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Review article

Combinatory effect of nisin antimicrobial peptide with bioactive molecules: A review

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A B S T R A C T

The global concern over the emergence of antibiotic-resistant pathogens is growing, demanding for new and more effective antimicrobial strategies. Antimicrobial peptides (AMPs) have been the focus of great interest as alternatives to antibiotics. Nisin, a naturally occurring AMP, possesses unique characteristics such as an uncommon structure, amphiphilic nature, low likelihood of promoting bacterial resistance, and strong bactericidal activity against a wide range of pathogenic microorganisms. Moreover, it displays low cytotoxicity and is considered safe for human consumption. Thus, nisin shows potential as an alternative antimicrobial treatment. However, certain physicochemical properties, including low solubility and stability at physiological pH, may hinder peptide's efficacy *in vivo*. For this reason, the combination of nisin with other bioactive molecules has received considerable attention as a promising approach to enhance the peptide's antimicrobial efficacy in clinical applications. This comprehensive review examines the effect of nisin when combined with other AMPs, traditional antibiotics, anticancer agents, natural extracts, biopolymers and nanomaterials. Additive and synergistic interactions are highlighted, as well as these molecules potential applications in the biomedical field, shedding a light on the future of these promising combinations.

1. Introduction

For many years, antibiotics were the most widely used antimicrobial agents for the management of infectious diseases. However, their excessive consumption has led to an alarmingly high development of resistance by microbial pathogens, raising a serious global, public-health problem [1,2]. Hence, the search for novel alternatives to conventional antibiotics has been highly demanded. Antimicrobial peptides (AMPs) are the most widespread evolutionarily conserved components of the innate immune system of plants and animals, acting as a primary line of host defense against infections by exogenous microorganisms [3]. Unlike antibiotics, which are enzymatically synthesized secondary metabolites, AMPs are gene-encoded and ribosomally synthesized extracellular proteins, distinguished by a broad spectrum of activity and quick response. They act by degrading the microbial membrane and/or preventing synthesis of the principal cell components, which reduce the risk of developing resistance. In addition, AMPs are also involved in a diversity of biological functions, such as immune regulation, angiogenesis, and tissue regeneration [4,5]. These molecules can be chemically modified to increase their therapeutic potential, thereby presenting novel prospects for the prevention and management of multidrug-resistant bacterial infections [6,7].

AMPs are not exclusive of multicellular organisms; bacteria (Gram-positive and Gram-negative) can also produce molecular components resembling AMPs known as bacteriocins [8]. These have a broad antibacterial activity spectrum mainly against species phylogenetically close to the producing strain, which exhibits specific protection mechanisms against their own bacteriocin products by means of a corresponding immunity protein [9,10]. Bacteriocins can be classified in four main groups, according to their way of action, stability against heat and enzymes, molecular mass, and presence of post-translational modified amino acids. They can also be distinguished in classes: class I (<5 kDa) or lantibiotics that comprise the heat stable and modified peptides known for their unusual amino acid composition, such as the lanthionine; class II (<10 kDa), which are relatively heat stable and unmodified peptides known as non-lantibiotics; class III (>10 kDa), the peptides sensitive to heat and categorized into two subclasses, bacteriolysins that cause cell lysis and the non-lytic bacteriocins that have the inverse effect; and, class IV that includes a variety of compounds made of lipids or carbohydrates [11,12]. The vast majority of the reported bacteriocins are originated from Gram-positive bacteria, particularly from the lactic acid bacteria (LAB). These are non-spore forming and catalase-lacking bacteria that produce lactic acid as a major final product of the fermentation of carbohydrates. LAB are widely used as starter-cultures

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in the food industry to produce fermented goods. In addition, they are used to develop new sensory properties (texture, flavor, color, etc.), improve the nutritional quality of foods, and to preserve and ensure food safety by preventing the growth of spoilage and pathogenic microorganisms. LAB and their metabolites have been processed and consumed worldwide without adverse side effects [9,13]. The genus includes mainly *Enterococcus*, *Carnobacterium*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, and *Streptococcus* [13]. Nisin is the most famous bacteriocin belonging to the class I lantibiotic group, mainly produced by *Lactococcus lactis* bacteria. Although nisin A was the first characterized variant, others have been identified in taxonomically distinct organisms across various environments. This peptide is known to exhibit great inhibitory effects towards Gram-positive pathogenic bacteria, while maintaining low toxicity in humans as long as its pure amount does not exceed 12.5 mg/kg [11]. Nisin has been increasingly used in food preservation applications. However, in recent years, research on nisin has expanded towards the biomedical field, with therapeutic applications against bacterial infections, cancer, and oral diseases [14]. Despite its effectiveness against clinically relevant pathogens, the generalized use of nisin as a therapeutic agent comes with limitations related to solubility and stability under physiological pH, which restricts its systemic administration. Therapies based on combinatorial or synergistic effect of nisin with other bioactive compounds, like AMPs, traditional antibiotics, anticancer agents, extracts, and nanomaterials, may circumvent this issue. This review explores the progresses made on this front for biomedical purposes.

2. Nisin

Nisin is a polycyclic peptide composed of 34 amino acids capable of forming dimers or tetramers that possess a complex structure made of dehydroalanine (Dha) and dehydrobutyrine (Dhb) amino acids, resultant from the dehydration of serine and threonine, respectively; and uncommon amino acids such as lanthionine and β-methyllanthionine groups arising from the post-translational modifications of cysteine residues, characterized by a ring formation obtained through covalent binding of amino acids via five-position thioether bridges (Fig. 1) [15,16]. These are responsible not only for the structural arrangement and rigidity of the peptide, but also for imparting functional properties, such as proteolytic resistance, oxidation tolerance, and thermal stability at low pH [17]. The N-terminal domain contains hydrophobic residues and three rings: the A ring, which is crucial for the bioactivity of nisin; the B ring, which function remains unknown; and the C ring, which governs the structural diversity of nisin. On the other hand, the C-terminal domain contains hydrophilic residues and intertwined D and E rings, important in the antibacterial activity of nisin. Between the C and D rings, it is located the hinge region responsible for the flexibility of the entire nisin molecule (Fig. 1) [18]. Nisin is made of a large amount of basic amino acids, which endow the peptide with a net positive charge [19]. In aqueous solutions, nisin is completely stable at pH 2.0 and can be stored for a long periods at temperatures ranging from 2 to 7 °C, whereas above pH 7.0, it is poorly soluble and its inactivation occurs even at room temperature, since the presence of nucleophiles (electron-donating chemical species) turn the Dha and Dhb residues more susceptible to modification [9].

Nisin occurs naturally in many dairy products, being first discovered in 1928 in fermented milk cultures by Rogers and Whittier [21]. It is a product of the fermentation of food-grade bacteria. The most common variants of this peptide are produced by several strains of the non-pathogenic bacteria *L. lactis* ssp. *lactic*. Yet, nisin can also be produced by other Gram-positive species, like *Blautia*, *Staphylococcus* and *Streptococcus* [22]. Nisin can be quickly digested by proteases of the mammalian gastrointestinal tract, without triggering side effects on the gut microflora. Considering that nisin does not enter into the systemic circulation, the likelihood of developing antimicrobial drug resistance is reduced [23]. Toxicity studies have shown that consumption of nisin does not produce any harmful effects in humans, with its estimated lethal dose (LD_{50}) being of 6950 mg/kg, comparable to that of salt [24]. In addition, its broad antimicrobial activity, thermal stability and odorless and tasteless characteristics have placed nisin within the European food additive list under the number E234. It also achieved the "Generally Recognize As Safe" (GRAS) status by the World Health Organization (WHO) in 1969 and by the US Food and Drug Administration (FDA) in 1988 for its use as a natural food preservative. Presently, nisin is the only commercially available bacteriocin used in permitted food products, licensed in over 50 countries [4,22,25]. Nisin is biosynthesized ribosomally from a genetically encoded precursor peptide, termed NisA, which is composed of an N-terminal leader peptide, essential for recognition by the modification machinery, and a C-terminal core peptide, where post-translational modification occurs. The unmodified nisin precursor is processed by a specific maturation machinery that is responsible for: (i) dehydration reactions of hydroxyl amino acids to produce Dha from serine and Dhb from threonine, via NisB; (ii) ring formation by NisC catalyzing the conjugate addition of cysteine residues to five of the Dha and Dhb residues to generate five cyclic thioethers; (iii) transport of the propeptide across the cytoplasmic membrane by NisT; and (iv) cleavage of the leader peptide by the NisP protease, which releases the biologically active nisin [26,27]. For its production, nisin is fermented from *L. lactis* cells grown in a supplemented whey or milk medium [28], following primary metabolite kinetics (exponential growth phase to stationary phase) [5]. After fermentation, the produced nisin is concentrated, separated and purified. Over time, several methodologies have been developed and tested to maximize its production, including control of temperature, time and pH; various culture media to ensure bacterial growth and reduce production costs; mixtures of microorganisms; and genetic modifications [13,15].

