



Enhanced Bactericidal Effects and Drug Delivery with Gentamicin-Conjugated Nanoparticles

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Abstract

Amid the rise of antibiotic-resistant bacterial strains in human populations, novel solutions are necessary. Nanoparticles, renowned for their adaptability, offer a diverse range of research avenues, notably as antibacterial agents. Coupling antibiotics with nanoparticles stands out as a potential strategy, effectively intriguing drug delivery challenges and circumventing antibiotic resistance. This review focuses on studies of nanoparticles in conjugation with the aminoglycoside antibiotic gentamicin and their effectiveness as novel nanoparticle conjugates, their applications in drug delivery and enhanced bactericidal effects. In most cases, the nanoparticle conjugates were found to efficiently kill different bacterial strains, which was mainly dependent on the size, stability and concentration of the nanoparticles. Despite the several limitations such as nanotoxicity, accumulation of intravenously injected nanomaterials in tissues and organs, the urge and potential for the advancement of nanoparticle-drug conjugates still demands more scientific advancements.

Keywords Gentamicin · Nanobiotics · Antimicrobial resistance · Antibacterial activity · Drug delivery · Nanoparticles

Introduction

Despite the scientific advancements of antibiotics, the ever-growing bacterial species gaining resistance to commercially available antibiotics are now a major threat throughout the world as antibiotics become more endangered in terms of efficiency [1, 2]. Ever since the use of antibiotics in therapeutic settings began in the 1930s, there has been an epidemic of antibiotic resistance [3]. Alexander Fleming warned that the overuse of antibiotics will lead to the development of drug-resistant microorganisms in the future following his discovery of penicillin in 1945 [4]. We are currently dealing with serious complications

that, according to many experts, would prevent the value of today's antibiotics from being used in 100 years, contrary to what he predicted decades earlier [5, 6].

Overuse and misuse of antibiotics mainly due to the inappropriate prescription of drugs by medical professionals is one of the leading causes of increasing microbial resistance [7]. Extensive use of antibiotics in the agricultural field and the availability of a few novel antibiotics also contribute to this crisis [8]. Thus, the need for novel bactericidal components is an alternative approach to address the current situation.

Nanobiotics, where nanoparticles themselves have manipulable antimicrobial properties, have been considered a promising approach [9–11]. Nanoparticles can evade drug resistance compared to commercially available antibiotics in certain cases [12]. Conjugation of nanoparticles with antimicrobial properties to certain antibiotics that are readily available in the market has shown promising outcomes towards antibiotic resistance, drug delivery, long-term imaging and tracking [13, 14].

The issue of organ toxicity linked with metallic nanoparticle conjugates remains a dynamic area of investigation and advancement. Researchers are actively engaged in comprehending the mechanisms of toxicity, enhancing nanoparticle design, and devising tactics to reduce their adverse impacts

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[15]. Multiple strategies have been employed, concentrating particularly on the modification of nanoparticle surfaces to alleviate their toxicity. Through the incorporation of biocompatible coatings for example polymers, a protective shield is established, effectively diminishing direct interaction between the nanoparticles and biological systems. Petrow et al. produced core-shell nanoparticles by enveloping Ag NPs with a cross-linked poly (L-lysine) coat containing doxorubicin. The study exhibited favorable biocompatibility and the induction of cancer cell death upon drug release [16]. The concern of nanoparticle biodegradability has also been acknowledged as a notable limitation. In response, liposomal nanoparticles have been introduced as a strategy to overcome this hurdle [17].

Gentamicin, an aminoglycoside that was first isolated from *M. purpurea* species in 1963, provided a breakthrough in treating infections caused by gram-negative aerobes such as *P. aeruginosa* [18]. Since then, gentamicin has been commonly used in a range of medical applications as a broad-spectrum antibiotic [19]. In the recent past, there have been good clinical outcomes of gentamicin as well as the opposite in some situations. It is still one of the most commonly used antibiotics in the medical field due to its low cost and broad-spectrum efficiency [20]. However, intramuscular or intravenous application is required since it is poorly absorbed by the gastrointestinal tract [21]. Moreover, its cationic structure and accumulation in tissues are linked to tissue-specific toxicities, including nephrotoxicity and ototoxicity [22]. Gentamicin also exhibits vestibulotoxicity, inducing harm to the vestibular system [23].

Gentamicin, an aminoglycoside antibiotic, is employed to treat severe infections caused by aerobic gram-negative bacteria. Instead of being a singular compound, gentamicin is composed of three main components - gentamicin C₁, C_{1a}, and C₂ - as well as several minor components. The major components differ in the level of methylation within the 2-amino-hexose (purpurosamine) ring. In this ring, gentamicin C_{1a} lacks methyl groups, while both C₁ and C₂ possess a methyl group at the 6' position. Additionally, gentamicin C₁ is N-methylated at this position, whereas C_{1a} and C₂ have free amines. The C₂ component consists of two stereoisomers [24]. The level of distinct gentamicin components varies depending on the drug's manufacturing method and the manufacturer, making it difficult to investigate aspects such as pharmacokinetics and bactericidal effects due to an unknown combination of chemically related compounds [25]. Gentamicin is accessible through diverse trade names, contingent on the pharmaceutical producer and geographic location. A few of the renowned trade names associated with gentamicin comprise Garamycin, Gentak, Cidomycin etc. It has been effectively employed in treating a wide spectrum of infections such as eye, ear, and

bloodstream infections caused by bacteria [15]. Gentamicin is also combined with other drugs to treat various infections. The combination drugs is available in diverse formats, including tablets, capsules, syrups, creams, gels, ointments, liquids, and injections. Presently, there are 26 brands listed in the drug directory (<https://www.medindia.net/drug-price/gentamicin-combination.htm>) that offer generic versions of gentamicin combination, each marketed under distinct brand names for example, Beclotis-CG, Pred-G, Valisone-G and Dermitop etc. Gentamicin is commonly used to treat various bacterial infections, including serious conditions like peritonitis (inflammation in peritoneum), endocarditis, sepsis and septic shock, pneumonic plague (infection in lungs), and meningitis (inflammation of meninges). (Fig. 1)

The trade names, composition, and specific formulations of gentamicin are compared in Table 1.

Nanoparticle Conjugates with Gentamicin

The chemical make-up and arrangement of nanoparticles assist in the active targeting of antibiotics, extended binding and protection from enzyme degradation. These properties eliminate the use of higher doses of antibiotics, leading to fewer side effects. Many scientists have produced novel classes of antimicrobial agents, such as nanoparticle conjugates, to narrow the complications and development of multidrug-resistant infectious bacteria. Nanoparticles that have bactericidal effects themselves, for example, gold, silicon dioxide, iron oxide, titanium dioxide, etc., and organic nanoparticles such as liposomes fused with commercially accessible antibiotics have been examined against many multidrug resistant bacteria [26]. These nanoparticle conjugates are synthesized by two classes of interactions: chemical and physical. Some of the chemical reactions include amine surface-functionalized nanoparticles adjoined to antibiotics containing succinimide ester or isothiocyanate groups, aldehyde functionalized nanoparticles conjugated with antibiotics containing a hydrazine group, etc. Similarly, physical interactions include electrostatic, hydrophobic and biotin-avidin interactions [27]. Possible applications of NPs-gentamicin conjugations are depicted in Fig. 2.

Nanoparticle-antibiotic conjugates represent a remarkable advancement in the field of targeted drug delivery and enhanced therapeutic efficacy. These conjugates involve the integration of antibiotics with nanoparticles, creating a synergistic approach to combat infections. The mechanism of action of nanoparticle-antibiotic conjugates revolves around several key aspects that collectively contribute to their effectiveness [15]. Schematic representation of mechanism of action of nanoparticle-antibiotic conjugates are shown in Fig. 3.

