Artykuł przeglądowy *Review*

Nanoparticles associated with antimicrobial peptides (AMPs) – a promising combination for biomedical and veterinary applications

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Summary

Bacterial resistance to classical antibiotics is a growing concern, and scientists are seeking alternative methods to combat bacterial infections. One promising option is the use of antimicrobial peptides (AMPs) with strong antibacterial activity provided with diverse mechanisms, including membrane disruption, intracellular interference, and inhibition of bacterial metabolic processes. They can also modulate inflammation, enhancing antimicrobial effects. Compared to antibiotics, AMPs can have a broader range of activity and are less prone to bacterial resistance. However, their use has limitations, such as a short half-life and proteolytic degradation. To address these challenges, researchers are developing strategies to enhance the stability and efficacy of AMPs, including the use of nanocarriers. Nanocarriers, such as inorganic particles, liposomes, polymeric particles, cubosomes, and quantum dots can improve the stability of AMPs, protect them from degradation, enhance their penetration into bacterial cells, and increase their antibacterial activity. Nanocarriers can also boost AMPs absorption by bacteria and stimulate their enzymatic function. While nanocarriers show promise in enhancing AMPs efficacy and stability, further research is necessary to determine their safety and effectiveness in practical medical applications. This review offers a succinct exploration of specific nanoparticles, their function as carriers for AMPs, and their potential significance in both medical and veterinary contexts.

Keywords: antimicrobial peptides, host defense peptides, nanoparticles, nanocarriers

Antimicrobial Peptides (AMPs), also known as Host Defense Peptides (HDPs), are evolutionarily conserved compounds with confirmed direct antibacterial, antifungal, and antiviral activity, produced by various organisms, including humans, animals and plants (31). In addition, AMPs indirectly affect the immune system and exhibit pro-inflammatory and anticancer effects (46). AMPs are grouped into several classes based on their structure, namely linear peptides with α-helical structure, peptides with β-sheet or hairpin structure, peptides with a loop structure containing a single disulfide bridge, and peptides rich in amino acids such as tryptophan or proline (31).

Indeed, the classification of AMPs based on their secondary structure and amino acid composition is a generalization, and there are exceptions to this categorization. For example, there are AMPs that have a mixed structure, combining elements of -helix, -strand, and/or loop structures. Some AMPs have a completely irregular structure, lacking any discernible secondary structure. Additionally, as mentioned, there are cyclic peptides, which contain a covalent bond between the N- and C-terminal of the peptide, resulting in a closed ring structure. These cyclic peptides often exhibit improved stability and activity compared to their linear counterparts due to resistance to proteolytic degradation and increased rigidity, respectively (31).

Based on the number of disulfide bridges and tertiary structure AMPs are divided into defensins and cathelicidins (87). Defensins usually contain 3-4 disulfide

bridges and consist of 30-50 amino acids, while cathelicidins contain one or two disulfide bridges and consist of 12-50 amino acids (18). In total, about 2900 natural AMPs are known, representing a potential resource for future antimicrobial and anticancer therapies (67).

Both cathelicidins and defensins were found in mammals, such as humans, horses, cattle, rabbits, sheep mice and pigs (57). In porcine neutrophils the most diverse range of catelicidins (namely, proline-phenylalanine-rich prophenins PF-1 and PF-2, proline-arginine-rich 39-amino-acid peptide (PR-39), and cysteine-rich protegrin-1 to 5 (PG-1 to PG-5)) was confirmed (86).

More than 50 defensins have been identified in mammals; some of them are derived from macrophages, neutrophils, and Paneth cells, while others are released from epithelial cells and keratinocytes. Defensins production can be constitutive, such as in case of human b-defensin-1 (hBD1) or inducible, such as expression of hBD2 induced by exposure to pathogen components, e.g., LPS during infection with *Salmonella* Typhimurium on murine model (57).

Cationic AMPs typically destroy cells by disintegrating the cell membrane or mitochondrial membrane. The mechanism of action is based on electrostatic interactions between the peptide (cationic load due to high content of lysine and arginine) and the cell membrane surface. The negatively charged cell membrane binds to the positively charged peptide. After binding to the membrane, peptides disrupt its structure and compromise its integrity. Depending on the structure of the peptide, there are four mechanisms of membrane destruction – „barrel-stave", „toroidal-pore", „carpet-like" (93) or detergent-like (Fig. 1) (8):

• The "barrel-stave" model involves peptides with an alpha-helix structure forming a beam in the membrane with a channel in the center. It resembles a barrel made of staves. The hydrophobic regions of the helix are positioned in the membrane lipid region, while the hydrophilic part of the peptide binds to the hydrophilic lipid heads and forms the inner part of the gap. Peptides that cause membrane destruction on the principle of the "barrel-stave" mechanism are gramicidin A and alameticin (4).

• The "toroidal-pore" model involves AMPs penetrating the lipid bilayer of the membrane and causing the lipid monolayer to bend inward and form a gap. In the gap, there are phospholipid heads and peptides. Peptides that cause membrane destruction on the principle of the "toroidal-pore" mechanism are melittin and magainin (4).

• The "carpet-like" model involves peptides accumulating on the surface of the membrane without penetrating the lipid bilayer. Peptides bind electrostatically to phospholipid heads, covering the surface of the membrane. This leads to destabilization of the membrane, which can cause its rupture, lowering of the membrane potential, and leaking of cytoplasm components from the cell. A peptide that causes membrane destruction on the principle of the "carpet-like" mechanism is cecropin (4).