2.1. Antimicrobial activity of nisin

Nisin exhibits wide inhibitory activity against Gram-positive bacteria, particularly against spore-forming bacteria like *Bacillus* [29] and *Clostridium* [30], since spores are more sensitive to peptide than vegetative cells. Nevertheless, nisin also shows great potential against some genera of non-spore forming bacteria, including *Listeria* [31], *Micrococcus* [32], *Staphylococcus* [14], *Streptococcus* [33], *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Mycobacterium*, and *Pediococcus* [8,11].

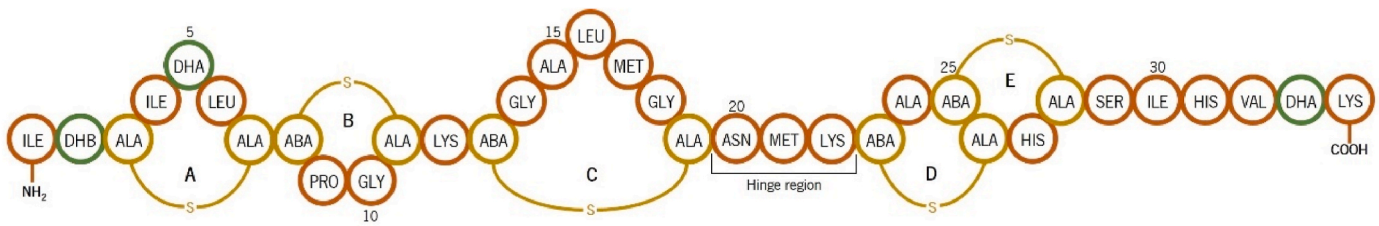

Fig. 1. Primary structure of nisin A. Dehydroalanine (DHA) and dehydrobutyrine (DHB) amino acids are represented in green circles. Lanthionine and β-methyllanthionine groups are represented by ALA-S-ALA and ABA-S-ALA, respectively. Adapted from Ref. [20] with CC BY 4.0 permission. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Additionally, this AMP acts against bacterial biofilms that form complex structures and confer increased resistance to antimicrobial agents, by disrupting or preventing their formation. Antibiotic-resistant strains, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE), also exhibit sensitivity to nisin [34]. However, this AMP shows little to none antimicrobial activity against Gram-negative bacteria, filamentous fungi, yeast cells, and viruses [11].

The peptide mode of action against vegetative cells has been extensively studied and is based on a dual action mechanism that occurs at the level of the bacterial membrane, a crucial structure for cell survival [35]. The positively charged nisin interacts with the negatively charged surface of the bacterial membrane by electrostatic bonds through a specific membrane target, Lipid II, an amphipathic peptidoglycan that functions as a precursor molecule in the bacteria cell wall synthesis, serving as a lipid anchor for many biomolecules. This interaction enables nisin to disrupt the multi-enzymatic production cycle of Lipid II, inhibiting the cell wall synthesis. In addition, the N-terminal domain of nisin binds with the carbohydrate-pyrophosphate moiety of Lipid II creating a nisin-Lipid II complex, which allows the C-terminal segment of nisin to permeate and insert into the cytoplasmic membrane, forming an ion channel or a pore (Fig. 2). This leads to an increase in cell membrane permeability with the proton motive force and pH balance being disrupted and causing ion leakage and ATP hydrolysis, ultimately leading to cell death [13,17,36]. On the other hand, the mode of action of nisin against spore-forming bacteria has not yet been fully elucidated. This peptide exerts a sporostatic function by controlling spore outgrowth in germinating spores through binding with Lipid II, preventing cells from becoming metabolically active after germination [23,37]. It has also been reported that heat treatment can increase the susceptibility of spores to nisin [38]. Treatments with CO_2 at high pressures can also aid with the penetration of nisin into the inner membrane of spores, resulting in greater inactivation [39].

Gram-negative bacteria are highly resistant to nisin since the structure and disposition of their membrane and cell wall are different from Gram-positive bacteria. They display a thick outer membrane that covers the cytoplasmic membrane and function as an additional highly permeable compound-selective barrier made of lipopolysaccharides (LPS) [41,42]. This insensitivity of Gram-negative bacteria to nisin could be due to the large size of the peptide, preventing its passage across the outer membrane, and the presence of LPS that act as a barrier to its action [43]. Yet, nisin can be combined with chelating agents, such as ethylene-diamine-tetra-acetic acid (EDTA) and Tween® 80, that are able to remove divalent magnesium and calcium ions from the outer membrane, which are essential for stabilizing the negative charge of the oligosaccharide chain of the LPS component. Thereby, about 50 % of the LPS molecules are released and the phospholipids of the cytoplasmic cell membrane are exposed, facilitating the permeabilization of nisin [43,44]. Other methods, such as heat treatment, hydrostatic pressure, freezing and lowering pH, can be combined with nisin to destabilize the outer membrane and promote its action against Gram-negative bacteria [15,19].

The antimicrobial activity of nisin is mainly dependent on its aqueous solubility and structural stability at varying pH; it increases with lowering pH. As the pH reaches or exceeds neutrality, nisin solubility reduces. Similarly, its antimicrobial activity decreases gradually with increasing pH, which can be explained by an irreversible modification of the molecular structure of nisin through the formation of multimers via intermolecular interactions [28,40]. The thermal stability of nisin is another factor that interferes with its antimicrobial activity and is also related to pH. At high pH values, nisin becomes increasingly less stable to heating even though it manages to resist heat under acidic conditions. At low temperatures, such as freezing, nisin activity is highly stable, but is significantly lost with increasing temperatures, which can be explained by the presence of the thioether bridges [13].

Apart from its direct antimicrobial action, nisin's ability to modulate the immune system is crucial for indirectly fighting microbial infections [8]. The multifactorial immunomodulatory effect of nisin includes the modulation of cytokine production, being among the peptides involved in regulating the immune response [45–47]; and modulation of innate immune cells, including monocytes, macrophages and neutrophiles [48,49], and adaptive immune cells, such as B and T lymphocytes [49,50]. It has been reported that nisin stimulates immune cells in a manner similar to host defense peptides, activating growth factor receptors and signal transduction pathways [45]. Nisin has also attracted interest as a potential anticancer agent due to its cytotoxic effect on cancer cells coupled with low somatic cytotoxicity. However, its exact anticancer mechanism has not yet been determined. It has been suggested that nisin, in head and neck squamous cell carcinoma (HNSCC) cells, induces apoptosis, cell cycle arrest in the G2 phase, and suppresses cell proliferation. These effects are attributed to the activation of CHAC1, a pro-apoptotic cation transport regulator, as well as to a concomitant influx of extracellular calcium, CHAC1-independent. Nisin is able to form pores in the HNSCC cells by altering their transmembrane potential and membrane composition, mediating the reorganization of phospholipids and the influx of ions, including calcium. These events are believed to