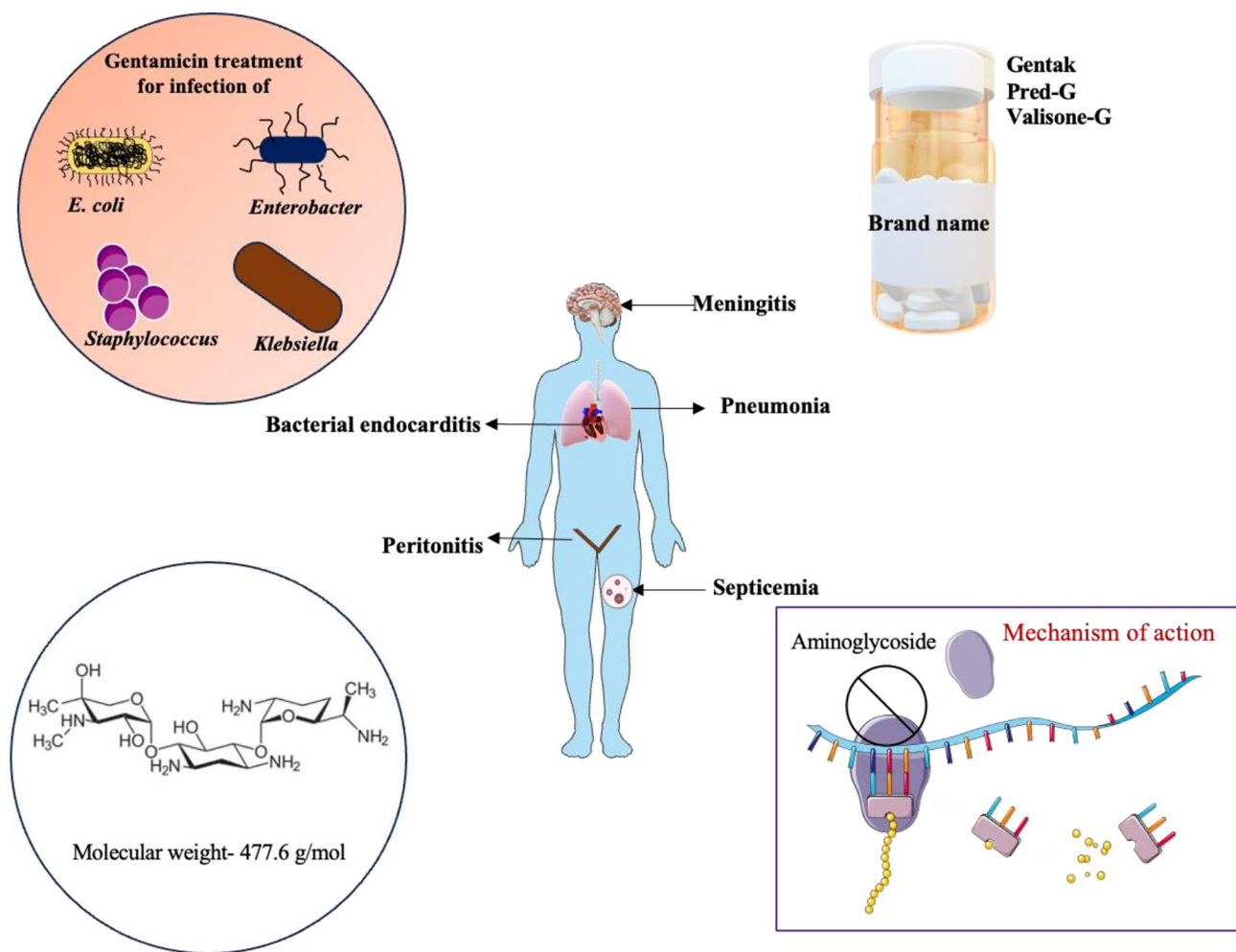


Fig. 1 The different infections and microbes treated using gentamicin. Parts of the figure were drawn by using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (<https://creativecommons.org/licenses/by/3.0/>)

The design of an effective therapeutic system necessitates the consideration of both spatial and temporal parameters. One widely utilized approach involves employing nanoparticles as carriers for drug delivery. During the process of transporting molecules from one location to another within an organism, it becomes crucial to comprehend the time frame associated with the delivery procedure, as well as to gain insight into how these molecules interact within the intricate biological milieu. This task becomes particularly challenging when dealing with targeted drug delivery systems, given the intricate nature of the system, which can impose limitations on the movement of cargoes within a living organism. A successful design for nanoparticle-based drug delivery hinges on the ability to precisely navigate the drug to its intended destination within the appropriate timeframe, even amidst the turbulent currents like blood flow and various layers of biological barriers [28]. Also, a

three-fold increase in biofilm reduction was observed, with silver-Polymyxin B nanoparticle conjugate systems, compared to neat Ag nanoparticles. This signifies how nanoparticle conjugates improve the efficacy of drugs [29].

This review emphasizes studies using various nanoparticle and gentamicin conjugates against different bacterial strains to analyse the increased antibacterial action in comparison to free gentamicin, as well as their applications in drug delivery.

Gold Nanoparticles

Gold nanoparticles have been used to treat various conditions, such as diarrhea, epilepsy, leprosy, mental diseases, plague and syphilis [30, 31, 32, 33]. However, in 1971, two researchers from Britain, Faulk and Taylor, introduced colloidal gold-antibody conjugation to visualize the surface

Table 1 Comparison of various brands of gentamicin*

Brand Name	Composition	Formulations	Infection
Garamycin	Gentamicin sulfate	injections, topical creams, and ophthalmic solutions	bacterial infections
Genoptic	Gentamicin	ophthalmic solutions	eye infections
Gentak	Gentamicin sulfate	ophthalmic ointments	bacterial infections
Cidomycin	Gentamicin	Injections	bacterial infections
Gentacin	Gentamicin	Eye/ear drops	bacterial infections affecting the eyes, ears, and other body parts
Septopal	Gentamicin-collagen sponge	Injection	orthopedic and trauma-related procedures

(accessed from <https://www.drugs.com/ingredient/gentamicin.html> on 29-08-2023.)

antigens in *salmonellae*. Thereafter, it has been successfully

used in several other applications, such as diagnosis, imaging, and cancer treatment [34].

Mixtures of gentamycin with gold nanoparticles (15 nm) were assessed for enhanced bactericidal activity. The formation of the conjugates was confirmed by UV spectroscopic analysis as well as the blue to red color conversion of the solution. Antimicrobial activity was evaluated by the disk diffusion method which exhibited no difference in antimicrobial activity between mixtures and free gentamicin. When compared with available literature, it is known that the nanoparticles should be stabilized, where in this study the color of the nanoparticle solution did not match with the corresponding single-particle non-aggregated colloidal color, which could be the reason that the conjugates did not show enhanced antibacterial activity [35].

Several studies have investigated the potency of gentamicin-gold nanoparticle conjugates to effectively target certain bacterial species. For example, gold nanospheres (10–12 nm) conjugated to gentamicin were successfully synthesized and used to assess their use in drug delivery to *staphylococcal* infected foci. Conjugation was confirmed by both UV–Vis spectroscopic analysis and by the color change

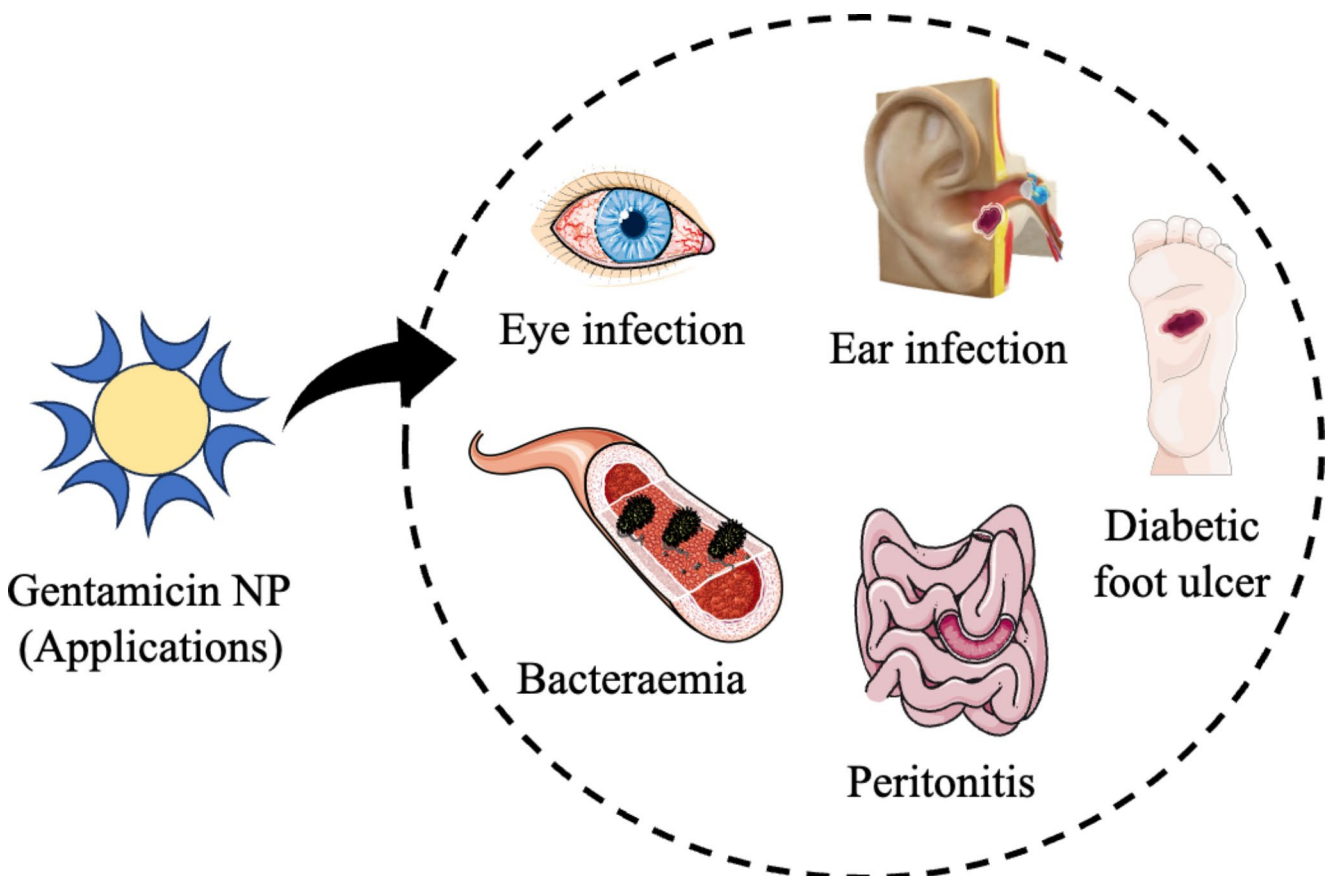


Fig. 2 Various applications of Gentamicin conjugated nanoparticles. Parts of the figure were drawn by using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Com-