• The "detergent-like" model is similar to the carpet model and interacts with negatively charged peptides on the external surface. Upon exceeding a certain concentration of AMPs, peptide molecules automatically rotate, increasing the fluidity of the membrane. As a result, the cell membrane begins to invaginate inward, forming a bilayer structure similar to detergent micelles. Peptides that cause membrane destruction on the principle of the "carpet-like" mechanism are cecropin and aurein (49).

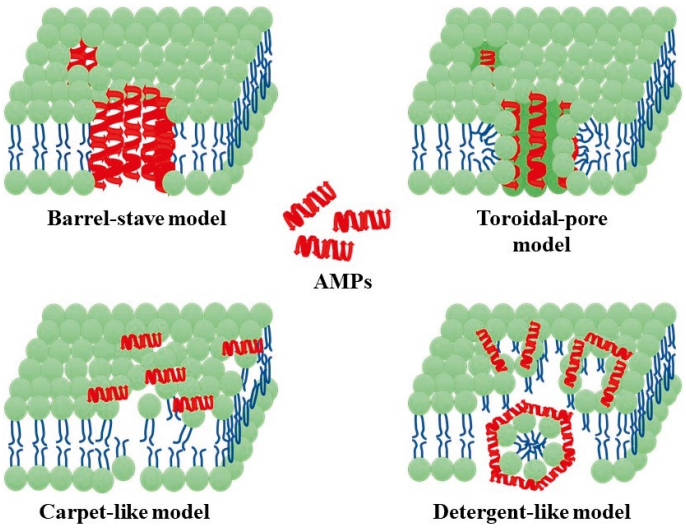

Fig. 1. Different mechanisms of bacterial membrane destruction by AMPs

Given the growing problem of antibiotic resistance, AMPs have become an area of active research for their potential as therapeutic agents against bacterial infections. Anionic AMPs, such as maximin-H5(42) and dermcidin (43), are a type of antimicrobial peptide that have a net negative charge due to their high content of aspartic and glutamic acids. They can also adopt an amphipathic structure, which allows them to interact with the bacterial cell membrane. Daptomycin is an example of an anionic AMPs that requires the presence of cations such as zinc for binding to the bacterial cell membrane. This is because the bacterial cell membrane is also anionic and requires the presence of cations for binding. Daptomycin inhibits ribonuclease and induces cell death, making it an effective antimicrobial agent against certain types of bacteria (31).

Despite their promising properties the limited therapeutic application of AMPs is due to challenges like systemic toxicity, susceptibility to proteolysis, rapid clearance, and short half-life. To overcome these issues, chemical modifications and delivery strategies have been proposed to improve their effectiveness (22). Delivery vehicles, including nanoparticles like gold and silver, liposomes, and polymeric particles, play a vital role in enhancing AMP properties. They protect against degradation, enhance bacterial cell penetration, and increase antibacterial activity. However, further

research is needed to ensure the safety and efficacy of AMP-nanocarrier complexes for medical applications (82). Simultaneously, research in human and animal health focuses on stabilizing AMPs, which often have limited bioavailability and environmental instability when used alone, thus reducing their effectiveness (10). Combining nanoparticles with AMPs allows controlled release, sustaining activity, extending half-life, and reducing cytotoxicity. This synergy may enhance both AMP and nanoparticle effectiveness through combinatorial formulations (53).

Nanoparticles

Nanoparticles are defined as structures with dimensions not exceeding 100 nm. They often exhibit unique physical, chemical, and biological properties that differ significantly from their macroscopic counterparts. Nanoparticles are characterized by a specific geometric structure with a high surface-to-volume ratio, which increases their activity and affects their absorbance and reactivity (34). Changes are also observed in physical properties such as thermal, electrical, and magnetic conductivity. Metal nanoparticles are a group of nanomaterials with potentially the widest range of applications. Their properties depend on the structure's shape. By properly controlling the synthesis conditions, the shape and size of nanoparticles can be controlled. The more developed the surface area, the more significant its impact on material reactivity, antimicrobial activity, and adsorption properties. For this reason, metal nanoparticles have become a valuable commodity, finding application in many fields, including medicine, pharmacy, cosmetics, dentistry, etc. (7). Due to their organic nature nanoparticles can interact with biological systems, making them valuable tools in biotechnology and biomedicine (70).

Nanomaterials can be represented as a matrix of nanoparticles (NPs) with various compositions, such as quantum dots, liposomes, polymer nanoparticles, cubosomes, inorganic NPs (Fig. 2) (63). To enhance their functionality, NPs can be modified by attaching different optically active compounds using covalent or non-covalent bonds. Covalent bonds provide greater stability in different environments, making them easier to use under various conditions. After synthesizing NPs, their surface can be modified with functional groups like amino (-NH_2), thiol (-SH), and carboxyl (-COOH) (26). Molecules containing fluorophore groups (e.g., fluorescein isothiocyanate, dansyl chloride) or drugs (e.g., doxorubicin, valrubicin) can be attached to these surfaces (39, 71). Noble metal NPs (such as gold and silver) can also be attached to enhance the optical signal. The presence of surface plasmon resonance (SPR) makes the optical properties of nanomaterials interesting. Metallic NPs have a gas of free electrons that circulate in their crystal lattice. When excited by light, the collective oscillation (vibration) of these electrons, known as plasmons, leads to SPR. SPR causes strong scattering of light, the creation of a local electromagnetic field, and absorption (37).