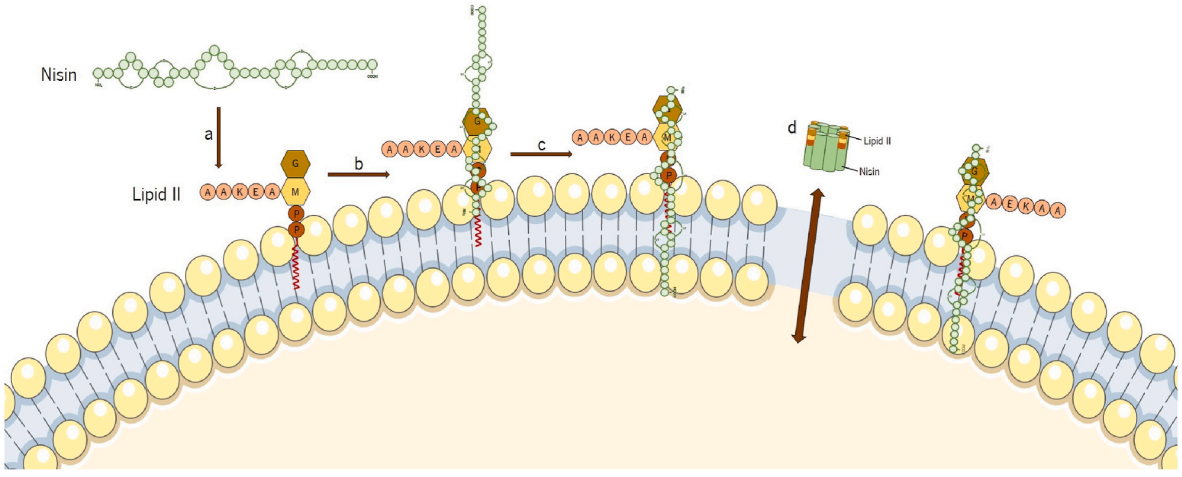

Fig. 2. Nisin's mechanism of action against bacteria. **(a)** First, nisin interacts with the bacterial cell membrane and **(b)** specifically targets the carbohydrate-pyrophosphate group of Lipid II through its N-terminal. **(c)** The C-terminal inserts into the membrane to form a pore. **(d)** The nisin-Lipid II complex pore has a stoichiometry of eight nisin molecules to four Lipid II molecules. The figure was produced using elements from Servier Medical Art. Adapted from Ref. [40] with CC BY 4.0 permission.

contribute significantly to the apoptosis and changes in cell proliferation. Furthermore, nisin can inhibit angiogenesis, HNSCC orasphere formation, and tumorigenesis *in vivo* [51,52].

2.2. Natural variants of nisin

Thus far, thirteen naturally occurring nisin variants have been discovered and characterized, with nisin A (Fig. 1) being the most extensively studied and the first to be purified. Genetic modifications in different strains of *L. lactis* have allowed new variants to be produced, including Z, F and Q, which differ in their properties because of the occurrence of small alterations in their amino acid sequences. nisin Z is considered the first natural variant of nisin A. Both are isolated from *L. lactis* strains used in milk and dairy production and share an identical structure except for a single amino acid, His27 found in nisin A is replaced by Asn27 in nisin Z [53] (Fig. 3). It has been demonstrated that both exhibit similar antimicrobial activity, although, at neutral pH, nisin Z shows a superior rate of diffusion and solubility [54]. Only these two variants are currently used in commercial applications. Nisin F producer, *L. lactis* F10, can be isolated from the intestine of freshwater catfish in South Africa and differs from nisin A in the positions Asn27 and Val30 (Fig. 3) [55]. *In vitro* [55] and *in vivo* [56,57] studies have demonstrated the anti-infective therapeutic effect of this variant. Nisin Q is isolated from *L. lactis* 61-14 from Japanese river water and differs from nisin A in four positions, Val15, Leu21, Asn27 and Val30 (Fig. 3) [58]. This variant has shown similar antimicrobial activity to nisin A, Z and F against *S. aureus* [59]. In addition, it demonstrates greater oxidative tolerance than nisin A [60], being considered an alternative to nisin A [61].

More distantly related nisin variants have been isolated from *Streptococcus* genus, such as nisin U, U2, P, H, E and G. Nisin U and U2 are produced by independent isolates of *S. uberis*, which frequently inhabits the lips, skin and udder tissues of cows and is present in raw milk. Nisin U and nisin U2 differ from nisin A at nine and ten amino acid positions, respectively, as well as for lacking three C-terminal amino acids, making them shorter variants. In nisin U and U2 the Lys4, Ile15, Dhb18, Pro20, Leu21, Gly27, His29, Phe30 and Gly31 are present (Fig. 3). Nisin U2 contains an additional substitution, the Val1, which replaces the Ile1 found in nisin U and A (Fig. 3) [62]. Nisin P was discovered by genome mining techniques in *S. gallolyticus* ssp. *pasteurianus,* an organism residing in the digestive system of ruminants. Its protein sequence resembles that of nisin U2, differing only in two amino acids positions, Ala20 and Ile21 (Fig. 3) [63]. Garcia-Gutierrez et al. identified and purified nisin P from *S. agalactiae* isolated from human feces, and compared its *in vitro* activity with nisin A and nisin H. They found that nisin P was less potent than the other two variants [64]. Nisin H, which sequence differs from nisin A in five amino acids (Phe1, Met6, Dhb18, Tyr21 and Lys31) (Fig. 3), can be isolated from a strain of *S. hyointestinalis* present in the porcine intestine. Nisin H appears to be an intermediate between variants of lactococcal and streptococcal origin, since it maintains the three terminal amino acids found in nisin A, Z, F and Q, while possessing a Dhb18 similar to nisin U, U2 and P [65]. Bhattacharya et al. employed *in silico* methods to explore the potential of different nisin variants to bind to the hACE2 receptor, the target site of the 2019-nCoV coronavirus. Nisin H and Z exhibited a significant binding affinity to the receptor, uncovering its potential as a coronavirus therapeutic agent [66]. Two *S. equinus* strains, APC4007 and APC4008, obtained from sheep milk, are responsible for producing nisin E, which has a very similar amino acid sequence to nisin U, except for two substitutions in Ala15 and Ile21 and an extra C-terminal amino acid, the Asn32 (Fig. 3) [67]. Nisin G, produced by *S. salivarius* DPC6487, isolated from human gut, presents seven amino acid substitutions compared to nisin A: Tyr4, Val15, Ala18, His20, Leu21, Asn27 and Ile31 (Fig. 3). This variant shows great potential for probiotics-related applications [68]. Other variants of nisin have also been identified, including nisin O found in *Blautia obeum* A2-162 isolated from a human gastrointestinal tract. It has been reported that the nisin O operon is somewhat unusual as it encodes four peptides, with the first three being identical between each other and similar to nisin U, while the fourth peptide exhibits the highest divergence from nisin A with 18 amino acid substitutions (Fig. 3) [69]. Nisin J is the first report variant produced by a human skin isolate of staphylococcal origin (*S. capitis* APC 2923). Compared to nisin A, this variant has eight amino acid substitutions (Lys4, Gln17, Dhb18, Phe20, Ala21, Gly30, His33, Thr34) and an extra C-terminal amino acid, the Lys35 [70]. Recently, a new variant produced by *Ligilactobacillus salivarius* P1CEA3, nisin S, was

Fig. 3. Comparison between the different natural variants of nisin. The orange circles represent the structure of nisin A. The amino acid substitutions are indicated by green circles. Adapted from Ref. [22] with CC BY 4.0 permission. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

identified. Seven amino acids substitutions were detected compared to nisin A, namely Tyr4, Thr20, Ser25, Gly27, His29, Val30 and Ile32 [71].

In addition to these natural nisin variants, there are bioengineered forms of nisin with improved physicochemical properties, antimicrobial effectiveness, stability in diverse physiological conditions, and enhanced its pharmacokinetic features for healthcare applications [17,40]. Extensive research has been conducted in modifications in the hinge region of nisin, which is important for the insertion of the peptide into the bacterial membrane. This region comprises three amino acids, Asn20–Met21–Lys22, positioned between the first three (A, B and C) and the last two (D and E) lanthionine-constricted rings of the peptide (Fig. 1) [72–75]. Furthermore, the fusion of anti-Gram-negative peptides with the C-terminus of nisin can enhance its activity against Gram-negative bacteria, improving its outer membrane-permeating capacity [76]. Several bioengineered strategies have been described, frequently involving Polymerase Chain Reaction (PCR)-based approaches, like site-directed, random and saturation mutagenesis [59,77,78]. The use of heterologous bacteriocin expression systems to produce bioengineered nisin variants offers advantages over natural producers, including the possibility of increasing yield, since the original hosts often show low production rates. Also, through this, improved peptide purity and safety is guaranteed as many producing cultures require complex broths for growth and the original producer may be pathogenic [79,80].