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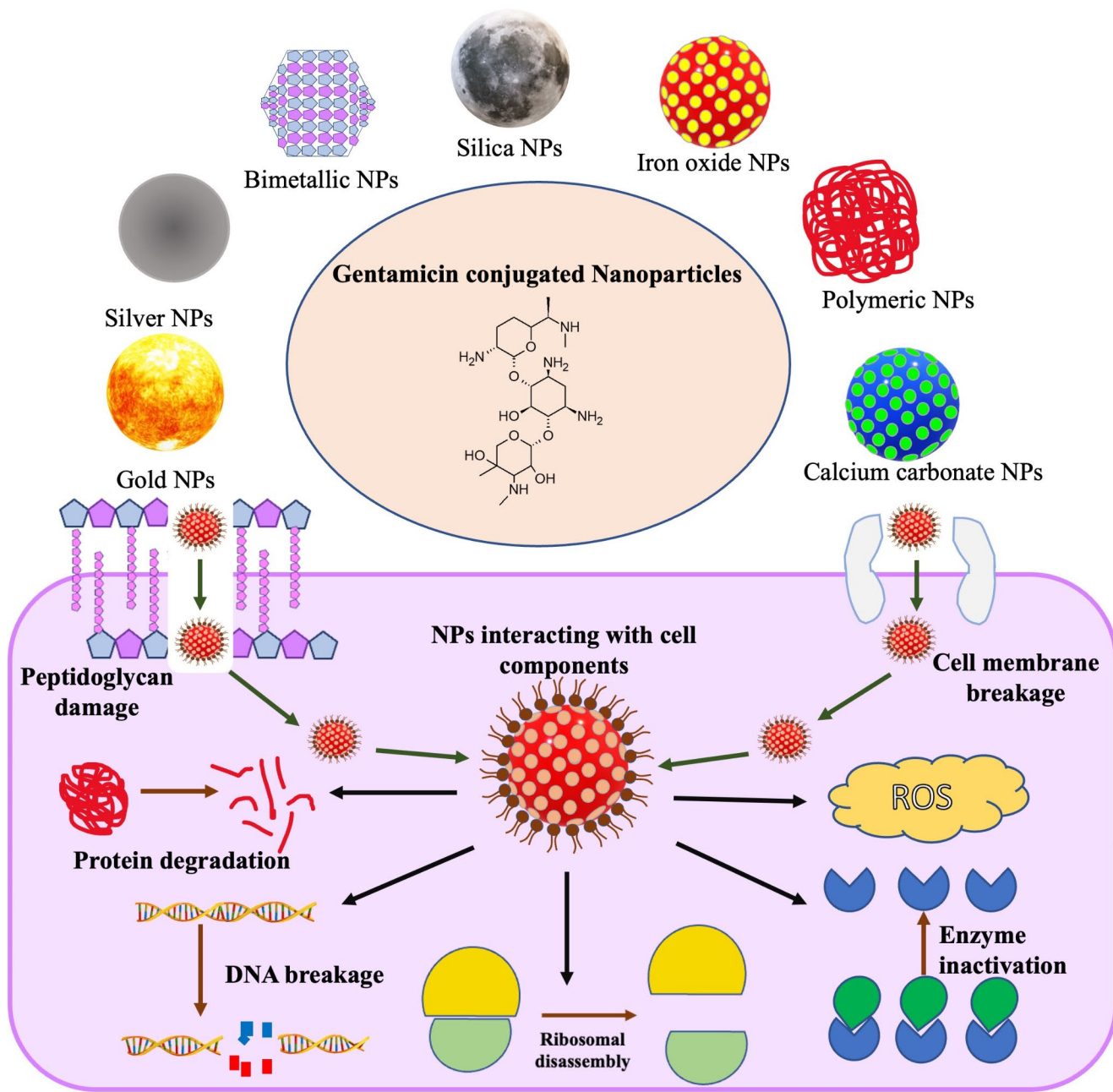


Fig. 3 Applications of gentamicin in various infections and mechanism of action of nanoparticle-antibiotic conjugates

from red to purple–blue. Approximately 347 gentamicin molecules were coupled to each gold nanosphere, and this combination showed a significantly enhanced antibacterial effect when compared to free gentamicin against *S. aureus* bacterial species. The effect of the conjugates on BALB/c mice indicates a considerable accumulation of conjugates in the site of infection compared to uninfected muscle after 30 min [36].

In another study, gentamicin was covalently bound to gold nanoparticles by the capping agents cysteine and glutathione and characterized using Fourier transform infrared

spectroscopy (FTIR), UV–Vis spectroscopy and transmission electron microscopy (TEM) analysis. Glutathione-capped nanoparticles showed higher antibacterial activity than cysteine, owing to the elevated amounts of gentamicin released from the nanocarrier. Moreover, glutathione-capped gold nanoparticles showed drug release for at least 48 h, whereas cysteine showed a maximum of 24 h. However, the conjugates showed a lower minimum inhibitory concentration (MIC) value against methicillin-resistant *S. aureus* and *S. aureus* strains [37].

Gold nanorods were conjugated with gentamicin using the Nanothink acid linker to facilitate the targeted delivery of gentamicin to infection sites caused by *Staphylococcus aureus*. The gentamicin-gold nanorod conjugate exhibited an enhanced antibacterial effect, as evidenced by the minimal inhibitory concentration and minimum bactericidal concentration values. Moreover, in a mouse model, a bio-distribution study demonstrated the specific localization of the gentamicin-GNR complex at the site of *staphylococcal* infection with a high level of sensitivity [38]. The efficacy of gold nanocages loaded with gentamicin along with other antibiotics was tested against *P. aeruginosa* in a study. The results showed the choice of antibiotic to be critical and that the gentamicin-loaded gold nanocages targeted a conserved superficial protein of the bacterial cell membrane, being highly effective against biofilms of *P. aeruginosa* [39]. In addition, another approach used gold nanoparticles functionalized with chitosan and loaded with gentamicin to demonstrate their antibacterial activity. Analysis was performed through a novel computational approach that could realistically describe controlled antibiotic release from complex hybrid systems. Based on modelling data, it was noted that the amount of gentamicin bound to the gold nanoparticles was minor, but the conjugates were able to show very efficient and slower release of the drug [40].

To investigate the antibacterial efficacy of gentamicin-attached gold nanoparticles against *E. coli*, another study was conducted. It was observed that in the case of larger gold nanoparticles, the ratios for inhibition regions with gentamicin were higher with respect to gentamicin alone. Gentamicin-conjugated gold nanoparticles were also more successful than the other categories in preventing bacterial growth [41]. Immediate antibacterial therapy of wounds

minimizes the risk of infection and enhances wound healing. On the other hand, the products used to treat wounds frequently fail to provide both sterilization (particularly for super bacteria) and hydration, and some may even induce additional wound harm. As a wound dressing, gold nanoparticles were combined with gentamicin sulfate (GS) and put into a mixture of konjac glucomannan (KGM) and gelatin (KGM/Gelatin@Au NPs/GS). Antibacterial tests revealed that gold nanoparticles enhanced the antibacterial activity of GS, with Au NPs/GS effectively eradicating bacteria, particularly super bacteria [42]. One-pot synthesis produces gentamicin-coupled gold nanoparticles (G-GNPs) that are easy, resilient and environmentally acceptable, with gentamicin acting both as a reducing and stabilizing agent. G-GNPs further demonstrated superior antibacterial properties when compared to pure gentamicin, and G-GNPs also showed tremendous effects towards gentamicin-resistant *E. fergusonii* ATCC 35,469 [43].

The chemical structures of different capping agents coupled with gold nanoparticles are given in Fig. 4.

Summary of size of the gold nanoparticles used in gentamicin conjugation, experiments conducted, bacterial species used and applications of the nanoconjugates are given in Table 2.

Silver Nanoparticles

Silver nanoparticles are used in numerous sectors, such as biomedicine, industrial purposes, and health care. The exceptional physical and chemical properties, such as electrical and optical properties, of silver nanoparticles allow them to be used in various fields [44].

