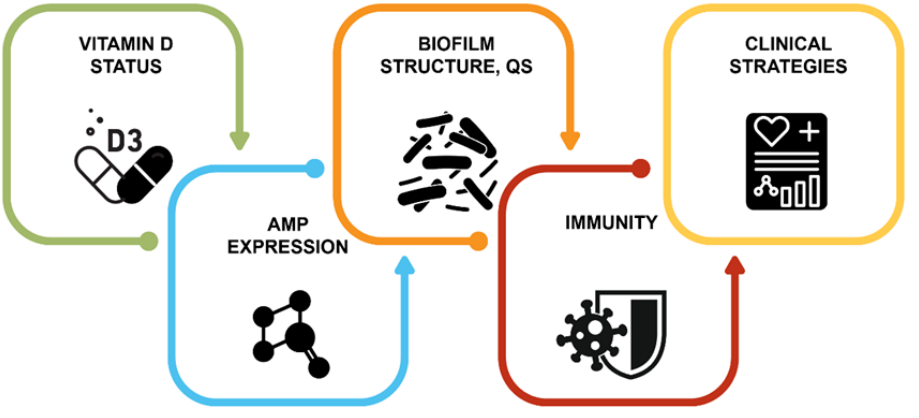

Fig. 4: Multiscale model of the vitamin D-AMP-biofilm axis and therapeutic opportunities. Graphic credit: Nuraly S. Akimbekov, 2025.

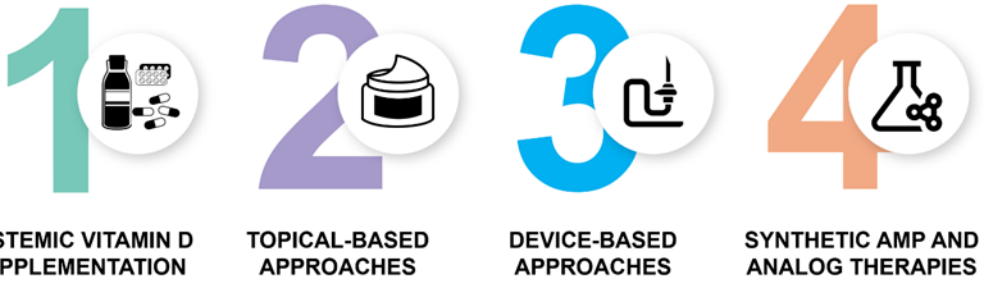

Fig. 5: Principal therapeutic strategies targeting the vitamin D-AMP-biofilm axis. Graphic credit: Nuraly S. Akimbekov, 2025.

8. Therapeutic strategies

The translation of the vitamin D-AMP-biofilm axis into clinical practice involves four primary approaches: optimizing systemic vitamin D status, implementing local or topical AMP-based interventions, and developing engineered delivery systems and synthetic or analog therapies (Fig. 5).

Each approach targets distinct stages of the biofilm life cycle and addresses specific clinical scenarios.

8.1 Systemic vitamin D repletion as an adjunct

Vitamin D status directly influences the production of cathelicidin and defensins in tissues; therefore, correcting vitamin D deficiency represents a logical and cost-effective adjunct strategy, particularly for populations with chronic or recurrent infections. Randomized controlled trials of vitamin D supplementation for the prevention of acute respiratory infections (ARIs) have demonstrated modest or context-dependent benefits. Some recent studies and meta-analyses report small protective effects, primarily in deficient individuals, while others observe minimal or no overall benefit.[112,113] These trials rarely assessed AMP levels or biofilm-related endpoints. However, mechanistic studies indicate that any observed benefit may be partially mediated by cathelicidin induction in airway and other epithelial tissues.[86,112]

The rationale for systemic vitamin D supplementation is particularly compelling in the urinary tract. Hertting *et al*. demonstrated that vitamin D induces cathelicidin expression in human bladder tissue ex vivo, and that vitamin D-deficient mice develop more invasive urinary tract infections characterized by increased intracellular bacterial communities.[86,114] Subsequent study by Mohanty *et al*. have shown that supplementation enhances the bladder epithelial barrier by upregulating tight junction proteins, thereby providing an additional defense against ascending uropathogens.[115] Although large randomized controlled trials have not yet evaluated vitamin D specifically for biofilm-mediated catheter-associated urinary tract infections, current evidence supports considering vitamin D repletion as a foundational strategy to optimize host AMP responses in organs susceptible to biofilm formation.

From a therapeutic design perspective, systemic vitamin D supplementation is unlikely to serve as a stand-alone anti-biofilm agent. The primary objective should be to ensure that patients at high risk, such as those with chronic catheters, recurrent respiratory infections, or impaired wound healing do not exhibit profound vitamin D deficiency. This approach enables endogenous cathelicidin and defensin responses to complement both local and systemic antimicrobial interventions.