3. Combinatory effects

Over the years, nisin has been extensively used with minimal evidence of resistance. Yet, some studies indicate that nisin-sensitive strains can develop resistance once repeatedly exposed to increasing concentrations of nisin [81–83]. The resistance mechanism employed by microorganisms against nisin involves cell wall thickening, change in surface charge, alterations in membrane phospholipid and fatty acid composition, enzymatic degradation of nisin, DNA mutation, and differential gene expression, particularly in Gram-positive bacteria such as *S. aureus*, *Listeria monocytogenes*, and *Streptococcus* [84]. Combinations of nisin with other compounds have been investigated for their additive or synergistic effects in order to address the issue of resistance and boost its therapeutic potential for treating bacterial infections [85]. Synergy is defined as a condition in which the combined action of antimicrobial agents has an impact greater than the sum of their individual effects. Its clinical importance lies in leveraging the different mechanisms of action of the agents involved, thereby offering an additional therapeutic option for challenging infections [86]. The following sections delve into the biological effects arising from the combination of nisin with biopolymers, nanoparticles, fibers, other AMPs, proteins, traditional antibiotics, anticancer agents, natural extracts and chelating agents (Fig. 4).

3.1. Combination of nisin with biopolymers

Among all biopolymers, polysaccharides have gained attention due to their biocompatibility, biodegradability, natural abundance, easy modification and functionalization, and non-immunogenic features, which has turned them into one of the most used biomaterial types in nanomedicine [87,88]. Because nisin has limited solubility and stability at physiological pH, which hinders its systemic application, keeping its integrity until the targeted location is reached is critical. Pectin, a neutral polysaccharide present in the cell wall of plants, was combined with hydroxypropyl methyl cellulose (HPMC), a polymer used as an excipient and as a controlled delivery component in oral medicines, for delivering drugs in the colon. While HPMC promoted mechanical protection, pectin was found to control nisin release, generating an enzymatically moderated delivery system. This system envisioned the treatment of colonic infectious caused by *Clostridium difficile* and VRE, being most effective in an 80/20 % ratio of pectin/HPMC loaded with 100 mg of nisin during a 6 h dissolution test. Nisin was found to be

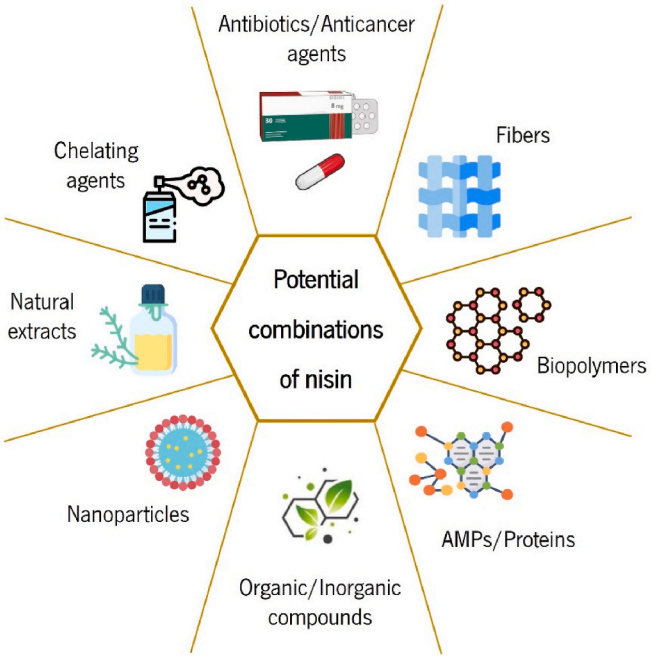

Fig. 4. Potential combinations of nisin with other bioactive agents, polymers and structures. The figure was produced using elements from free collection Flaticon.

active/stable during processing and *in vitro* testing [89]. Starch possesses the capability to resist digestion in the upper gastrointestinal tract and subsequently be fermented by colonic bacteria. Consequently, delivery systems based on this polysaccharide were proposed for the targeted release of nisin in the colon. Gough et al. assessed the impact of nisin on the murine microbiota in mice using two starch-based administration matrices: starch dough and starch gel. The results revealed that the mice fed with a diet containing starch gel guaranteed the integrity of nisin in their feces. Moreover, this group exhibited a larger variety of bacterial taxa in the lower gastrointestinal tract compared to the mice fed with the starch dough-based matrix. This could be attributed to the fact that nisin was released more rapidly and at an earlier stage from the starch dough-based matrix. As a result, a larger amount of nisin was digested in the upper gastrointestinal tract by the digestive enzymes secreted there, resulting in a reduced impact on the microbiota of the lower gastrointestinal tract [90].

Cellulose is a commonly used polysaccharide with immense relevance in the biomedical field. With the advancement of nanotechnology tools, nanocelluloses have emerged as a new family of natural-based materials, with unique properties (i.e., large surface area to volume ratio) [91]. Weishaupt et al. investigated the possibility of enhancing the antimicrobial activity of nisin by combining it with a nano-fibrillated cellulose and generate a nisin-containing biocomposite suspension. Data reported a significant improvement of the antimicrobial profile of nisin against *Bacillus subtilis* and *S. aureus* strains in the conjugate composite compared to its free form [92]. In another study, nisin immobilized onto nanocrystalline cellulose was seen to retain its antimicrobial activity for longer than in its free form (inactivation occurred after 24 h exposure to the bacterial strains), suggesting that the immobilization was effective in controlling release and improving the peptide mechanisms of action against bacteria, making it a promising candidate for developing antibacterial wound dressings [93].

A polyelectrolyte multilayer system based on natural polysaccharides, carrageenan and chitosan, was also investigated to assess the antimicrobial activity of the variant nisin Z through layer-by-layer technique. The results revealed significant efficacy against *S. aureus* and MRSA strain, with over 90 % and 99 % of planktonic and biofilm

cells being eliminated, respectively, compared to control films. Remarkably, the location of nisin Z within the multilayer did not compromise its antimicrobial efficacy. These systems have been investigated for a variety of applications, including to accelerate wound healing or reduce infection rates due to biofilm growth on implantable stents [94]. Tayeferad et al. developed chondroitin sulfate (CS)–nisin nanogels (CS–N NGs) to fight MRSA and methicillin-sensitive *S. aureus* (MSSA) on soft tissues. CS is a multifunctional glycosaminoglycan composed of alternating sugar units, including N-acetylgalactosamine and glucuronic acid, that is non-toxic, biodegradable, biocompatible, easily modified and with tunable size. The enzyme and pH-sensitivity inherent to the CS–N NGs, due to the presence of susceptible bonds in CS, resulted in an effective and controlled release of nisin in the simulated infectious medium, eradicating the clinical strains with low cytotoxicity [88]. In another study, the biocompatible anionic polysaccharide gellan gum (similar to the agar used in microbiological cultures) was conjugated with nisin to enhance its antibacterial activity and stability over time. It was found that the conjugate extended the antibacterial activity of nisin against *Staphylococcus epidermidis*. Under acidic conditions, the duration of its action increased from 96 h–216 h, and under alkaline conditions, it increased from 48 h to 144 h. In addition, hemolysis and cytotoxicity tests confirmed the excellent biocompatibility of the conjugate and its thermal resistance at 80 °C [95].

ε-poly lysine (ε-PL) is an attractive FDA-approved biopolymer with recognized GRAS status, which has shown strong antimicrobial activity against a diverse group of microorganisms and stability in a broad range of pH. Najjar et al. demonstrated the strong antimicrobial synergism of nisin with ε-PL against *Streptococcus mutans*, an important oral pathogen responsible for dental caries in humans and serious infectious diseases, making these active ingredients promising for oral care products [96]. Liu et al. also evaluated this synergistic effect against *Escherichia coli*, *S. aureus* and *B. subtilis*, concluding that nisin can instigate the penetration of ε-PL within bacterial cells, this way facilitating its interaction with the bacteria DNA to prevent their replication [97]. Glycol chitosan (GC) is a water-soluble form of chitosan that can be chemically modified and/or covalently crosslinked to itself or other polymers to generate hydrogels or colloidal structures for drug encapsulation and controlled release. Flynn et al. uncovered optimal synergistic antimicrobial activity between nisin (15 μg/mL) and GC (3/6 %, w/v) against *S. aureus*. Here, a versatile platform was presented by incorporating GC into an injectable polysaccharide gel for encapsulation and extended release of nisin for at least 10 days [98]. Another work by Flynn et al. evaluated the synergisms between nisin and ε-PL and/or GC demonstrating that nisin's antimicrobial activity can be enhanced in fasted state simulated intestinal conditions [99].