Fig. 4 Chemical structures of capping agents coupled with gold nanoparticles (a) Cetyltrimethylammonium bromide (CTAB) (b) Chitosan (c) Glutathione and (d) Polydopamine

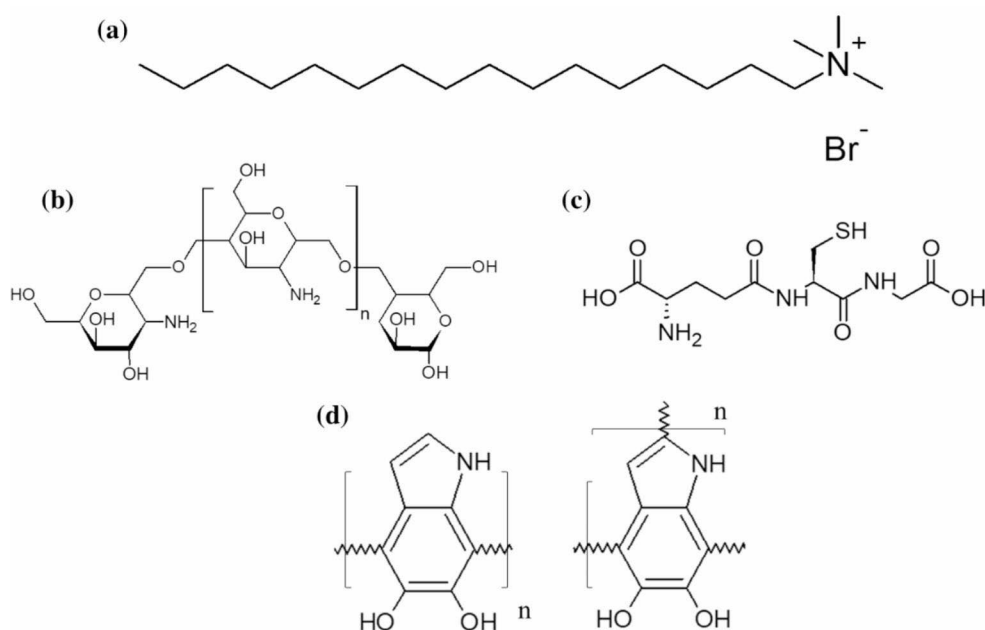


Table 2 Summary of gold nanoparticle-gentamicin conjugates and their outcomes in antibacterial activity and drug release experiments

Size (nm)	Synthesis method	Bacteria	Experiments	Results	Application	References
15	Turkevich method	<i>E. coli</i> <i>K-12</i>	-Disk diffusion -MIC -MTC	Showed no significant difference	Antibacterial effects	[35]
10–12	Purchased from Sigma	<i>S. aureus</i>	-Liquid broth dilution -MIC -MBC	Due to the small size, the higher localized delivery inside the cell	Drug delivery, Antibacterial effects	[36]
5.2/ 7.8	Turkevich method	Methicillin-resistant <i>S. aureus</i> <i>S. aureus</i>	-MIC	Continuous release of drug for few days. Glutathione acts as a better capping agent compared to L-cysteine	Drug release	[37]
41 × 10	Purchased from Sigma	<i>S. aureus</i>	-Liquid broth dilution -MIC -MBC	Higher concentration of gentamicin bound to the rod compared to spheres leading to higher efficiency	Drug Delivery Antibacterial effects	[38]
36–41	Turkevich method	<i>S. aureus</i> <i>P. aeruginosa</i>	-Photothermal microscopy -Biofilm assay -Catheters	Highly effective against <i>P. aeruginosa</i> biofilm	Antibacterial effects	[39]
12–20	Turkevich method	<i>S. aureus</i> <i>E. coli</i>	-Agar well diffusion	Controlled and sustained release of gentamicin is observed	Targeting efficiency, Antibacterial effects,	[40]
-	Turkevich method	<i>E. coli</i>	-Disk diffusion	Gentamicin conjugated gold nanoparticles were more effective bacterial growth.	Antibacterial effects	[41]
3 ± 1	Turkevich method	<i>E. coli</i> <i>S. aureus</i>	-MIC -MBC -Zone inhibition -Wound healing assay	KGM/Gelatin@Au NPs/GS can be used for killing superbugs, inhibiting bacterial growth, and promoting wound healing	Wound healing	[42]
15	One pot synthesis	<i>E. coli</i> , <i>S. aureus</i>	-Well diffusion MIC -Membrane lipid peroxidation	The developed G-GNPs hold a great potential against gram positive, gram-negative and drug resistance bacteria.	Antibacterial effects	[43]

*NA: not available

Several approaches have employed antibiotic-conjugated silver nanoparticles for antimicrobial applications. For instance, in a 2019 study, silver nanoparticles 60–80 nm in size were biosynthesized from *P. glomerata* species, and the combined effect of these nanoparticles with gentamicin was tested against *P. aeruginosa*, *S. aureus* and *E. coli*. The combination of silver nanoparticles with gentamicin showed increased inhibition for all three species. The study also stated that the synthesis of nanoparticles using *P. glomerata* can certainly eradicate the problem of using harsh chemicals, which can have unfavorable effects in its applications and implementations, hence allowing the nanoparticles to become more biocompatible [45].

In another study, silver nanoparticles, 5–30 nm in size, were biologically synthesized using (*A. flavus*) and conjugated with 5 different antibiotics, including gentamicin, and these were tested against 8 different strains of bacteria, namely, *K. pneumoniae*, *E. coli*, *M. luteus*, *S. aureus*, *P. aeruginosa*, *E. faecalis*, (*B. subtilis*) and *A. baumannii*. A potent range from 12 to 25 mm zone of inhibition was indicated by gentamicin infused with nanoparticles, while free gentamicin showed an average of 11.3 mm zone of inhibition and regular silver nanoparticles showed an average of 14.8 mm zone of inhibition [46]. Similarly, a combination

of gentamicin and biosynthesized silver nanoparticles from *Bacillus species*, with a diameter range of 14–42 nm, showed a slight increase in antibacterial activity when tested against several bacterial species. Gentamicin alone showed a zone of inhibition of 14 mm, whereas gentamicin with silver nanoparticles showed a 15 mm inhibition zone, resulting in a fold increase of 0.15, particularly against the *S. epidermidis* strain 73. The mixture also showed a 1.04-fold increase in the zone of inhibition against *S. epidermidis* strain 145, with inhibition areas of 7 and 10 mm for gentamicin alone and the mixture, respectively. Gentamicin loaded silver nanoparticles have not significantly boosted the antibacterial activity [47].

Magnetic nanoparticles (MNPs) composed of polyacrylic acid (PAA) were fabricated. These MNPs were then coated with multidimensional films comprising gentamicin along with tannic acid (TA) and silver nanoparticles (Ag NPs). This intricate synthesis aimed to evaluate the antibiofilm characteristics and controlled drug release properties of the particles, ultimately enhancing biocompatibility. In vitro drug release models showed that the MNP + gentamicin + Ag NP complex showed pH-responsive release properties, proving that the complex may be used to deliver gentamicin molecules on demand to the infection foci. The complex also

showed a significant increase in biofilm disruption in both *E. coli* and *S. aureus* due to the use of magnetic field navigation that guided the nanoparticles to penetrate the biofilm, showing the dual responsive release of gentamicin. The proposed mechanism of action was excess production of reactive oxygen species (ROS) produced by the complex, which aided in biofilm disruption and eradication [48]. Another study was conducted to observe the antimicrobial properties of AgNPs generated by *Bacillus* sp. (SJ14) in conjunction with ciprofloxacin, methicillin, gentamicin and rifampicin. Multidrug-resistant and biofilm-forming coagulase-negative bacteria were evaluated with the conjugates, namely, isolates of *S. epidermidis* and *S. haemolyticus*. Almost all of the conjugates' MICs (minimum inhibitory concentrations) were lowered, except for methicillin, which was lowered from 250 g/mL to 7.8 g/mL against *S. epidermidis*. Except for rifampicin, the synergistic effect of nanoparticles and antibiotics could be demonstrated by fractional inhibitory concentration (FIC) measurement [49].

A summary of the size of the silver nanoparticles used in gentamycin conjugation, experiments conducted, bacterial species and applications of nanoconjugates are given in Table 3.

Bimetallic Nanoparticles

Nanoparticles comprising two dissimilar metals are called bimetallic nanoparticles. Their constituent metals and nanometric size determine their properties. These are increasingly gaining interest because they allow optimization of

the plasmon absorption band energy that can be utilized as a versatile tool for biosensing [50].

Bimetallic nanoparticles of silver and gold were synthesized biologically by *P. veronii* in a study with a size range of 5–50 nm, and their antibacterial effects were tested. It was reported that the combination of nanoparticles with gentamicin showed an 11.15% increase in antibacterial effect compared to the antibiotic alone against *S. aureus*, *B. subtilis*, *E. coli* and *K. pneumoniae* bacterial strains [51].