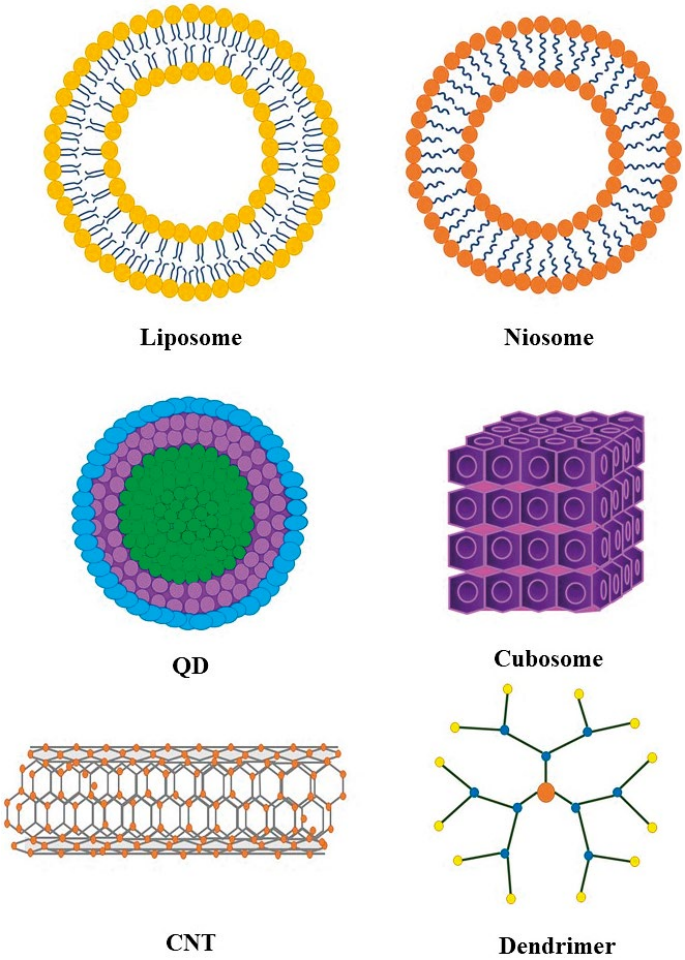

Fig. 2. Schematic representation of some NPs

Quantum dots (QDs)

Quantum dots (QDs) are semiconductor nanocrystals with ultrasmall sizes (1.5-10 nm) that exhibit unique optical properties due to changes in the bandgap energy caused by quantum confinement effects. QDs can be used as carriers for AMPs due to their unique photophysical properties and chemical stability. This allows for precise visualization and treatment specifically within the site of infection (14).

Carbon-based quantum dots (CQD) are small carbon nanoparticles known for their exceptional properties, including good conductivity, high chemical stability, low toxicity, and strong photoluminescence. They have attracted attention for their antimicrobial properties (59). HSER, a 22-amino acid peptide derived from the retinoic acid receptor protein in humans, exhibits a defensive response against both Gram-positive and Gram-negative bacteria. By conjugating HSER with CQD, the antimicrobial activity of HSER is enhanced. The interaction between HSER-CQD and bacteria leads to the disruption of cell walls and inhibition of DNA amplification. Moreover, HSER-CQD has shown compatibility with red blood cells and non-toxicity to

normal human epithelial cells, making it a potential alternative to antibiotics (54).

Cubosomes

Cubosomes are formed from the self-assembly of molecules with amphiphilic (both hydrophilic and hydrophobic) properties in a liquid crystalline phase. These molecules are typically surfactant-like in nature, meaning that they have a polar (hydrophilic) head and a non-polar (hydrophobic) tail. In the liquid crystalline phase, the amphiphilic molecules organize themselves into a cubic crystallographic structure, with two zones of water separating the surfactant-controlled cubosome structures. This structure is characterized by a solid lipid crystalline cubic symmetry that is comparable to a honeycomb or cavernous structure (77). The cubic structure provides excellent opportunities for trapping hydrophobic, hydrophilic, and amphiphilic components, as well as forming bi-continuous water and oil channels. The bi-continuous channels refer to two distinct (but non-intersecting) hydrophilic regions separated by the bilayer (83).

The release of drugs from cubosomes is based on the principle of drug diffusion, where the concentration gradient of the drug in the cubosomes drives diffusion. The rate of drug release from cubosomes is influenced by various factors such as drug solubility, diffusion coefficient, partition coefficient, cubic liquid crystalline geometry, pore size and distribution, interfacial curvature, temperature, pH, and ionic strength of the medium. To prevent hydrophobic drugs from being trapped in the hydrophobic domain of the cubic phase, cubosomes are additionally loaded with a digestive substrate (0.1 M HCl) (28).