Polyethylene glycol (PEG) combined with molybdenum disulfide (MoS_2) (PEGylated MoS_2) can act as a drug delivery vehicle, however,

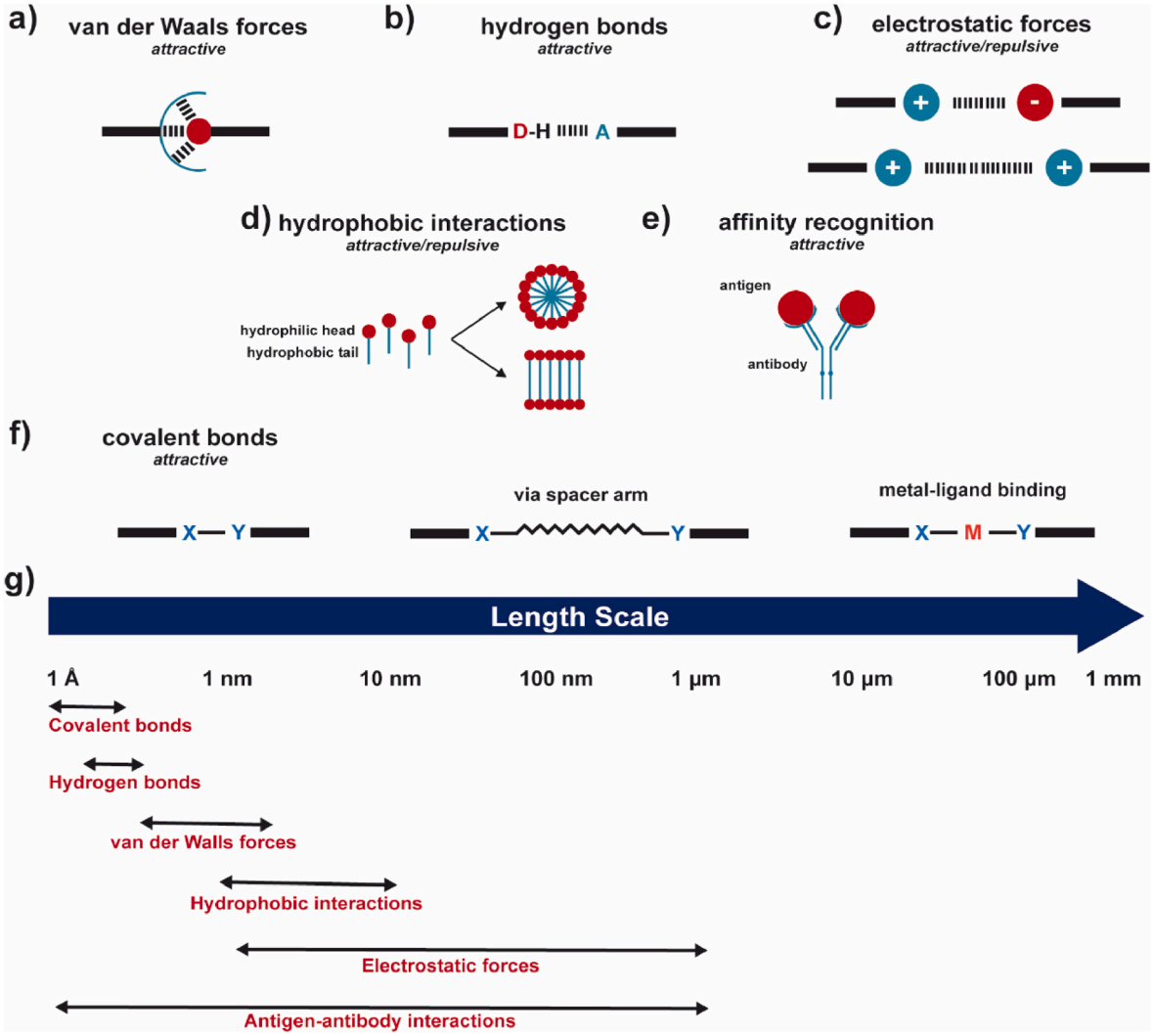

Fig. 5. Forces involved in nisin combined with nanoparticles or polymeric structures. **(a)** van der Waals forces; **(b)** hydrogen bonds; **(c)** electrostatic forces; **(d)** hydrophobic interactions; **(e)** affinity recognitions; **(f)** covalent bonds; and **(g)** length scale of the forces involved (used with CC BY 4.0 permission from Ref. [3]).

with poor antibacterial activity. To overcome this issue, a nisin@PEGylated MoS_2 conjugate was engineered. This conjugate was found to increase reactive oxygen species (ROS) production and, through that, instigate bacteria apoptosis. The nisin@PEGylated MoS_2 facilitated peptide penetration through the cell membrane of *E. coli*, above the *S. aureus*, favoring intracellular ROS production. Consequently, the antibacterial activity of nisin was improved against the Gram-negative bacteria. Additionally, the toxicity of the conjugate was found to be minimal against HeLa cells [100].

3.2. Combination of nisin with nanoparticles

Since nisin is extremely sensitive to enzymes and is, therefore, rapidly metabolized by the human body, protective strategies by means of nanoparticles (NPs) or polymeric structures that ensure mechanical and chemical resistance are viable. These bonds are established through intermolecular forces of varying degrees of intensity (Fig. 5). Nanoparticle assisted co-delivery of multiple drugs seems to be a clever, promising and elegant approach to overcome the different pharmacokinetics, biodistribution and membrane transport limitations and costs of individual drugs. Goudarzi et al. investigated a novel drug delivery system for uses against gastrointestinal (AGS and KYSE-30), hepatic (HepG2) and blood (K562) cancer cell lines based on nisin-loaded poly (lactic acid) (PLA) NPs, the nisin-PLA-PEG-PLA NPs. The system was examined for its cytotoxic effect against the cancer cell lines showing improved effectiveness over the nisin in its free form. The NPs were also deemed a successful protective barrier for nisin, controlling its liberation overtime and preventing bacteria from developing resistance [101]. On the inorganic front, Arakha et al. investigated the functionalization of nisin onto silver NP (AgNP) via interfacial interactions, predominantly electrostatic, against *B. subtilis*, *E. coli*, *S. aureus* and *Proteus vulgaris* and determined that the interaction energy and interfacial ROS generated played an important role in destabilizing the bacteria membrane, facilitating the permeabilization process. The cavity formed on the membrane and the intracellular ROS reduced cell viability, showing this antibacterial formulation to be capable of extending the efficacy of nisin [102].

3.3. Combination of nisin with fibers

Fiber-based scaffolds have been employed as carriers for delivering various compounds, including drugs, growth factors, proteins, NPs, and AMPs (Fig. 5) [103]. Depending on the intended purpose, different techniques can be used for fiber production. One of the most widely used techniques for producing fibers at the nanoscale is the electrospinning. This is a simple and cost-effective method that produces nanofibers with a large surface-to-volume ratio [104]. In the context of wound healing, Heunis et al. produced nanofibers from poly(ethylene oxide) (PEO) and poly(D,L-lactide) (PDLLA) polymers (50:50 w/w) loaded with nisin. The dressing was tested against viable cells in a murine excisional skin infection model, showing a significant reduction on *S. aureus* proliferation: bacterial burden of nisin-treated wounds was 4.3×10^2 colony forming unit (CFU), whereas the control wounds reported 2.2×10^7 CFU after 7 days culture. Additionally, the wound dressings promoted excisional wound closure, with histological analyses showing no adverse side effects [105]. Ahire et al. investigated the antimicrobial potential of PEO/PDLLA electrospun nanofibers loaded with nisin and AgNPs and demonstrated their ability to hinder the growth of various microorganisms, including Gram-positive and Gram-negative bacteria. It was also observed that nanofibers containing only nisin were unable to inhibit the growth of Gram-negative bacteria. Therefore, the combination with AgNPs was found essential for achieving a broad-spectrum antimicrobial wound dressing [106]. Coaxial and triaxial fibrous membranes, encapsulating nisin at their core, have also been explored using electrospinning. The coaxial fibers consisted of a polyvinylpyrrolidone (PVP)/nisin core surrounded by a hydrophobic polymer shell made of polycaprolactone (PCL). Results showed an antimicrobial activity of 5 log against *S. aureus* after 24 h culture. However, the efficacy of the fibers decayed with time (3 log reduction after another 24 h), suggesting their suitability only for applications requiring burst releases of nisin. On the other hand, the triaxial fibers, consisting of a PVP/nisin core, an intermediate PCL layer, and a hygroscopic cellulose acetate sheath, exhibited excellent antimicrobial activity, achieving a log reduction above 4 for exposures exceeding 5 days. In comparison to the coaxial fibers, these displayed stronger and longer-lasting antibacterial effects, providing antimicrobial protection up to 7 days of culture. Furthermore, the nisin-encapsulated triaxial fibers demonstrated excellent antimicrobial activities against other Gram-positive bacteria, namely *Bacillus anthracis Sterne* and *Micrococcus luteus* [107].