Silica Nanoparticles

Silica (silicon dioxide, SiO₂) is yet another inorganic compound with a wide variety of biomedical applications. It possesses flexible surfaces that can be easily modified and easily synthesized and has inert chemical properties. Mesoporous silica particles are one of the three highly manufactured nanomaterials. However, continued exposure to silica nanoparticles poses adverse health effects affecting the immune system [52].

SiO₂ nanoparticles sized 327 nm, when conjugated to gentamicin and used as carriers for the antibiotic, aided in the prolonged release of gentamicin. The key purpose of the study was the chemical synthesis of SiO₂ nanoparticles, conjugation with gentamicin and characterization of the conjugates for their potential antimicrobial administration, especially in orthopaedic applications [53]. Moreover, antimicrobial assays conducted on SiO₂ nanoparticles, free gentamicin and SiO₂ gentamicin nanoconjugates separately against three bacterial species (*B. subtilis*, *P. fluorescens*

Table 3 Summary of silver nanoparticle-gentamicin conjugates and their outcomes in antibacterial activity and drug release experiments

Size (nm)	Synthesis method	Bacteria	Experiments	Result	Applications	References
60–80	extracellular synthesis from <i>Phoma glomerata</i>	<i>E. coli</i> <i>P. aeruginosa</i> <i>S. aureus</i>	-Disk diffusion	Slight increase in ZOI compared to free gentamicin	Antibacterial effect	[45]
5–30	extracellular myco-synthesis using culture filtrate of <i>Aspergillus flavus</i>	<i>E. coli</i> <i>S. aureus</i> <i>M. luteus</i> <i>P. aeruginosa</i> <i>E. faecalis</i> <i>A. baumannii</i> <i>K. pneumoniae</i>	-Disk diffusion	12–5 mm Zone of inhibition	Antibacterial effect	[46]
14–42	Green synthesis using soil <i>Bacillus</i> sp.	<i>S. epidermidis</i> <i>S. aureus</i> <i>S. typhi</i> <i>S. paratyphi</i> <i>V. cholerae</i>	-Disk diffusion	Conjugates showed slight increase in diameter compared to free gentamicin	Antibacterial effect	[47]
-	Hydrothermal method	<i>S. aureus</i> <i>E. coli</i>	-Biofilm eradication	Enhanced biofilm eradication	Anti-biofilm activity assay	[48]
8–21	<i>Bacillus</i> Sp. SJ14	<i>S. epidermidis</i> <i>S. haemolyticus</i>	-MIC	Significant therapeutic application to manage organisms associated with device-related infections.	The results thus indicate the potential of silver nanoparticles to enhance the efficacy of antibiotics	[49]

and *E. coli*) showed that the conjugate systems showed a higher antibiotic effect towards gram-positive bacteria. SiO₂ nanoparticles themselves did not show any antimicrobial effect, and the conjugates had a higher MIC value than free gentamicin [53].

In another study, thin films embedded with mesoporous silica nanoparticles and filled with gentamicin were used to assess their antibacterial efficiency as well as antibiofilm activity for a prolonged period. The persistence of the inhibition zone around the thin film for up to 103 days proved that the thin film complex could prevent bacterial growth for a long period. Additionally, the antibiofilm activity of the nanocomposites against *S. aureus* species remained active for more than two months [54].

Iron Oxide Nanoparticles

Iron oxide nanoparticles possess unique magnetic properties, in addition to their nontoxic and biodegradable behavior. These properties enable them to be used in various fields, mainly in diagnosis and therapy [55].

For instance, magnetic mesoporous bioactive glass nanoparticles (MMBG) were prepared in a study from iron oxide (Fe₃O₄) with a particle size of 15–30 nm and a pore size of 5.4 nm. MMBG showed a higher loading efficiency of 65.7–68.6% than nonmesoporous nanoparticles, which were 14.3–19.6%. This meant that the higher drug encapsulation and high surface area were due to the mesoporous property of the nanoparticles. Additionally, MMBG had a higher drug release rate during the first 12 h and showed prolonged release for more than 6 days [56].

Another investigation employed iron oxide nanoparticles with a size of 10–14 nm and coated them with gentamicin to analyse their bactericidal effects. The nanoparticles showed superparamagnetic character and a coercivity close to zero according to the magnetization curve. Furthermore, an initial outburst of release followed by a sustained release for approximately 250 h was observed. Simultaneously, antibacterial experiments against *S. aureus*, *B. subtilis*, *P. aeruginosa* and *E. coli* indicated colony reduction at a concentration of 2–3 mg/ml, leading to 99% bacterial cell death. Amino acid leakage studies depicted that cellular RNA and DNA were distorted by the conjugated systems [57].

Similarly, Fe₃O₄ nanoparticles were capped with sodium alginate, and gentamicin was loaded into these magnetic nanoparticles in a 2018 study. On average, 56.7% of gentamicin was loaded into the sodium alginate-capped magnetic nanoparticles. The MIC values of the conjugates showed that they could inhibit all visible growth against *P. aeruginosa* and were relatively more effective than free gentamicin [58].

A different investigation analysed carboxyl surface-functionalized iron oxide nanoparticles conjugated with gentamicin for their antibiofilm properties and checked the importance of the homogenous distribution of the drug throughout the thickness of the biofilm. The nanoparticles had an average diameter of 60 nm. This small size allowed prevention of reticular rejection of the complex. Additionally, a clear scattered distribution of nanoparticles along the biofilms was noted, which depended on the time of exposure to the magnetic field. Five minutes of exposure to an external magnetic field showed a homogenous distribution of the drug as well as higher antibacterial efficiency when compared to the complex without exposure. On assessing the conjugate-mediated eradication of biofilms of bacterial species *E. cloacae*, *S. aureus*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa* and *E. faecalis*, similar minimum bactericidal concentration (MBC) values of the conjugates and free gentamicin were obtained. Therefore, clinical applications of magnetic nanoparticle complexes are feasible once the magnetic field exposure time is optimized and drug-loaded nanoparticles are distributed uniformly throughout the biofilm [59].

A technique was employed wherein gentamicin was mixed with iron oxide nanoparticles and a polymer known as polyethylene glycol along with Fe₃O₄ NPs. The results indicated a remarkable enhancement in the antibacterial activity of the nanoparticles against the tested bacterial strains. In particular, the Fe₃O₄ NPs + PEG + Gen formulation exhibited significant inhibitory effects on *P. mirabilis* and *S. epidermidis* [60]. The agar well diffusion method was used to study the antibacterial activity of chloramphenicol and gentamicin and their formulation with encapsulated iron oxide nanoparticles. *Escherichia coli* and *Staphylococcus aureus* strains were resistant to the antibacterial effects of drug-encapsulated Fe₂O₃ NPs, possibly in a dose-dependent manner [61].

A summary of the size of the iron oxide nanoparticles used in gentamicin conjugation, experiments conducted, bacterial species used and applications of nanoconjugates are given in Table 4.

Polymeric Nanoparticles

Synthetic and natural polymers are commonly used nanomaterials to construct nanoparticle-based drug carriers. These are also used in imaging and detection of apoptosis [62].

Poly lactic-co-glycolic acid (PLGA) nanoparticles loaded with gentamicin were prepared to assess the enhanced antimicrobial activity against *P. aeruginosa*. PLGA nanoparticles 251 nm in size were chemically synthesized by a water-in-oil-in-water (w/o/w) emulsion, each encapsulating

Table 4 Summary of iron oxide nanoparticle gentamicin conjugates and their outcomes in antibacterial activity and drug release experiments

Size (nm)	Synthesis method	Bacteria	Experiments	Result	Application	References
-	Chemical	<i>S. aureus</i> <i>S. epidermidis</i>	-Drug loading and release study - Bactericidal effect assay	Better excellent drug loading release	Drug delivery property Bactericidal property and biocompatibility	[56]
10–14	Co-precipitation	<i>S. aureus</i> <i>E. coli</i> <i>P. aeruginosa</i> <i>B. subtilis</i>	-Disk diffusion -Amino acid leakage -Colony forming unit's analysis	Enhanced bactericidal effect	Antibacterial effect	[57]
12–40	Co-precipitation	<i>P. aeruginosa</i>	-MIC	Relatively more effective than pure gentamicin	Antibacterial effect	[58]
60	Chemical	<i>E. cloacae</i> <i>S. Aureus</i> <i>K. pneumonia</i> <i>A. baumannii</i> , <i>P. aeruginosa</i> <i>E. faecalis</i>	-Bacterial staining and observation under confocal laser scanning microscope	Homogenous distribution and antibacterial effect similar to free gentamicin	Antibacterial effect	[59]
21.09 to 55.54	Co-precipitation	<i>P. mirabilis</i> , <i>S. epidermidis</i> , <i>A. baumannii</i>	Zone inhibition, MIC and MBC	Remarkable enhancement in the antibacterial activity of the nanoparticles against the tested bacterial strains.	Antibacterial effect	[60]
34	Co-precipitation	<i>S. aureus</i> <i>E. coli</i>	Disk diffusion, Zone inhibition	Increased antibacterial activity observed in a dose dependent manner	Antibacterial effect	[61]

approximately 22.4 µg of the drug at pH 7.4. The PLGA-gentamicin complex showed higher MIC, MBC and minimum biofilm eradication concentration (MBEC) values than free gentamicin. This is more likely due to the interaction of the biofilm anionic polysaccharide with the cationic gentamicin, which limits the availability of the drug to act against the resident bacteria. Moreover, the PLGA-gentamicin complex provided continuous and controlled release of the drug for up to 16 days [63].