Cubosomes have been utilized as drug delivery carriers for three AMPs (AP114, DPK-060, and LL-37). AP114 is a plectasin-like variant of defensin found in the saprophytic fungus *Pseudoplectania nigrella*. It exhibits high *in vitro* activity against methicillin-resistant *Staphylococcus aureus* (MRSA) pathogens and shows potential for treating skin and soft tissue infections. Unlike other AMPs that disrupt bacterial membranes, plectasin derivatives directly bind to the cellular lipid II precursor, inhibiting membrane biosynthesis (32). DPK-060 has been developed for the treatment of skin infections. It is a chemically synthesized peptide structurally derived from human kininogen protein. Compared to its endogenous analogue, DPK-060 exhibits greater resistance to enzymatic degradation by infection-associated proteases, without showing any signs of increased cytotoxicity. DPK-060 demonstrates strong *in vitro* antimicrobial activity against a broad spectrum of bacteria, with both Gram-positive and Gram-negative bacteria, including MRSA (27). LL-37 is a cationic α-helical peptide with broad antimicrobial properties against Gram-positive and Gram-negative bacteria. It also exhibits antifungal, antiviral, and endotoxin-binding capabilities, and promotes wound healing. LL-37 is resistant to degradation and remains effective when interacting with bacterial membranes. At sub-MIC levels, it causes morphological changes in cell membranes, while at MIC, it leads to complete lysis of bacterial cell membranes (19). Proteolytic protection was investigated by incubating the preparations with two elastases, and different efficiency of association onto cubosomes was observed among the AMPs, with LL-37 showing the highest affinity. Cubosomes loaded with AP114 exhibited preserved antimicrobial activity, while in the case of LL-37, broad-spectrum bacterial killing was reduced to only Gram-negative bacteria. Interestingly, cubosomes loaded with DPK-060 showed a slight enhanced effect against *S. aureus* and *Escherichia coli* strains. Furthermore, it was found that cubosomes protect LL-37 from proteolytic degradation, resulting in significantly improved bactericidal activity after elastase exposure compared to the unformulated peptide (11).

Liposomes

Liposomes are the most well-known, commonly used, and easy-to-produce LNP (lipid nanoparticle) drug delivery system, as they were the first nanomedicine approved by regulatory agencies. They are spherical, self-closing double-layered lipid structures that can spontaneously form in an aqueous suspension. The main component of liposomes is phospholipids, although other types of lipids, namely cholesterol (Chol), are often included. They are biodegradable, biocompatible, and exhibit very low toxicity and immunogenicity (78). The use of liposomes as drug carriers was first proposed in 1970 by Gregory Gregoriadis. Two decades later, the anticancer PEGylated liposomal doxorubicin (Doxil®) was the first nanodrug approved by the FDA. Liposomal preparations are currently the leading nanocarrier platform in medicine (24). Liposomes are a suitable delivery system for encapsulating amphiphilic peptides, such as AMPs, as they protect them from proteolytic degradation due to the presence of the phospholipid bilayer. There is no universal method for preparing liposomes, and their composition, size, and surface properties must be tailored based on their intended application. The most commonly used method for preparing liposomes is the "thin-film hydration" method, followed by extrusion or sonication to narrow the size distribution. However, the use of organic solvents in these methods makes them unsuitable for pharmaceutical applications (48).

Polymyxin B (PB) is an antimicrobial lipopeptide that was responsible for the first successful liposomal antimicrobial preparation. Polymyxins were discovered in 1940, but their clinical use declined in the 1970s due to their nephrotoxicity. The first attempts to encapsulate polymyxin B in liposomes were made in 1990. Early studies showed that encapsulation of PB in charged liposomes was not harmful to its antimicrobial activity (5).

Niosomes

Non-Ionic surfactant based vesicles (niosomes) are promising drug carriers due to their ability to encapsulate both hydrophilic and hydrophobic substances. Niosomes, similar to liposomes, consist of a bilayer formed by a non-ionic surfactant and stabilized with cholesterol. They demonstrate excellent stability in the stomach and high permeability in the intestine (16). Encapsulation of substances inside niosomes can improve the stability and bioavailability of the drug, as well as reduce toxicity or side effects (25). Niosomes offer several advantages over liposomes, including improved stability, lower cost, ease of formulation, and scalability. Niosomes are more stable than liposomes due to the use of non-ionic surfactants, which provide greater physical and chemical stability. There are many different methods for producing niosomes, including hydration-filtration methods, hydration-mixing methods, ultrasonic methods, genetic engineering methods, and microfluidic methods. Each of these methods has its advantages and disadvantages, which must be considered when choosing the proper method of fabrication (61). The studies on niosomes also include the influence of various factors on their stability and biological properties, such as pH, temperature, active substance content, type of surfactant, etc. These studies aim to understand the behavior of niosomes in the body and their impact on the bioavailability and toxicity of the active substance. Much research on niosomes focuses on the use of these carriers in the treatment of diseases such as cancer, skin diseases, infectious diseases and autoimmune diseases (9, 61). There are already niosomes on the market containing active substances such as hyaluronic acid, retinol, vitamin C and coenzyme Q10, which are used in cosmetics (52). Polymyxin B niosomes, prepared using sorbitan monostearate (Span® 60) and cholesterol, exhibit comparable pharmacokinetic profiles to intravenously administered polymyxin B sulfate (16). Niosomes containing LL-37 offer prolonged antibacterial activity with lower dosages. The development of a niosomal delivery system containing LL-37 and other antimicrobial agents shows promise for treating severe infections (72). However, further clinical trials are still needed to confirm the effectiveness of niosomes as drug carriers in the treatment of various diseases.

Polymer nanoparticles

Antimicrobial polymers comprise cationic and hydrophobic components. Cationic groups like ammonium, sulfonium, and phosphonium ions aid in binding to bacterial membranes, while hydrophobic groups penetrate lipid bilayers, causing membrane damage and bacterial death (47). Chain length influences antimicrobial activity; longer chains may lead to reduced efficiency and increased hemolysis (66). To mitigate toxicity, hydrophilic and neutral motifs like PEG and polysaccharides are used (6). The balance between cationic and hydrophobic groups determines antimicrobial efficacy and biocompatibility. Different antimicrobial polymer topologies impact bacterial activity. Main-chain and side-chain cationic polymers, homopolymers, copolymers, oligopeptides, and dendritic polymers are utilized (66, 85).