Wet spinning is a very versatile technique for producing microfiber structures of large intrinsic porosity and interconnected pores. Microfibers composed of a blend of sodium alginate and gelatin polymers were functionalized with the nisin Z variant by immersion and tested against *S. aureus*. The results showed that the fibers chemically stabilized by crosslinking with glutaraldehyde and functionalized with nisin Z were capable of progressively eliminating the bacterium, reaching an inhibition superior to 99 % after 24 h of culture. These findings highlighted nisin Z as an effective agent in combating *S. aureus*-induced infections when loaded into biodegradable, crosslinked polymeric scaffolds [14].

3.4. Combination of nisin with AMPs

The use of multiple AMPs with different mechanisms of action allows for combinatory or synergistic effects to manifest against targeted microorganisms, reducing the required dose and thereby minimizing toxicity and preventing microbial resistance. This is known as the multiple hurdle approach. Here, the greater the diversity of the "hurdles", the more effective they are in lowering the probability of targeted cells surviving treatment and developing resistance [96,108]. P10 is a cationic peptide, a synthetic derivative of the AMP LL-37, that exhibits a broad-spectrum antimicrobial profile, being lethal against many antibiotic-resistant bacteria strains, and strong anti-biofilm activity. The minimum inhibitory concentration (MIC) of P10 and nisin combination was determined against Gram-negative bacteria. Synergisms were observed against antibiotic-resistant standard strains ranked in the global priority list of WHO, such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii* [108]. Chi and Holo tested the combination of Garvicin KS, an antimicrobial bacteriocin produced by *Lactococcus garvieae* capable of inhibiting the action of Gram-negative bacteria, with the AMPs Farnesol and nisin, observing a rapid eradication of all *S. aureus* strains tested. Each individual agents were unable to achieve such outcome even after 48 h of exposure. The combination of nisin and Garvicin KS resulted in complete bacterial elimination within approximately 12 h, with no regrowth observed after 48 h. Similar observations were made with the nisin and Farnesol mixture [109]. Gomes et al. developed a strategy to fight multidrug-resistant and biofilm-producing strains of *S. aureus* and *P. aeruginosa* on diabetic foot ulcers (DFUs), a major complication of Diabetes *mellitus*, through the combination of Pexiganan and nisin. The drug delivery system included guar gum, a natural polysaccharide, to ensure the peptides' protection against degradation or inactivation. Data demonstrated the ability of the system to reduce local therapeutic needs by requiring smaller amounts of Pexiganan for achieving the envisioned outcome [110].

3.5. Combination of nisin with proteins

Clinically relevant antimicrobial proteins such as lactoferrin and lysozyme, the two most abundantly secreted in the human respiratory tract, can be combined with nisin for improved antimicrobial efficacy. De Kwaadsteniet et al. reported synergisms between the variant nisin F with these proteins against one of the most important pathogens in

upper and lower respiratory tract infections, *S. aureus*, in immunocompromised rats. Notably, the proteins action was amplified in the presence of small concentrations of nisin F (80–320 AU/mL). Histological tests confirmed the absence of toxicity introduced by nisin F in the lungs, trachea and bronchi of rats, as well as in the production of white cells, lymphocytes and neutrophils [57]. Sodium caseinate, a pH-responsive protein derived of milk protein casein, is a suitable candidate for nutraceuticals delivery and serves as a natural vehicle for bioactive compounds. Niaz et al. developed a nano-carrier system using mucoadhesive protein (sodium caseinate)–polysaccharide (sodium alginate) coacervates to encapsulate nisin. This system demonstrated high encapsulation efficiency of nisin at pH 5 and effectively control of the growth of *Enterococcus* and *Staphylococcus* species, which are responsible for biofilm-forming oral diseases. Additionally, it was found to inhibit and eradicate pre-formed oral biofilms, suggesting its potential use in pharmaceutical products and oral mouthwash. Due to their efficacy in releasing antimicrobial agents in the oral cavity, triggered by pH, these systems may offer promising preventive and therapeutic solutions for controlling biofilm-associated oral infections [111]. With equal purpose, Tong et al. evaluated the effect of nisin combined with 18 D-amino acids, which form one of the most striking features of the peptidoglycan composition in bacteria and play a key role in the regulation and disassembly of bacterial biofilms. These agents were explored independently and in combination against cariogenic *S. mutans* bacterium and biofilms. The findings from crystal violet biofilm assays revealed that mixtures of Glu, Asp or Cys amino acids with nisin improved its effectiveness against *S. mutans* and prevented biofilm formation during early stages of culture [112].

3.6. Combination of nisin with antibiotics

The rise of microbial resistance to traditional antibiotics is a significant threat to the successful treatment of various infectious diseases. AMPs have emerged as a promising option to combat this problem by replacing certain antibiotics or working side-by-side with them. Nowadays, there are numerous studies exploring the effectiveness of combining nisin with conventional antibiotics. Polymyxins are a class of antibiotics used as a drug of last resort to treat numerous infections caused by Gram-negative bacteria that are resistant to all remainder available antibiotics. However, polymyxins may cause serious toxic effects, mainly on the kidneys and nervous system. To mitigate these risks and reduce concentration while enhancing efficacy without increasing bacterial resistance, several researchers have investigated the combination of polymyxin B and/or E (colistin) with nisin [16,108,109,113–116]. These combinations were tested against clinically relevant Gram-negative bacteria, such as *P. aeruginosa* [108,113,114,116], *A. baumannii* [108,109,115], *E. coli* [109,116] and *Salmonella choleraesuis* [116], assessing their antimicrobial and anti-biofilm effects. Nisin and polymyxins have different targets in bacterial cells, indicating that potential synergisms may involve polymyxins acting on the outer cell wall followed by nisin acting on the cell membrane. All studies have demonstrated the potential of these synergistic approaches as novel therapeutics for treating multi-drug resistant bacteria [16,108,109,113–116].

β-lactam antibiotics are amongst the most common due to their effectiveness, low cost, easy administration, and minimal side effects. These antibiotics contain a β-lactam ring in their chemical structure and specifically target transpeptidase enzymes involved in bacterial cell wall synthesis. However, bacteria tend to develop resistance to these antibiotics by producing a β-lactamase enzyme, which attacks the β-lactam ring [117]. The effectiveness of combining nisin with three β-lactam antibiotics (ampicillin, ceftriaxone and cefotaxime) against *Salmonella enterica* serovar Typhimurium, a microorganism responsible for gastrointestinal tract infections, was evaluated by Singh et al. [86] and Rishi et al. [118]. *In vitro* and *in vivo* studies demonstrated a strong synergistic effect between nisin and ceftriaxone as well as nisin and cefotaxime, whereas only an additive effect was observed with nisin and ampicillin. This difference can be attributed to the presence of β-lactamase, which renders ampicillin ineffective. However, this enzyme is incapable of inactivating higher-generation cephalosporins, such as ceftriaxone and cefotaxime, which possess an oxyimino side chain [86,118]. Another study from the same authors aimed at exploring the mechanisms responsible for the nisin/β-lactam combinatory effect against *Salmonella*. Multiple modes of action were reported, including permeabilization of the bacterial outer membrane due to the metal chelating properties of β-lactam antibiotics; inhibition of DNA, RNA and protein synthesis; and interference with the immune-modulatory response [119]. Alves et al. studied the combination of nisin with the β-lactam antibiotic oxacillin against MRSA. The action of oxacillin in bacterial cell wall biosynthesis facilitated and enhanced the effects of nisin on the plasmatic membrane, leading to lysis and cell death. These combined actions showed a promising effect at ¼ of the MIC of the agents, potentially increasing microbiological safety [120]. Over the last few years, vancomycin, an extensively administrated antibiotic for treating MRSA infections, experienced an increase in its usage and subsequent emergence of intermediate and resistant pathogens, such as VRE and vancomycin-intermediate *S. aureus*. Angelopoulou et al. studied the efficacy of combining nisin with vancomycin against *S. aureus* strains isolated from subclinical and clinical mastitic lactating women. The aim was to assess their effectiveness in inhibiting biofilm formation and eradicating pre-formed biofilms. The results revealed that the idealized therapy outperformed significantly the nisin or vancomycin used alone for the majority of the tested strains [121]. Similarly to nisin, vancomycin operates by targeting Lipid II, beginning with non-covalent binding to this essential precursor of cell wall synthesis. However, the molecular mechanisms of action differ significantly between these two antimicrobial agents [122]. Still, vancomycin may decrease access to Lipid II by competing with nisin. Consequently, a more effective strategy to achieve synergistic effects might involve combining nisin with an antibiotic that employs a distinct pathway of attack [123].