8.2 Topical LL-37 and AMP-based therapies

The most direct clinical exploitation of vitamin D-induced AMPs so far is topical LL-37. In a randomized, placebo-controlled trial in patients with hard-to-heal venous leg ulcers, Grönberg *et al*. reported that LL-37 treatment significantly improved healing rates when added to compression therapy, and was well tolerated.[116] A larger phase IIb trial (HEAL LL-37) confirmed safety but showed more nuanced efficacy, underlining that dosing, wound characteristics and patient selection are critical determinants of response.[117]

A more recent double-blind trial evaluated LL-37 cream in the treatment of diabetic foot ulcers. Although the overall benefit was less pronounced compared to venous ulcers, subgroup analyses indicated potential advantages in specific wound types.[118] While these trials did not explicitly quantify biofilm presence, chronic wounds of this nature are generally biofilm-dominated. LL-37's capacity to promote re-epithelialization and angiogenesis, as well as to modulate microbial communities, positions it as a promising adjunct to debridement and standard care.

Preclinical work extends these ideas to other sites: LL-37 and truncated mimetics (*e.g*. KE-18, KR-12) inhibit and disrupt biofilms of Candida albicans, *S. aureus and E. coli in vitro*, and show good activity in models of ventilator-associated pneumonia and device-related infections.[77,119] These data support the concept of site-directed LL-37-based therapies (gels, sprays, or dressings) designed to flood a biofilm-containing niche with high local peptide levels without large systemic exposure.

Collectively, these studies demonstrate that topical LL-37 and related peptides are safe and biologically active; however, their clinical effects remain heterogeneous and context dependent. The phase IIb HEAL LL-37 trial confirmed safety but did not show a clear benefit across all wound types, necessitating subgroup analyses to identify potential responders.[117] Furthermore, none of the published LL-37 trials have directly quantified biofilm burden, so improvements in wound closure cannot be conclusively attributed to antibiofilm effects. Larger, well-stratified trials with standardized biofilm assessments are required to determine when LL-37-based interventions significantly modify biofilm-driven pathology.

8.3 Device coatings and engineered delivery systems

Given the significant challenges posed by biofilms on implants and catheters, there is considerable interest in

incorporating AMP-like molecules into device surfaces. Recent research by Pennone *et al*. on LL-37-derived peptides for orthopedic infections indicated that carefully optimized fragments can maintain antibiofilm activity against multidrug-resistant pathogens with acceptable cytotoxicity, making them suitable candidates for cement or coating formulations in fracture fixation and prosthetic joints.[120] Concurrently, studies of urinary catheters coated with novel biofilm preventive agents have shown that surface-bound antibiofilm molecules can substantially reduce colonization by multiple uropathogens. This platform could potentially be adapted for cathelicidin mimetics or defensin-inspired peptides.[119,121]

Hybrid systems that combine AMPs with nanomaterials are also under development. For instance, LL-37 combined with silver nanoparticles exhibits enhanced activity against biofilm-forming *S. aureus* and *P. aeruginosa* and is being investigated for use in orthopedic device coatings.[122] Nanocarriers offer additional advantages, such as protecting peptides from proteolysis, improving penetration into dense biofilm matrices, and enabling stimulus-responsive release based on factors like pH or enzymatic activity. These features may be particularly beneficial for targeting mature biofilms.

8.4 Synthetic antibiofilm peptides and vitamin D-aware trial design

A broader class of synthetic antibiofilm peptides, many of which are inspired by LL-37 or defensins, is being developed with specific biofilm targets in mind. De la Fuente-Núñez *et al*. reviewed several peptides that either prevent biofilm formation or induce dispersal across a range of microbial species, with some candidates advancing toward clinical application.[119] Although most of these peptides are not directly induced by vitamin D, their design frequently incorporates structural motifs from cathelicidin and defensins, thereby extending the vitamin D-AMP paradigm into the realm of synthetic therapeutics.[85]

Future therapeutic development in this field will require clinical trial designs that are specifically attuned to biofilm-related outcomes. This includes the adoption of endpoints beyond traditional clinical cure, such as imaging or molecular markers of biofilm burden, as well as stratification by baseline vitamin D status or mucosal cathelicidin levels. Incorporating these parameters is expected to clarify the circumstances under which vitamin D repletion, topical LL-37 or defensin analogs, or AMP-coated devices provide meaningful benefits in the management of biofilm-associated diseases.

9. Challenges, limitations, and safety considerations

Although promising, strategies targeting the vitamin D-AMP-biofilm axis encounter several significant limitations.