In another study, PLGA nanoparticles loaded with gentamicin were used to assess their efficacy as a drug delivery system to treat osteomyelitis infection. Zeta sizer analysis showed that the PLGA-loaded gentamicin molecules had a diameter in the range of 200–400 nm, as well as a low polydispersity index, showing that all the nanoparticles had a uniform size distribution. The size of the nanoparticle was also seen to decrease with increasing surfactant concentration (polyvinyl alcohol). Drug release analysis showed an initial burst of release, where 26% of the drug was released during the first 12 h, followed by sustained release for more than 30 days. When the antibacterial effects against *S. aureus* and *S. epidermidis* species were analysed, both species showed similar average zones of inhibition, and MIC values for *S. epidermidis* were 0.064 µg/ml and *S. aureus* had a higher value of 0.500 µg/ml [64].

In the same manner, a study was conducted on PLGA nanoparticles loaded with gentamicin (with a diameter of 227 nm) for the treatment of intracellular *K. pneumoniae* infection in the lungs. Evidence proved that *K. pneumoniae*

could survive inside macrophages both in vitro and in vivo. Since gentamicin alone was less permeable through the cell membrane, it limited the clinical effectiveness of the drug when treating intracellular pathogens. The overall objective of this study was to examine the competence of PLGA-loaded gentamicin complexes to deliver their antibiotic payload more efficiently than free gentamicin. However, the results of the antimicrobial assay showed a reduced effect when compared with free gentamicin, as determined by the MIC values. It was postulated that this was due to the encapsulation of the drug inside the nanoparticle, preventing immediate exposure to the bacteria. Drug release analysis showed that over a 120-hour period, 80% of the drug was released, further validating the hypothesis [65]. A double emulsion approach was used in a separate study to combine PLGA and GS. Gentamicin-loaded PLGA nanoparticles exhibited outstanding antibacterial characteristics and many prospects in making smart wound dressings [66].

A different study prepared gentamicin loaded with polydopamine nanoparticles to assess its antibacterial efficiency. Gentamicin was added to polydopamine using in situ polymerization techniques at various monomer and gentamicin concentrations. Gentamicin's amine group and polydopamine's hydroxyl group formed hydrogen bonds, resulting in conjugation. Additionally, conjugates made with a monomer to drug ratio of 1:1 demonstrated the maximum loading capacity. Gentamicin had an MIC value of 1.90 g/ml, and the polydopamine-gentamicin complex had an MIC value of 0.6 mg/ml when the bactericidal activity was tested

against *P. aeruginosa* and *S. aureus*. By themselves, polydopamine nanoparticles did not exhibit any bactericidal properties. The possibility that certain drug molecules were trapped inside the nanoparticles appeared to be the cause of the higher MIC value. Therefore, further possible release mechanisms needed to be implied to increase gentamicin release from polydopamine nanoparticles [67].

Another work focused on nanoparticles composed of maleic anhydride-comethyl vinyl ether to assess their efficiency in ophthalmic drug delivery. These polymeric nanoparticles were created using the solvent evaporation approach. The amine groups of gentamicin facilitated ionic interactions with the nanoparticle, yielding an average nanoparticle size of 150 nm. The efficiency of encapsulation also dramatically increased with increasing ionic strength and pH. In vivo drug release analysis of the complex showed a significantly extended release of gentamicin when injected into rabbit cornea [68]. Chitosan nanoparticles have a positive surface charge and mucoadhesive properties, which help them adhere to mucus membranes and continuously release the drug. They are used in the treatment of various conditions, such as pulmonary diseases, cancer and gastrointestinal diseases [69].

In a study, gentamicin- and salicylic acid-loaded spherical chitosan nanoparticles were synthesized with a high zeta potential ($> +30$ mV) and an average size of 148–345 nm. In vitro drug release results indicated that in phosphate buffer saline at pH 7.4, initially, an outburst of release was observed, followed by slow release [70]. In a 2016 study, a chitosan/fucoidan nanoparticle conjugate system for the pulmonary delivery of gentamicin was developed with a size range of 270–300 nm. Drug release results showed biphasic release with zero-order kinetics during the initial 10 h of the experiment and sustained release for the next 75 h [71].

Chitosan nanoparticles, when prepared by the gelation method, could also be used as a nanocarrier for gentamicin. Cotton fabrics and blends with polyester were treated with gentamicin and chitosan conjugates to obtain increased antibacterial activity. Testing of the antibacterial properties was performed on *S. aureus* and *E. coli*. The disk diffusion test revealed that, due to a larger concentration of the drug at the bacterial cell membrane, the antibiotic's activity shifted from bacteriostatic to bactericidal as the antibiotic load increased. Due to the nature of the cell membrane, the composites had stronger antibacterial activity against gram-positive bacteria [72].

In another study, gentamicin-loaded phosphatidylcholine-chitosan nanoparticles were synthesized to assess their effects on biofilms and intracellular bacteria. These nanoparticles varied in size from 76 to 137 nm. Through FTIR analysis, it was seen that phosphatidylcholine was absorbed

onto the chitosan nanoparticles and that gentamicin occupied the outermost layer of the system. The standard amount of gentamicin per nanoparticle was 20 $\mu\text{g/ml}$. The MIC values were further obtained against four strains of bacteria, namely, *P. aeruginosa*, *L. monocytogenes*, *E. coli* and *S. aureus*. A dramatic decrease in biofilm formation was also reported by *L. monocytogenes* species when treated with the conjugates compared to free gentamicin, and similar effects were observed with the *P. aeruginosa* biofilm [73].

In a 2020 study, cranberry proanthocyanidin-chitosan nanoparticles with gentamicin were synthesized and assessed for their antibacterial efficiency. Different ratios of proanthocyanidin, chitosan and gentamicin were used to form four different complexes with a size range of 242–277 nm. Antimicrobial assays showed that the nanoparticles with gentamicin could agglutinate *E. coli*, *S. aureus*, and *P. aeruginosa* species and that the proanthocyanidin-chitosan nanoparticles with gentamicin showed greater antibacterial activity than gentamicin alone [74].

Ascorbic acid (AA) and gentamicin sulphate (GM) were delivered using chitosan nanoparticles (CSNPs) as a carrier. The in vitro antibacterial activity and cytotoxicity of the produced particles were assessed. The produced particles (GM-AA-CSNPs) demonstrated encapsulation effectiveness and loading capacities of 89% and 22%, respectively, under the ideal conditions of 4:1:1 of chitosan to drug ratio [75].

A summary of the size of the polymer nanoparticles used in gentamicin conjugation, experiments conducted, bacterial species used and applications of nanoconjugates are given in Table 5.

Calcium Carbonate/Calcium Silicate Nanoparticles

Researchers extensively utilize calcium carbonate (CaCO_3) in biomedical applications due to its well-known characteristics, including widespread availability, affordability, safety, biocompatibility, pH sensitivity, and slow biodegradability. Recently, there has been a surge of interest in using it as a drug delivery system for various categories of medications [76].

Gentamicin-loaded calcium carbonate (CaCO_3) nanoparticles were synthesized in a study to assess their drug delivery against the bacterium *S. aureus*. The nanoparticles were prepared by the microemulsion method with a particle size of 113–120 nm and characterized by dynamic light scattering, SEM, XRD analysis, differential scanning calorimetry, FTIR and drug release profiles. An in vitro drug release study showed slow release for 12 h. Additionally, both free gentamicin and the conjugate systems, on the basis of their MIC values, showed similar bactericidal effects against *S. aureus* species [77].