MRSA strains are a major cause of hospital and community-associated infections. Their ability to develop resistance to most antibiotics and to form strong biofilms greatly amplifies their threat. Several combinations of antibiotics and nisin have been studied to fight these pathogens and their biofilms, including methicillin [124], vancomycin [122], ramoplanin [125], ciprofloxacin [122,126], daptomycin, teicoplanin, linezolid and azithromycin [126]. The mechanism of action of most of these antibiotics is similar to that of nisin, attacking the cell wall synthesis pathway at different points, which results in enhanced cell wall disruption when used together [120,124]. However, ciprofloxacin, linezolid, and azithromycin differ in their mechanism of action, as they interact with intracellular pathways, such as protein synthesis inhibition or DNA gyrase enzyme interference. Thus, by disrupting bacterial cell membranes, nisin enables increased intracellular absorption of these antibiotics [126]. Enterococci, commonly found in the gastrointestinal tract, are associated with various clinical problems. A study conducted by Tong et al. demonstrated that the presence of nisin can reduce the MIC and minimum bactericidal concentration (MBC) values of 18 different antibiotics against *Enterococcus faecalis*. Notably, nisin showed significant synergy with chloramphenicol, ciprofloxacin, and β-lactam antibiotic penicillin, resulting in high efficacy against *E. faecalis* bacterium and its biofilms [123]. Antibiotic-resistant enterococci, namely VRE, are one of major causes of hospital-acquired infections. El-Kazzaz and El-Khier examined the antimicrobial impact of nisin in combination with commonly used antibiotics against VRE, such as ampicillin, gentamicin, ciprofloxacin, imipenem, vancomycin, cefuroxime, cefazolin, chloramphenicol, linezolid and cefepime, and observed improved antibacterial activity. Synergism evaluations specifically focused on the nisin/ampicillin and nisin/chloramphenicol combinations. Ampicillin is a β-lactam antibiotic that hinders peptidoglycan biosynthesis by binding with penicillin-binding proteins. On the other hand, chloramphenicol leads to cell wall thickening due to peptidoglycan accumulation. Data

reported an antimicrobial effectiveness of 91.3 % and 82.6 % against the tested VRE isolates, respectively with ampicillin and chloramphenicol [127]. Brumfitt et al. also tested the nisin/chloramphenicol against VRE isolates, confirming the occurrence of synergistic effects in approximately half of the examined isolates, while the remaining isolates showed an additive effect [125]. Persister bacteria constitute a subgroup within the bacterial population that can survive lethal doses of antibiotics through phenotype changes, rather than relying on heritable resistance mechanisms, like antibiotic-resistant bacteria that survive due to genetic mutations. Persister bacteria survive by adopting a dormant state, ceasing their regular metabolic functions, and appearing inactive within the body. Consequently, the presence of persister bacteria is recognized as a significant contributor to the failure of antibiotic treatment for infections [128,129]. *L. monocytogenes*, the causative agent of listeriosis, possesses the ability of becoming a persister cell and tolerate high concentrations of antibiotics. In the context of this pathogen, the effectiveness of combining nisin with antibiotics, such as ampicillin, gentamicin and ciprofloxacin, was examined to assess their antipersister potential. Although these combinations did not achieve complete eradication of persister cells, they notably reduced their survival rates [129]. Rishi et al. reported a significant reduction (approximately 78 %) in *Salmonella* persister cells when nisin was combined with ampicillin in the presence of mannitol, a carbon source that potentially reestablishes the susceptibility of persister cells to antibiotics by stimulating their metabolic activity [128].

The effectiveness of bioengineered nisin variants combined with conventional antibiotics to generate new formulations with therapeutic potential for human diseases has also been analyzed. In a study conducted by Field et al., two enhanced nisin derivatives (nisin M21V and nisin I4V) were examined in conjunction with a selection of common antibiotics to combat biofilm formation by *Staphylococcus* spp. Potent inhibitory effects were achieved with the nisin variants compared to the nisin/antibiotic equivalent or to each antimicrobial administered on its own. In particular, nisin M21V/penicillin effectively inhibited biofilm forms of *S. aureus* SA113. Conversely, when dealing with the planktonic cells of the same strain, the nisin derivative showed remarkable efficacy in combination with chloramphenicol. In the case of nisin I4V, significant efficiency was achieved with chloramphenicol against biofilms of *S. pseudintermedius* DSM21284, while nisin I4V/penicillin combination displayed notable potency against DSM21284 planktonic cells [130]. Desmond et al. investigated natural-origin nisin and two nisin derivatives, namely nisin PV and nisin K12A-PV, in combination with various antibiotics against antibiotic-resistant strains of *Staphylococcus capitis* and *Streptococcus agalactiae*. These microorganisms are commonly associated with neonatal infections. Among the combinations tested, nisin PV/ampicillin proved to be the most effective against *S. capitis* AV80, an isolate that showed significant resistant to both antimicrobials. In the case of *S. agalactiae* strains, the combination nisin/ampicillin demonstrated enhanced potency against *S. agalactiae* CIT 85, while nisin/erythromycin proved to be highly effective against *S. agalactiae* CIT 67 [131].

3.7. Combination of nisin with anticancer agents

In recent times, many reports have surfaced highlighting the potential of nisin as an anticancer agent [130]. Its combination with conventional chemotherapeutic agents may enhance the effectiveness of these treatments, prevent cancer recurrence, and potentially reduce instances of chemotherapy resistance [51,132,133]. In this context, Preet et al. studied the anticancer capacity of nisin combined with a conventional chemotherapeutic drug, doxorubicin (Dox), functionalized onto gold NPs (nisin/Dox–GNPs), against 7,12-dimethylbenz(a)anthracene (DMBA)-induced skin carcinogenesis. They demonstrated that when administered alone, nisin and Dox reduced the average tumor volumes by 14 and 51.3 %, respectively, after 4 weeks of treatment. However, when combined, nisin and Dox synergistically decreased tumor volume by 66.82 %. It was hypothesized that nisin interacted with cancer cell membranes leading to pore formation, causing an increased Dox uptake. Hence, nisin/Dox-GNPs were released intracellularly in larger concentrations causing inhibition of DNA synthesis or interfering with other intracellular events. This was followed by an increase in ROS production, which led to tumor inhibition, and attested to the synergistic activity between nisin and the chemotherapy drug [134]. Avand et al. also studied the combination of these two agents against breast cancer cell lines (MCF-7) and observed that at subinhibitory concentrations side effects associated with Dox were reduced, while its therapeutic index was improved [135]. Rana et al. combined nisin with 5-Fluorouracil (5-FU) and oligomeric chitosan coated AgNPs to form a composite nanostructure to act against DMBA/Phorbol 12-myristate 13-acetat (TPA)-induced murine skin cancer. The system was tested *in vivo* reporting a reduction in both tumor volume (68.34 %) and tumor burden (82.39 %). Interestingly, the anticancer therapeutic potential of nisin and 5-FU was found to be enhanced *in vivo* when bound to single composite nanoconstructs. The findings of this study provided a basis for creating innovative synergetic platforms to combat a variety of cancers [136]. The effect of combinations of the variant nisin Z with conventional anticancer agents, namely 5-FU, etoposide and hydroxyurea was also evaluated by Lewies et al. on melanoma cells. All combinations demonstrated increased cytotoxicity in melanoma cells compared to mono-treatment with the anticancer agent alone [137]. More recently, the combination of thioridazine, a drug originally used for antipsychotic treatments but then proven effective against cancer cells [138], with nisin was investigated by Ibrahim et al. against the human liver cancer HepG2 cell line, showing the synergistic effect between the two agents by increasing ROS levels, and inhibiting PI3K/AKT proliferation pathway and anti-oxidative SIRT1/NRF2 mRNA expression [139].