Polymeric antimicrobial agents shows promising results for controlled release, improved bioavailability, and protective properties (72). Nanocapsules and nanospheres are reservoir and matrix systems, affecting drug association. Surface modifications influence drug delivery rates, and techniques like ultracentrifugation and analytical methods assess drug association (60, 65). Understanding drug association modes aids in optimizing drug delivery systems for desired therapeutic effects.

Dendrimers

Dendrimers (from Greek "*dendron*" meaning tree) are nanostructures with a size of approximately 20 nm. Chemically, dendrimers are polymers with a highly branched, three-dimensional structure that resembles a sphere. Studies of antimicrobial dendrimers (AMPDs) containing lysine and leucine attached to functional groups at the ends of branches, such as G3KL and T7, have demonstrated *in vitro* activity against various Gram-negative bacterial strains, including multidrug-resistant and extensively drug-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa* isolates. G3KL shows promising antibacterial properties while exhibiting low toxicity towards human red blood cells (12, 64). T7 is a small-molecule peptide that offers advantages such as easy chemical synthesis, stability, low steric hindrance, and good potential for clinical applications (41). Like many other AMPs, polymers, peptidomimetics, and folds, peptide dendrimers use a mechanism that disrupts the bacterial membrane. In the case of these dendrimers, this mechanism consists of a α-spiral folding of the amphiphilic dendrimer core in contact with the bacterial membrane. Such properties make peptide dendrimers potentially useful in antimicrobial therapy (12).

Gold nanoparticles (AuNPs)

Gold nanoparticles (AuNPs) have unique properties due to their small size and quantum effects. They have been extensively studied for biological and biomedical applications, including bioimaging, drug delivery, and therapeutics. The synthesis of AuNPs has a long history dating back to Roman times, but it was Michael Faraday who first exploited the scientific potential of gold colloids. The excellent AuNPs surface chemistry allows for functionalization with various biomolecules, such as peptides and antibodies, enabling targeted drug delivery and therapeutics. Additionally, their small size allows for increased cellular uptake, which is crucial for effective drug delivery. In summary, AuNPs have

emerged as versatile tools for various biomedical applications due to their unique optical and surface properties (15).

Research on AuNPs is growing thanks to their numerous advantages, such as ease of synthesis and conjugation with biomolecules, their ability to maintain their structure in circulation, and their increased efficacy against bacteria, demonstrating their high potential in the field of nanomedicine (1).

Among various products, photoluminescent Au nanodots (AuNDs) were functionalized with hybridized ligands, an antimicrobial peptide (surfactin; SFT), and 1-dodecanethiol (DT). Ultra-small SFT/DT-AuNDs (size ≈ 2.5 nm) showed highly effective antimicrobial activity, especially against multidrug resistant bacterial strains. *In vitro* cytotoxicity and hemolysis analyses showed acceptable biocompatibility. Moreover, *in vivo* studies of wound healing in MRSA-infected rats showed improved healing and better epithelialization (78). This study suggests that SFT/DT-AuNDs could be a promising antimicrobial agent for preclinical applications in the treatment of skin wounds and infections. Rai et al. also reported a one-step methodology for generating conjugated AMPs (AuNP). The prepared AuNPs conjugated with AMPs showed controlled size (14 nm) and low polydispersity and allowed for incorporation of high concentrations of AMPs (65). Furthermore, these systems exhibited higher antimicrobial activity and stability in serum and in the presence of non-physiological concentrations of proteolytic enzymes than soluble AMPs, as well as low cytotoxicity against human cells (80). It is worth mentioning that AuNPs have been investigated as a promising platform for novel anticancer treatments. This report describes the improvement in internalization of AuNPs by cells, with higher cytotoxicity and cellular uptake for smaller NPs compared to larger nanospheres and nanorods, suggesting that the anticancer activity of selected peptides was modulated by the size and shape of AuNPs (91).

Studies on murine models found the potential of loading AMPs onto DNA aptamer-functionalized gold nanoparticles (AuNP-Apt) as an innovative delivery system for treating intracellular bacterial infections in different species of mammals. Intravenous administration of AuNP-Apt conjugates effectively eradicated intracellular *S.* Typhimurium cells, leading to the survival of infected mice (92). In turn, the antimicrobial peptide HPA3PH is based on aptamer-targeted gold nanoparticles appeared useful for complete inhibition of *Vibrio vulnificus* colonization in infected mice (13).

Silver nanoparticles (AgNPs)

Silver nanoparticles (AgNPs) are another group of nanomaterials, which are characterized by high biological activity and potential use as antibacterial and antiviral agents. As with AuNPs, their physical, chemical and biological properties are strongly dependent on the size and shape of the particles. AgNPs can be obtained in many ways, including chemical reduction, laser ablation and electrochemical synthesis. Depending on the method of synthesis, they can have different sizes, shapes and surface properties (62).

The ability of AMPs to permeabilize of bacterial membranes might help AgNPs to access internal target sites, thus their combined activities showed synergistic effects against gram-negative bacteria (69).

AgNPs coated with a multifunctional peptide (MFP) (MFP@AgNPs) were developed and tested for their antimicrobial properties. MFP is a sequence that can absorb physically onto AgNPs through electrostatic interactions. It consists of a matrix metalloproteinase (MMP) fissile sequence (PVGLIG), an antibacterial peptide (tachyplesin-1) and a target peptide (PGP-PEG). MFP@AgNPs showed antibacterial activity against both gram-positive and gram-negative bacteria. SEM images showed that MFP@AgNPs-1 induced cell disruption by damaging the cell membrane. The developed MFP@AgNPs-1 reduced the cytotoxicity of AgNPs and enhanced antimicrobial activity against multidrug-resistant *A. baumannii* (MDR-AB) *in vitro* and *in vivo*, providing a possible solution against multidrug-resistant bacterial infections (45).