First, vitamin D possesses a relatively narrow therapeutic window when administered pharmacologically, as opposed to correcting deficiency. High-dose supplementation may result in hypervitaminosis D, hypercalcaemia, nephrocalcinosis, and kidney injury, especially with large bolus doses or dosing errors.[123-125] Consensus guidelines recommend that doses exceeding established upper limits should be administered only under medical supervision. Consequently, long-term high-dose regimens intended to enhance antimicrobial immunity require careful evaluation in clinical trials before widespread empirical use.[124]

Second, cathelicidin LL-37 exhibits both protective and potentially harmful biological effects. While it is essential for barrier defense, aberrant or excessive LL-37 expression is associated with several inflammatory diseases, such as psoriasis and rosacea, where dysregulated processing and overexpression promote NF-κB activation, leukocyte recruitment, and angiogenesis.[126-128] Overexpression has also been linked to altered lipid metabolism and increased cardiovascular risk in psoriasis, raising concerns regarding chronic systemic elevation of LL-37 as a therapeutic strategy.[129] Therefore, interventions targeting LL-37 must carefully balance antibiofilm efficacy with the risk of exacerbating inflammation or inducing unintended tissue remodeling.

Third, modulation of the vitamin D-cathelicidin axis can alter host-microbiota interactions in complex and context-dependent ways. LL-37 has demonstrated the ability to ameliorate barrier dysfunction and microbiota disturbances in experimental colitis.[130,131] However, elevated LL-37 levels in chronic obstructive pulmonary disease (COPD) are associated with increased bacterial load, neutrophilic inflammation, and airway microbial imbalance, particularly among patients treated with inhaled corticosteroids.[132] These findings indicate that pharmacological manipulation of AMP levels may produce divergent effects depending on anatomical site and disease context.

Although antimicrobial peptides (AMPs) are frequently characterized as “resistance-proof” due to their targeting of essential microbial envelope features, accumulating evidence demonstrates that bacteria can develop tolerance or resistance to both host-derived and therapeutic peptides. Documented resistance mechanisms include: (1) cell-envelope remodeling, such as increased incorporation of positively charged amino acids into phospholipids (mprF-mediated lysinylation of phosphatidylglycerol) or D-alanylation of teichoic acids, which diminish electrostatic attraction of cationic peptides; (2) lipopolysaccharide (LPS) modification and outer-membrane

remodeling in Gram-negative bacteria; (3) upregulation of multidrug efflux pumps; (4) secretion of exoproteases that degrade LL-37 or defensins; and (5) enhanced production of extracellular DNA (eDNA) and other matrix components that sequester AMPs within biofilms.[59,66,133-136] Laboratory evolution studies have identified strains of P. aeruginosa, S. aureus, and Enterococcus spp. exhibiting reduced susceptibility to LL-37 or LL-37-like peptides, occasionally accompanied by altered susceptibility to conventional antibiotics.

In comparison to classical antibiotics, the selection of AMP resistance may proceed more slowly, as it often requires multiple structural and regulatory adaptations. Nevertheless, chronic low-level exposure, such as that resulting from prolonged use of AMP-releasing coatings or repeated topical applications, can exert sustained selection pressure and may reduce the efficacy of endogenous host peptides at affected sites. These factors support the implementation of dosing regimens that achieve high local concentrations for brief durations, rigorous monitoring for the emergence of AMP-tolerant phenotypes, and the preferential use of AMP-based approaches in combination with other agents, such as antibiotics or dispersal enzymes, to mitigate the risk of resistance development.

From a drug-development standpoint, antimicrobial peptide therapeutics face generic hurdles: susceptibility to proteolysis, off-target cytotoxicity, potential immunogenicity, and high manufacturing costs.[133-136] Nano-formulations and surface coatings can mitigate instability but raise additional safety concerns about long-term accumulation of carrier materials and organ toxicity.[135] Bacteria can also adapt to AMP pressure via surface charge modification, protease production, or efflux, so resistance is not impossible even if it is less common than with classical antibiotics.[133,134]

Finally, clinical and regulatory challenges include difficulties in establishing robust biofilm-specific endpoints beyond standard clinical cure, as well as inter-patient variability in vitamin D status and VDR genetics. Furthermore, chronic wounds, device-associated infections, and mucosal biofilms exhibit considerable heterogeneity. These factors suggest that vitamin D-AMP-based interventions may be effective in certain contexts but not others, highlighting the necessity for carefully stratified, biofilm-focused clinical trial designs prior to widespread adoption in clinical practice.