Table 5 Summary of polymer nanoparticle gentamicin conjugates and their outcomes in antibacterial activity and drug release experiments

Size (nm)	Synthesis method	Polymer	Bacteria	Experiments	Result	Application	References
227–240	water/oil/water, emulsion evaporation	PLGA	<i>P. aeruginosa</i>	-Disk diffusion -MBC -MBEC	No enhanced effect	Antibacterial effect	[63]
-	water/oil/water		<i>S. aureus</i> <i>S. epidermidis</i>	-Disk diffusion -MIC	Sustained release for 35 days	Antibacterialeffect Drug release	[64]
-	water/oil/water		<i>K. pneumoniae</i>	-Drug release analysis	80% drug release after 120 h	Drug delivery antibacterial effect	[65]
200–400	water/oil/water		<i>E. coli</i>	-Disk diffusion	Drug delivery	Antibacterial effects	[66]
250–550	oxidative self polymerisation	Polydopamine	<i>S. aureus</i> <i>P. aeruginosa</i>	-MIC -MBC	Increased MIC value compared to free gentamicin	Antibacterial effect	[67]
< 150 nm	solvent evaporation-precipitation	Poly (maleic anhydride-methyl vinyl ether)	-	-Release Studies - Toxicity Studies -Gentamicin concentration in cornea and anterior chamber in rabbits after drug instillation -Sustained gentamicin release into the rabbit's eye	Immediate release of drug Significant, sustained release profile and better drug availability	This system can be applied for oral and topical delivery of charged drug molecules	[68]
270–300	Ionic cross linking	Chitosan	-	-Drug release analysis	Initial burst of release followed by slow release	Drug release	[70]
148–345	Ionotropic crosslinking		<i>K. pneumoniae</i>	-Drug release analysis	Sustained release for 72 h	Drug delivery Antibacterial effect	[71]
3–4	Ionic gelation method		<i>S. aureus</i> <i>E. coli</i>	-Disk diffusion	Higher effect against <i>S. aureus</i>	Antibacterial effects	[72]
76–137	Ionic cross linking		<i>L. monocytogenes</i> <i>P. aeruginosa</i> <i>S. aureus</i> <i>E. coli.</i>	-MBEC -Fluorescence microscopy	Enhanced antibiofilm action	Antibiofilm potential	[73]
242–277	Chemical method	Chitosan- Proanthocydin	<i>P. aeruginosa</i> <i>S. aureus</i> <i>E. coli</i>	-Bacterial agglutination assay	Enhanced bactericidal effects than free gentamicin	Antibacterial effect	[74]
278	Ionic gelation method	chitosan	<i>P. aeruginosa</i> <i>S. aureus</i>	-Zone of inhibition -cytotoxicity	Lower toxicity to the normal cells	Drug delivery Antibacterial effect	[75]

In another study, mesoporous calcium silicate nanoparticles were prepared by a sol-gel method and loaded with gentamicin. These were then used to assess and increase the osteogenic drug delivery efficiency for endodontic materials. The nanoparticles had an average diameter of 200 nm, with an average pore size of 3.05 nm and a high surface area to volume ratio. In vitro, drug release experiments indicated that the nanoparticles maintained sustained release [78].

Another study was conducted to assess the synergistic effect of gentamicin-loaded CaCO₃. The synthesized nanoparticles exhibited a chain-like structure with a good polydispersity index and a size range from 60 to 80 nm. The interaction between gentamicin and the nanoparticles was assessed by FTIR analysis, where a hydrogen bond interaction between the two molecules was speculated. In vitro drug release results showed that 50% of the drug was released within the first hour, and the total amount of drug was released after 24 h, showing that the release of the drug was slower compared to the free form of gentamicin. When assessing the antimicrobial activity of the conjugates, the conjugate system showed enhanced antibacterial effects compared to free gentamicin against *B. subtilis*. The proposed mechanism of action of the conjugates was that, due to the overall negative charge and size, the conjugates are absorbed into the cell wall by intermolecular forces, increasing cell wall damage and permeability [79].

A summary of the size of the calcium carbonate/calcium silicate nanoparticles used in gentamicin conjugation, experiments conducted, bacterial species used and applications of nanoconjugates are given in Table 6.

Liposomes

Liposomes are phospholipid vesicles containing more than one concentric lipid bilayer surrounding distinct aqueous spaces. It can trap lipophilic and hydrophilic compounds.

This property of liposomes allows them to encapsulate a wide range of various drug molecules. Therefore, they are well-studied nanocarriers for targeted drug delivery with excellent cellular and tissue uptake and enhanced biodistribution of compounds [80].

Various studies have investigated the improved antibacterial efficacy of antibiotics, specifically gentamicin-conjugated liposomes. For instance, in a study, liposomes made from different lipid compositions and cholesterol were encapsulated with gentamicin, resulting in nanoparticles having an average diameter range from 408 to 418 nm with 4–5% drug encapsulation efficiency. These liposomal complexes could retain 70% of the drug up to 48 h, where liposomes incubated at 4 °C could retain more antibiotics compared to liposomes incubated at 30 °C [81]. Gentamicin-encapsulated liposomes were compared with free gentamicin to assess the antibiotic activity against *P. aeruginosa*. The liposomes had an average size of 420–440 nm and an encapsulation efficiency of 4.51%. The MIC values for the complex were significantly lower than those for free gentamicin [82].

In another study, liposomes encapsulated with gentamicin and gallium metal were used to assess their enhanced antimicrobial effects. The synthesized liposomes had a diameter of 310–335 nm. The MIC and MBC showed that the complex of liposomes and gentamicin was more effective than free gentamicin against *P. aeruginosa* biofilms. The cell viability assay using A549 cells suggested that encapsulated gentamicin was much safer than free gentamicin [83].

Liposomes were synthesized to evaluate the stability of the composite as a nanocarrier of gentamicin in another study. The outcomes demonstrated prolonged drug release for smaller gentamicin liposomes [84].

The efficacy of gentamicin and liposomal formulations with distinct surface charges was tested against *K. oxytoca* and *P. aeruginosa* in a different investigation. The liposomal

Table 6 Summary of Calcium carbonate / Calcium silicate nanoparticles gentamicin conjugates and their outcomes in antibacterial activity and drug release experiments

Size (nm)	Synthesis method	Bacteria	Experiments	Result	Application	References
113–120	Microemulsion	<i>S. aureus</i>	-Drug release MIC	Antibacterial effect and drug delivery	Similar bactericidal effects as free gentamicin	[77]
200	Sol-gel	<i>E. faecalis</i>	-Drug loading and release kinetics -In vitro soaking -Cell viability -Alkaline phosphatase activity assay -Enzyme linked immunosorbent assay -FGF-2 loading -Alizarin red S stain	-Better loading efficiency -Potent drug carrier -Drug delivery -Bacterial growth inhibition	Can be used as endodontic materials as dental pulp tissue regenerative materials	[78]
62.5	Carbonization method	<i>B. subtilis</i>	-Drug loading -Entrapment efficiency -Drug release -Zeta-potential analysis -Microscopic investigation of bacteria	High drug loading and entrapment efficacy towards gentamycin sulfate Increased antibacterial activity	Potential carriers in drug delivery system with improved antibacterial activity	[79]

drug complex was synthesized by the dehydration method, which yielded a liposomal structure size of 620–840 nm. A 1.8–43.6% drug encapsulation efficiency was seen by the complex, which could retain 60% of the drug content during the initial 48 h. The MIC values of neutral liposomes were less than those of gentamicin alone, and the MBC values were twofold-fold lower than those of gentamicin alone. Moreover, negatively charged liposomes showed similar results as free gentamicin [85].

A summary of the size of liposomes used in gentamicin conjugation, experiments conducted, bacterial species used and applications of nanoconjugates are given in Table 7.

Selenium Nanoparticles

Selenium, an essential nutrient element with significant biological roles, presents an intriguing opportunity for integration with antibacterial agents. It is a trace element necessary for maintaining health and promoting growth through dietary intake. Elemental selenium, being the least toxic form of selenium, has gained considerable interest in its nano form. Recent research has highlighted the anticancer, antioxidant, antibacterial, and antibiofilm properties of selenium nanoparticles. Notably, these nanoparticles have exhibited remarkable antimicrobial activity against pathogenic bacteria, fungi, and yeasts in various studies [86].

The antibacterial and antibiofilm properties of biogenic selenium nanoparticles (Se NPs) created by green technologies that are both cost-effective and environmentally acceptable were analysed in a study. The production of Se NPs was discussed in this work using 2 distinct techniques: a biogenic procedure employing *Penicillium chrysogenum* filtrate and a gentamicin medication after gamma irradiation. In comparison to biogenic Se NPs, synthetic Se NPs-CN have a

promising antibacterial ability against all urinary tract infections (UTIs) caused by the microorganisms tested. Se NPs-CN has shown antibacterial activity against *C. albicans* with a zone of inhibition (ZOI) of 26.0 mm, 23.0 mm for *E. coli* and 20.0 mm for *S. aureus* [87].

Mechanism of Action

Understanding the mechanism of action of nanoparticle-antibiotic conjugates aids in the improvement and development of more viable composites and predicts more efficient designs to mitigate antimicrobial resistance. However, the exact mechanism of action of the nanoparticle composites contributing to the enhanced bactericidal effects remains unknown [88]. It is important to know both the mechanisms of action of antibacterial nanoparticles alone and their action in conjugation with gentamicin. To predict the exact mechanism of action of different nanoparticle-conjugated gentamicin, many studies have been conducted exploring the bactericidal effects of nanoparticles. The ability of nanoparticles to infiltrate biofilms and alter bacterial metabolism are two of their most potent strategies for causing cell death [11]. Oxidative stress, nonoxidative processes, and metal ion release in metal oxide nanoparticles are the other often hypothesized modes of action [89].