A novel nanocomposite comprising AMPs, Polydopamine (PDA), and Silver Nanoparticles (AgNPs) was developed. Notably, treatment utilizing the AMP@PDA@AgNPs formulation showcased remarkable anti-biofilm efficacy. Rigorous quantitative analysis of biofilms substantiated the heightened anti-biofilm activity of AMP@PDA@AgNPs in comparison to AgNPs and AMPs alone, effective against both Gram-negative and Gram-positive bacteria. The collaborative antibacterial prowess of AMPs and AgNPs substantially elevated the nanocomposite's anti-biofilm potential, leading to profound biofilm eradication (89).

The antifungal activity of AgNPs was shown against *Candidaalbicans*, *Candida glabrata*, *Candida krusei*, and *Candida parapsilosis*; however, there is no significant effect against *Candidatropicalis*. In the light of fact that candidiasis is an opportunistic mycosis that can occur in different animal species and different *Candida* species can cause disease, it seems that the results of research on biosynthesized Ag NPs will soon find clinical application (68).

Magnetic nanoparticles (MNPs)

Magnetic nanoparticles (MNPs) exhibit a response to an applied magnetic field. There are five main types of magnetic materials: ferromagnetic, paramagnetic, diamagnetic, antiferromagnetic, and ferrimagnetic. Each of these types of materials exhibits different magnetic properties, depending on the arrangement of the atoms within the material (17). Ferromagnetic materials consist of aligned magnetic domains, resulting in a net magnetic moment even in the absence of an external field. Paramagnetic materials have

unpaired electrons that align weakly with an external field but do not retain magnetic moment. Diamagnetic materials have a net magnetic moment of zero and only slightly respond to an external magnetic field. Antiferromagnetic materials have opposite magnetic moments that cancel each other out, resulting in a net magnetic moment of zero. Ferrimagnetic materials have atoms with antiparallel magnetic moments, resulting in a net magnetic moment that is not as strong as in ferromagnetic materials. They also exhibit hysteresis, meaning there is a residual magnetic moment after the external field is removed (33).

MNPs are promising carriers for AMPs due to their magnetic properties, which allow for controlled direction and accumulation in target tissues or cells using an external magnetic field. Additionally, MNPs provide convenient methods for separation and removal after use, which reduces toxicity and minimizes potential side effects. Examples of AMPs delivered using MNPs include nisin, magainin, cecropin A, LL-37, defensins, and recombinant proteins. Methods used to prepare MNP-AMPs complexes include electrostatic adsorption, chemical binding, biotinylation, and immobilization on the surface of MNPs. Some studies also use additional ligands or polymers to stabilize MNP-AMPs complexes and to prevent premature degradation (88).

Carbon nanotubes (CNTs)

Carbon-based nanoparticles, such as carbon nanotubes (CNTs), have attracted significant attention from researchers due to their unique physicochemical properties. CNTs are cylindrical structures formed by rolled graphene sheets, with high aspect ratios and metallic or semiconductive properties based on their rolling angle. Single-walled carbon nanotubes (SWCNTs) have higher antimicrobial activity than multi-walled carbon nanotubes (MWCNTs) due to their smaller size and greater surface area (40). The antimicrobial mechanism of CNTs involves direct contact with bacterial membranes, compromising their integrity, morphology, and metabolic activities. Additionally, oxidative stress and mechanical properties, such as low wear rates, low friction coefficients, favorable tribological characteristics, and high corrosion resistance, also play a role in the antimicrobial properties of CNTs. Overall, CNTs have the potential to be used as effective antimicrobial agents in biomedical applications (73).

The studies on novel antimicrobial nanocomposites based on CNTs and AMPs, such as epsilon-polylysine, poly (L-lysine), and poly (L-glutamic acid), have demonstrated their strong antimicrobial and anti-adhesive effects. One of the advantages of these composites is their low susceptibility to the development of resistance to antibacterial drugs. Furthermore, the immobilization of natural antimicrobial peptide nisin with PEG has enhanced the antimicrobial and anti-adhesive properties of MWCNT. The MWCNT/nisin composite has shown significantly higher antimicrobial activity against various bacteria such as *E. coli*, *P. aeruginosa*, *S. aureus*, and *Bacillus subtilis*, as well as much higher antibiofilm activity compared to the control film. The strong antimicrobial activity of nisin disrupts the synthesis of the cell wall, which increases the permeability of the cell membrane, leading to its potent antimicrobial properties. These findings suggest that CNT/AMPs nanocomposites have potential as antimicrobial agents in biomaterials, including medical implants and other medical devices that are prone to bacterial infections. However, further research is necessary to assess the safety and effectiveness of these composites in clinical settings (81).

Merits and limitations of nanocarriers

As previously discussed, NPs have found diverse applications in biomedicine, serving as effective drug delivery vehicles and potential alternative for antimicrobial agents. Various nanomaterials such as MNPs, liposomes, dendrimers, polymers, and carbon nanotubes have been harnessed to enhance AMPs' activity against multidrug-resistant microorganisms (50).

The efficacy of utilizing poly-ε-caprolactone nanoparticles as carriers for AMPs (HHC-8 and MM-10) has been demonstrated in treating *Mycobacterium* infections. Notably, a synergistic effect was observed when combining AMP-NPs with the conventional antibiotic rifampicin. This synergy may arise from the protective role of AMP encapsulation, promoting improved membrane penetration and enhanced antibiotic accumulation within mycobacteria (74). This discovery holds significant implications from an epizootic standpoint, given the prevalence of mycobacterial infections among domestic animals, including bovine and avian species (55, 56). Companion animals, not subject to mandatory tuberculosis control, could potentially disseminate mycobacteria, contributing to epizootic outbreaks and posing a public health risk. Consequently, continued research on nanoparticles is warranted, with potential applications in both medical and veterinary practices.