3.8. Combination of nisin with natural extracts

Essential oils (EOs) are often applied in the food industry to prevent biofilm formation by microorganisms that cause food spoilage, such as *Bacillus cereus*, *E. coli*, *L. monocytogenes*, *Salmonella* Typhimurium and *S. aureus* [140]. However, recently, because of the growing interest and wager in naturally-derived agents with reduced environmental impact, their relevance in biomedical applications has been boosted. The EO cinnamaldehyde and the natural extract curcumin were combined with a nisin-like bacteriocin GAM217, purified from the *L. lactis* strain GAM217 that was isolated from goat milk. Their antimicrobial and anti-biofilm activity against reference and clinical bacterial strains was evidenced by synergistic effects capable of destroying and impairing more than 80 % of the biofilm formed by antibiotic-resistant, *E. coli*, *P. aeruginosa*, *Enterobacter intermedium*, *Citrobacter diversus* and *Klebsiella pneumonia* strains. Cytotoxicity data against *Vero* cell lines indicated that the combinations displayed low cytotoxic, while being effective in reducing the adhesion of bacteria to mammalian cells, particularly when compared with the individual action of each compound [141]. In a similar approach, nisin was explored with gallic acid and different EOs, such as finger root, kaffir lime, holy basil and lemongrass, against the Gram-positive streptococcal bacterium, *S. mutans*. Gallic acid and kaffir lime leaf oil were deemed the most important instigators of the activity of nisin against the bacterium by lowering MICs, thus suggesting that they can be potentially used as adjunctive therapies for controlling *S. mutans* infection [142]. Pourhajibagher et al. investigated the incorporation of 5 % curcumin–nisin–PLA NPs (CurNisNps) in orthodontic acrylic resin. After 60 days of aging, the modified acrylic resin demonstrated a significant reduction in the microbial population and metabolic activity of *S. mutans* and *Candida albicans* biofilms. Furthermore, the inclusion of CurNisNps did not display any adverse effects on the mechanical properties of the acrylic resin, making it suitable for clinical uses for preventing dental caries, periodontal diseases, and candidiasis during removable orthodontic treatments [143].

3.9. Combination of nisin with chelating agents

The action of nisin against Gram-negative bacteria is limited due to its inaccessibility to the plasma membrane, requiring the addition of some chelating agents [144]. EDTA, a metal chelating agent, is able to bind to metal cations located in the outer membrane of Gram-negative bacteria, inducing their destabilization and allowing nisin accessibility to the peptidoglycan cell wall [132]. The potential of nisin as a natural preservative in cosmetics and topical products was explored by Maurício et al. The action of nisin was examined alone and in synergy with EDTA and similar synthetic preservatives. Acquired data indicated that nisin alone was effective in inhibiting Gram-positive microorganisms (*S. aureus* and *Bacillus* sp.), yet against Gram-negative bacteria (*E. coli* and *P. aeruginosa*) and fungi (*Aspergillus brasiliensis* and *C. albicans*) only the combination of nisin/EDTA/synthetic preservatives at 125 ppm/0.1/0.35 %, respectively, showed antimicrobial activity [145].

3.10. Combination of nisin with other organic/inorganic compounds

Organic and inorganic compounds can be used alone or in combination with peptides to enhance their antibacterial/antifungal activity. These conjugates have proven their effectiveness in combating biofilms, particularly in the periodontal area [146]. Kajwadkar et al. evaluated the antimicrobial efficacy of the variant nisin Z with sodium hypochlorite (NaOCl), an irrigating solution used in endodontic therapy capable of dissolving organic matter, eliminating loose debris, and inhibiting microbial activity, against planktonic and biofilm populations of *E. faecalis*, one of the most frequent bacterial species isolated from persistent endodontic and apical periodontal infections known attacking the gums and forming biofilms. The results showed that nisin Z alone exerts activity against *E. faecalis*. However, combined with low concentrations of NaOCl, the peptide activity against the biofilm was significantly improved, revealing its potential for uses in oral irrigating solutions [18]. In a similar study, Karunakar et al. joined nisin with NaOCl or chlorhexidine, a root canal irrigant and observed an increase in the antimicrobial activity of the mixture, particularly in the presence of NaOCl [147]. Nisin was also mixed to the common intracanal irrigant MTAD, which consists of 3 % doxycycline, 4.5 % citric acid, and 0.5 % polysorbate 80 detergent, showing a successful inhibition *E. faecalis* biofilms. Although MTAD exhibits inhibitory effects against *E. faecalis* in root canals, its bactericidal effectiveness was limited against the biofilms [148,149]. Additionally, nisin was found to enhance the post-antibacterial effect of MTAD at sub-MIC levels and to increase its susceptibility to alkaline environments [150]. Tong et al. studied the synergistic action between nisin and the anti-caries agents sodium fluoride or chlorhexidine against the cariogenic pathogen *S. mutans,* showing the effectiveness of the first combination but the unsuccess of the second [151].

In what concerns to organic compounds, the combinatory antibacterial effects of nisin and selected licorice polyphenols (glabridin, licoricidin, licochalcone A) were evaluated against planktonic and biofilm-embedded *E. faecalis*. The combination of nisin with each polyphenol individually, after a contact period of 30 min, resulted in a significant elimination of the biofilm [152]. The organic compound 2,3-Dihydroxybenzoic acid (DHBA), a non-toxic, plant-derived siderophore [153], was also tested with nisin against biofilms formed from a MRSA strain. This combination was incorporated into nanofibers of PEO/PDLLA, leading to a reduction of biofilm formation by 88 %, within 24 h exposure. For the same period, nanofibers with DHBA alone reduced biofilm formation by 63 %, and nisin only showed a 3 % reduction. The proposed formulation increased local iron concentration, which reduced biofilm formation, and highlighted the synergy between the two compounds [154].

4. Final remarks

Bacteriocins are a group of AMPs produced by bacteria that has attracted interest as a potential alternative to traditional antibiotics. Nisin, a naturally occurring bacteriocin, has been extensively studied in the biomedical field due to its unusual structure, amphiphilic nature, low toxicity, low probability of promoting bacterial resistance, and potent antimicrobial activity against various bacteria, namely pathogenic strains. Its effectiveness lies in its ability to disrupt the integrity of bacterial cell membranes, leading to cell death. In addition, nisin also exhibits anticancer and immunomodulatory effects through the modulation of cytokine production and innate immune cells. Natural variants of nisin have been discovered and characterized, confirming their antimicrobial profiles. Moreover, bioengineered forms of nisin have been developed, showing improved potency, stability, and extended antimicrobial activity. These advancements offer promising opportunities for the creation of novel antimicrobial agents tailored for biomedical applications. However, the clinical use of nisin may present challenges due to its limited solubility and stability at physiological pH. Consequently, the combinatorial effects of nisin with other bioactive molecules, polymers and other antimicrobial agents have been explored, offering a promising approach for the development of effective antimicrobial therapies. Significant additive and synergistic antimicrobial activities have been revealed, with synergistic effects being more desirable as they allow the use of lower concentrations of antimicrobials, potentially reducing treatment costs, toxicity, and the development of bacterial resistance. By combining nisin, which targets the cell membrane, with antimicrobials that act on intracellular targets through different mechanisms, a more effective treatment strategy against pathogens, especially drug-resistant bacteria, can be achieved.

This review has highlighted the versatility and potential of nisin as an antimicrobial agent, focusing on its natural variants and the range of molecules with which nisin can prosper. Additionally, the development of delivery systems or formulations that ensure controlled release and stability of nisin is crucial for the success of the therapy. Further research and innovation in this field will be most important for combating microbial infections and addressing the growing challenges of antimicrobial resistance.

CRediT authorship contribution statement

Tânia D. Tavares: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **Ana R.M. Ribeiro:** Investigation, Writing – review & editing, Writing – original draft. **Carla Silva:** Supervision, Writing – review & editing. **Joana C. Antunes:** Supervision, Writing – review & editing. **Helena P. Felgueiras:** Conceptualization, Supervision, Writing – review & editing.

Declaration of competing interest

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Data availability

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