To achieve bactericidal effects, nanoparticles should be in contact with bacterial cell walls by electrostatic attraction, van der Waal forces, receptor–ligand or hydrophobic interactions. The nanoparticles then cross the bacterial membrane and gather along the metabolic pathway, which influences the function and shape of the cell membrane. This is followed by the nanoparticles interacting with the bacterial cells' basic components such as DNA, lysosomes,

Table 7 Summary of liposome gentamicin conjugates and their outcomes in antibacterial activity and drug release experiments

Size (nm)	Synthesis Method	Liposomal composition	Invitro/invivo	Bacteria	Experiments	Result	Application	References
408–418	Sonication	DMPC, DCCP, DSPC and cholesterol	In vitro	<i>P. aeruginosa</i>	-MIC	Antibiotic effects	Pharmaceutical use	[81]
426.25±13.56	Evaporation	DMPC: cholesterol	In vitro	<i>P. aeruginosa</i>	-MIC	Antimicrobial effect	Clinical use	[82]
-	Sonication	DPPC: DMPG	In vitro	<i>P. aeruginosa</i>	-MIC -MBC	Antibiotic effects	Clinical use	[83]
12–80	dehydration–rehydration method	DPPC; Cholesterol	In vitro	<i>S. aureus</i>	-Diffusion assay	Antimicrobial effect	Clinical use	[84]
645–806	dehydration–rehydration method	DMPG, cholesterol and DPPC	In vitro	<i>P. aeruginosa</i> <i>K. oxytoca</i>	-MIC -MBC	Antibiotic effects	Drug delivery	[85]

*1,2-dimyristoyl-sn-glycero-3-phosphocholine- DMPC

*1,2-dipalmitoyl-sn-glycero-3-phosphocholine - DPPC

*1,2-distearoylsn-glycero-3-phosphocholine – DSPC

* 1,2-dimyristoyl-sn- glycero-3-phospho-(1'-rac-glycerol) – DMPG

ribosomes and enzymes that lead to oxidative stress, changes in the permeability of the membrane, heterogeneous alterations, enzyme inhibition, protein deactivation and changes in gene expression [90]. The bactericidal mechanisms of action of nanoparticles are generally described as one of three models, namely, the induction of oxidative stress [91], the release of metal ions [92] and nonoxidative mechanisms [93], with the possibility of all three mechanisms occurring simultaneously. The major processes underlying the bactericidal effects of nanoparticles are (1) cell membrane disruption, (2) generation of reactive oxygen species, (3) bacterial cell membrane penetration, and (4) intracellular induction of antibacterial effects-interaction with proteins and DNA.

The antibacterial effects of nanoparticles depend on their stability, size, and concentration in the medium [94]. Smaller nanoparticles typically exhibit greater antibacterial activity; the reason for this, according to one concept, is that they have a higher surface area to volume ratio than larger nanoparticles, which leads to an increase in the production of ROS that damage DNA, lipids, and proteins essential for bacterial cell growth [95]. However, some studies have shown that large nanoparticles are equally as effective, showing that size is not the most important factor in inducing bacterial toxicity [96]. The charge of the nanoparticle is another important factor that affects the antimicrobial activity. A study showed that positively charged amine-functionalized nanoparticles were able to change the function of the electron transport chain of bacteria. Nanoparticles with a positive charge can electrostatically attach to the negatively charged bacterial cell wall, causing them to exhibit enhanced toxicity [97, 98]. The acidic nature of nanoparticles has been found to aid in attachment to the bacterial cell wall, which can further enhance the bactericidal effects [99].

The chemical structure of aminoglycosides also facilitates their antibacterial effects. They are considered polycationic species; that is, they contain multiple electrolyte groups. Due to this property, they show binding affinity to the negatively charged moieties in the outer membrane of gram-negative aerobes and nucleic acids. The bactericidal activity is mainly due to the inhibition of protein synthesis where the drug binds to the 16 S rRNA and eventually disrupts the integrity of the cell membrane of the bacterium where the Mg^{2+} bridges between the adjacent lipopolysaccharide molecules are disrupted [100].

Nanoparticles influence cellular functions such as cellular differentiation and adhesion in bacterial cells [101]. Metal oxide nanoparticles such as copper, zinc, iron, titanium, etc., with smaller particle sizes have been reported to show promising bactericidal effects. Similarly, the antibacterial effect of SiO_2 nanoparticles is more significant at smaller sizes due to the increased surface area [102]. Metal oxide nanoparticles follow two major mechanisms of action

when exhibiting bactericidal effects. The first mechanism is the direct contact of the nanoparticles with the cell wall of the bacteria and their destruction by the oxidation reaction, which produces ROS. The second mechanism involves nanoparticles acting as free radical scavengers [103].

A study conducted on silver-polyethyleneimine nanoparticles showed that the composite attached to the bacterial cell wall and some fused within the bacterial cell wall. In contrast, negatively charged silver-citrate nanoparticles showed no attachment. Silver nanoparticles exhibit bactericidal effects by disrupting the disulfide linkages of the proteins present in the transmembrane layer of the bacterial cell wall following the decreased rate of metabolic processes that eventually lead to cell death [104]. Silver metal ions catalyze the formation of free radicals that can oxidize cellular macromolecules. These free radicals can diffuse and travel through the bloodstream. As the microbes are not exposed to the radicals for a long period, the ability of the bacteria to gain resistance is reduced. However, gold nanoparticles act by breaking down the bacterial cell wall, which can aid some antibiotics that are less penetrative through the cell membrane. Gold nanoparticles, when combined with a small amount of gentamicin, showed that the mixture of nanoparticles and antibiotic showed a significant enhancement in its antibacterial effect when compared to gentamicin alone [105]. Another experiment carried out on gold nanoparticles showed that these nanoparticles exhibit toxicity as a result of cell wall rupture induced by direct contact between the nanoparticle and the cell wall, rather than the generation of ROS [106]. Gold nanoparticles also induce cytoderm damage, which results from the electrostatic attraction between the negatively charged cytoderm and the positively charged nanoparticles [107]. The results of scanning electron microscopy of gold nanoparticles labelled with cefradine showed damage to the cell wall through direct physical contact. Furthermore, fluorescent probe analysis of the nanoparticles ruled out the possibility of ROS formation leading to the loss of cell viability [108].

Discussion

The need for novel antibiotic agents that are effective against resistant bacterial strains is gaining much attention around the world [109, 110]. Many scientific groups have tried to create potential commercial antibiotic or antimicrobial peptides by combining antimicrobial nanoparticles with a drug [111].

In this review, we describe nanoparticle conjugate systems with one of the aminoglycoside antibiotics, gentamicin, and its application in drug delivery and enhanced antibacterial activity. Gentamicin is hydrophilic and tends to

penetrate less through the hydrophobic bacterial cell wall; thus, higher concentrations are prescribed, which leads to harmful side effects such as nephrotoxicity and ototoxicity [100]. When gentamicin is conjugated with nanoparticles, the nano hybrids have a higher antimicrobial activity than the free antibiotic itself [53]. The produced composites might have some mechanism that could enhance the bactericidal activity, such as their potential to attach to or penetrate the cell wall, resulting in the delivery of a greater number of gentamicin molecules into a highly localized volume as each nanoparticle is surrounded by several drug moieties acting as a sole group [112]. A wide field of novel antimicrobial agents can offer a considerable outlook for the expansion of therapeutic agents in the treatment of bacterial infectious diseases that have an alternative mechanism of action, thus overcoming the current mechanism of antibiotic resistance [113].

Many conjugate systems have a good bactericidal effect against infectious bacteria, but the exact mechanism of many is not known [10]. Understanding the mechanism of the bactericidal effects of existing antibiotics and their nanoconjugate systems is one of the salient parts to originate more modern and biocompatible nano hybrids that may aid in coping with the difficulty of bacteria gaining resistance in the future [114, 115]. The tracking and movement of the conjugates can be aided using fluorescent peptides and nanoparticles, which will be useful in understanding their binding sites, parameters that need to be well analysed for high efficiency and mode of action [116, 117]. Proper understanding and advanced research on nano hybrids can lead to the progress of a novel, triumphant plan of action as a cure for human bacterial infections.

Conclusion

Antibacterial resistance is a great concern, and it can be handled by enhancing the drug efficiency using novel nanotechnology methods. Many studies summarised in this review indicated that gentamicin-nanoparticle conjugates shown better anti bactericidal effects and also efficient drug delivery strategies. Understanding the mechanism of enhanced activity may lead to discovery of novel antibiotics which will address the growing problem of antibacterial resistance. However, a thorough research on safety is essential for the success of these approaches.

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Declarations

Conflict of interest The authors declare that there is no conflict of interest.

Ethical Approval Not Applicable.

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