The potential of AMP-NPs extends beyond bacterial and fungal infections. HeLa cell line experiments revealed that concurrent treatment with a PEGylated liposomal epirubicin formulation and hepcidin 2-3 led to a notable increase in epirubicin's cytotoxicity. Incorporating hepcidin 2-3 into liposomes substantially heightened epirubicin uptake within cells, predominantly in the nucleus. Moreover, this preparation induced apoptosis in HeLa cells, evident through increased expression of p53, Bax, caspase-3, and caspase-9. This breakthrough broadens our understanding of AMPs' mechanisms and offers fresh insights into potential anticancer therapies for both veterinary and human medicine applications (36).

Derived from frog skin mucus, brevinin-2R (B2R) is a non-hemolytic defensin membrane peptide with potent antimicrobial activity against both Gram-

negative and Gram-positive bacteria. Intriguingly, B2R also displays partial selectivity in eradicating cancer cells. This effect is achieved by triggering an increase in reactive oxygen species (ROS) production within the cells, prompting programmed cell death. An innovative application lies in the conjugation of B2R with cerium oxide nanoparticles (CNP), showcasing the synergy between AMPs and NPs in anticancer therapies. CNP-B2R displayed heightened cytotoxicity against cancer cell lines compared to normal ones. This strategy of utilizing nanoparticles linked with antimicrobial peptides holds promise in addressing tumors resistant to conventional chemotherapy, offering potential advancement in modern therapeutic approaches for veterinary oncology (29).

However, drawbacks persist for AMPs, including cytotoxicity, conjugation challenges, stability concerns, and limited shelf-life. Different nanocarriers have been explored for AMP delivery, each with its limitations. Carbon nanotube synthesis is costly, and these NPs exhibit poor solubility. Liposomes offer biodegradability and versatility in loading hydrophobic and hydrophilic drugs, but they face issues with loading capacity and potential immune responses.

Dendrimers offer precise control over molecular design but suffer from synthesis costs and nonspecificity. Polymeric NPs display biocompatibility and tunable drug release, though drawbacks include low cell affinity and potential by-product toxicity. Addressing these challenges is crucial for harnessing the full potential of nanocarrier-assisted AMPs in combating drug-resistant pathogens (Tab. 1) (22, 79).

The toxicity of NPs is an area of intense scientific research and deliberation. It is not possible to generalize the toxicity of all NPs, because it is highly diverse, and there is no common criterion that can be established. However, there are certain factors that can influence their potential harm, among others their size, shape, chemical composition, surface area, stability and dose, since each NP, depending on its material composition, exhibits different structures, shapes, surfaces, physical and chemical properties, solubility, and cytotoxicity (21).

The assessment of toxicity of NPs is conducted in reference to specific organisms, including mammals, bacteria, protozoa, crustaceans, algae, and plants (21, 44). Bacteria, such as *E. coli*, are commonly used for NPs toxicity studies. Zinc oxide (ZnO) NPs exhibit mutagenic effects on bacteria and cause deformations of cytoplasmic membranes. NPs can reduce the fertility capability of fish due to their accumulation in sperm. Certain NPs, including ZnO and cerium dioxide (CeO_2), exhibit toxic effects on plants by inhibiting germination and root growth (76, 90).

NPs designed for various applications exhibit diverse behavior in different environments. In aquatic settings, their solubility, reactivity with the chemical environment, and interactions with biological processes determine their fate. Due to their low mass, NPs tend to remain suspended in water for extended periods, posing a potential risk to aquatic organisms (30, 44). In soil, NPs display a wide range of behaviors. Some become chemically inert upon absorption, while others retain their toxic properties. Environmental conditions can trigger biodegradation and chemical transformations in designed NPs, although the mechanisms involved are not yet fully understood. Many NPs used in nanotechnology are non-degradable, including ceramics, metals, and metal oxides. Certain NPs undergo chemical changes, such as the oxidation of nanoFe to FeO, while others, like Zn, Cu, and Si, may become more toxic as they oxidize in the air (62, 84).

Toxicity studies in mammals are conducted on rodent and nonrodent species. In experimental rodents,

Tab. 1. Merits and Limitations of Nanocarriers

Nanocarriers	Merits	Limitations
QDs	High stability Significant fluorescence Diagnostic potential	Cd and Se toxicity Health and environmental risks
Cubosomes	Stability Easy modification Transport of hydrophobic and hydrophilic drugs	Complicated production process Require safety studies
Liposomes	Compatibility with various substances Biodegradability Potential for targeted therapies	Short stability in blood serum Possible immunogenicity issues
Niosomes	Stability Controlled release potential Improved bioavailability	Complex production Possible aggregation Long-term stability challenges
Polymeric NPs	Enhancing Biocompatibility Controlled Drug Release Degradability	Low cell affinity Byproduct Toxicity Challenges
Dendrimers	Controlled structure Multiple functionalization possibilities Drug transport	Potential toxicity High production costs Limited membrane penetration ability
AuNPs	Unique optical properties Functionalizable surface Potential for targeted therapy	Aggregation potential Potential toxicity at high doses Long-term stability issues
AgNPs	Strong antibacterial properties Wide application range Functionalization potential	Potential toxicity at high doses Aggregation potential Long-term stability challenges
MNPs	Strong magnetic properties Controlled navigation via magnetic field Hyperthermic therapy	Aggregation potential Potential toxicity Magnetic stability challenges
CNTs	Unique mechanical and electrical properties Drug transport	Potential toxicity Controlled functionalization challenges Potential long-term stability issues

AuNPs induce changes in lymph nodes, liver, and cardiac muscles. AgNPs damage cellular mitosis and accumulate in internal organs, easily crossing the blood-brain barrier. Limited studies conducted on human cell lines using the comet assay have revealed clear DNA damage (21, 44).