These factors indicate that vitamin D-AMP-based interventions may demonstrate efficacy in specific clinical contexts, but not universally. Deployment should account for clinical uncertainty and the risk of bacterial adaptation. This underscores the need for rigorously stratified, biofilm-focused clinical trial designs before these interventions are widely implemented in clinical practice.

10. Emerging directions and future prospects

The vitamin D-AMP-biofilm axis is transitioning from foundational immunological studies to a stage characterized by precision pathway modulation, advanced peptide engineering, and systems-level analysis, which are expected to define research in the coming decade.

1. A promising avenue involves developing vitamin D analogs that maintain immunoregulatory properties while minimizing calcemic side effects. Several analogs, initially designed for bone and skin disorders, are being re-evaluated for their capacity to modulate innate immunity.[137,138] Certain compounds alter AMP expression distinctly from native $1,25(OH)_2D$, such as normalizing β-defensin levels in psoriatic skin and reducing excessive inflammation.[139] Strategic selection or design of analogs that selectively enhance cathelicidin or defensin responses at barrier sites, without inducing hypercalcemia or psoriatic pathology, may enable more precise manipulation of the vitamin D-AMP axis in tissues susceptible to biofilm formation.

2. Concurrently, artificial intelligence and machine learning (ML) are revolutionizing AMP discovery. Initial ML models for antibiofilm peptides have been deployed as predictive tools, while recent pipelines incorporate structural descriptors, sequence features, and experimental data to optimize potency, antibiofilm efficacy, and low toxicity within a unified framework.[140,141] Recent advances employ explainable ML and generative models to design novel anti-biofilm peptides, and comprehensive AI platforms are analyzing microbial genomes and metagenomes to identify millions of potential antimicrobial candidates.[142-145] Applying these computational approaches to vitamin D-responsive scaffolds, such as LL-37 and β-defensins, may expedite the development of next-generation cathelicidin-inspired antibiofilm therapeutics.

3. Another emerging area is systems biology and multi-omics. Vitamin D influences the expression of hundreds of genes across various immune cell types, and recent transcriptomic and epigenomic analyses in T cells and monocytes demonstrate that vitamin D status modulates entire signaling networks rather than isolated pathways.[14,146-148] Systems immunology methods that integrate transcriptomic, proteomic, metabolomic, and microbiome data can elucidate how vitamin D-induced AMPs contribute to broader host responses to infection.[149] Ultimately, these comprehensive analyses may facilitate personalized interventions, such as identifying patient subgroups with specific VDR variants or AMP expression profiles who are most likely to benefit from

vitamin D supplementation, topical LL-37, or AMP-coated medical devices.

4. Additionally, interactions with other innate immune pathways and microbiome-targeted interventions present further opportunities. Recent evidence indicates that inflammasome components, such as NLRP3, regulate AMP release in bladder epithelium, intersecting with sex hormone signaling and potentially affecting biofilms associated with urinary tract infections.[150] CRISPR-based strategies are also under investigation to modify both pathogenic and commensal organisms, enabling the engineering of microbiota that secrete protective AMPs or the direct editing of virulence and biofilm-associated genes.[151,152]

In summary, the advancement of this field depends on the integration of vitamin D biology, peptide engineering, AI-driven design, and systems-level analytics. If these approaches are effectively combined, while addressing the associated safety and complexity challenges, the vitamin D-induced AMP pathway could progress from a mechanistic concept to a foundation for innovative, biofilm-targeted anti-infective therapies.

11. Conclusion

Vitamin D-induced antimicrobial peptides, notably LL-37 and β-defensins, play a critical role at the intersection of host immunity and microbial ecology. By disrupting microbial membranes, interacting with extracellular polymeric substances, modulating quorum sensing, and regulating immune responses, these peptides inhibit biofilm formation, destabilize established biofilms, and facilitate clearance by both host immune mechanisms and conventional antibiotics. Findings from molecular, cellular, animal, and clinical studies indicate that vitamin D status modulates susceptibility to biofilm-associated infections by regulating the expression and function of antimicrobial peptides. Translational strategies involving vitamin D supplementation, topical delivery of antimicrobial peptides, synthetic peptide analogs, and advanced nanocarriers show promise, yet face challenges related to stability, toxicity, and the emergence of resistance. Future research that incorporates vitamin D analog development, precision medicine approaches, and artificial intelligence-driven peptide engineering may enable the development of effective therapies that leverage this innate immune pathway. In summary, the vitamin D-induced antimicrobial peptide pathway provides a promising foundation for the development of novel strategies to address biofilm-mediated infections, which continue to present significant challenges in contemporary infectious disease management.

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Conflict of Interest

There is no conflict of interest.

Supporting Information

Not applicable.

CRediT Statement

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