Prospects in clinical applications, importance in veterinary medicine

Functional nanomaterials have undergone rigorous preclinical and clinical investigations, resulting in the emergence of nanomedicines in today's market. Notably, clinically approved liposomal drug formulations and metallic imaging agents have paved the way. Within this realm, antimicrobial therapy stands as a key clinical focus, driving nanomedicine's progress. For instance, MNPs not only serve as carriers but also exhibit potential as antimicrobial agents. Specifically, AgNPs can permeate microbial cell membranes, releasing Ag^+ ions and inducing toxicity (3, 23).

Despite the potential of AMPs, their transition to clinical trials has been limited. AMP-based formulations in clinical trials are categorized based on their mechanisms of action: cell membrane disruption (e.g., Ruminococcin C), immune system modulation (IDR-1002), and intracellular function (e.g., HB-107, Buforin II). Tailoring modifications to AMPs offer a pathway to enhance delivery, biological activity, stability, and reduce toxicity. The successful implementation of nanotechnology in refining drug delivery systems provides a promising precedent. Likewise, applying nanotechnology to AMP delivery holds potential for creating novel antimicrobial agents to combat multi-drug resistance. Furthermore, it is imperative to thoroughly explore the fundamental biological effects, biodistribution, and pharmacokinetics of MNPs, particularly silver and other nano-based nanocarriers, in clinical contexts (22).

Microorganisms outpace antibiotic discovery, fostering drug resistance. AMPs, an alternative to traditional antibiotics, encounter challenges rooted in untapped potential and limited pharmacokinetics. Advanced delivery systems can facilitate AMP advancement into clinical trials. Antibiotics focus on specific components, inviting resistance. In contrast, AMPs penetrate bacterial membranes rapidly due to their amphipathic nature. This mechanism evades mutations and impedes resistance. Thus, immune-regulating AMPs offer comprehensive defense (2, 58).

Antibiotics face challenges in detoxification and complete renal clearance. In contrast, AMPs undergo metabolic degradation into essential amino acids, reducing clearance concerns. With increasing resistance to current antimicrobials, AMPs offer an alternative by disrupting pathogen membranes, limiting resistance due to repair needs. Despite their potential, AMPs are hindered by instability and degradation, impeding implementation. Drug delivery systems, particularly using nanomaterials, offer a solution by enhancing uptake, release, and protection against proteases, addressing MDR challenges while improving therapeutic efficacy and minimizing side effects (22, 35).

Given the broad therapeutic potential of NPs, understanding the effects of their conjugation, functionalization, encapsulation, and complexes on bacterial populations becomes essential. Versatile nanocarriers such as MNPs, polymers, and liposomes have demonstrated effectiveness as drug carriers, offering favorable therapeutic outcomes. Notably, NPs possess the advantage of precise infection site targeting, fostering synergistic interactions with AMPs (51). Together, they penetrate cell walls, aggregate particles, generate Reactive Oxygen Species, and hinder cellular activities, all of which are vital for combating pathogens and MDR infections. Challenges in AMP-carrier systems include carrier selection, entrapment efficiency, and conjugation chemistry. Ongoing research aims to optimize the association of AMPs with nanocarriers. While basic *in vitro* studies involving AMP-nanocarriers outweigh clinical trials, the increasing discoveries related to AMPs necessitate additional *in vivo* research. Understanding physiological barriers and immunological responses is crucial for simplifying challenges in clinical trials. The potential of nanotechnology to revolutionize medicine, especially in the context of AMP nanocarriers, justifies investment for effectively addressing MDR pathogens (20, 22, 38).

Research on NPs in the veterinary medicine is still relatively new, necessitating further investigation into their mechanisms of action and potential effects on animal, human, and environmental health over the short and long term. Despite this, the undeniable significance and promising applications of these nanostructures in the future of veterinary medicine remain evident (68).

Conclusions

Alternatives to traditional antibiotics are of paramount importance in the light of growing bacterial antibiotic resistance. Natural antimicrobial compounds, such as AMPs, have emerged as a promising avenue. These innate molecules, produced by the body's immune system, exhibit a broad range of inhibitory effects against diverse microorganisms. However, challenges such as high toxicity, susceptibility to degradation, and limited bioavailability have hindered the clinical application of AMPs. To overcome these obstacles, attention has turned to nanoparticles as carriers for AMP delivery, aiming to enhance their antimicrobial potential. Nanoparticles offer controlled release mechanisms, maximizing efficacy while minimizing toxicity. Additionally, they can improve bioavailability by mitigating undesired binding to other components. In the realm of veterinary medicine, these advancements hold significant potential. Applying AMP-nanoparticle complexes could yield targeted and improved therapies for animal health. With veterinary medicine confront-

ing antibiotic resistance challenges akin to human health, innovative approaches are essential. Integrating AMPs and nanoparticles could offer enhanced antimicrobial strategies, ultimately contributing to elevated standards of animal care and well-being.

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