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Review Article

Application and Challenge of Nano-Systems against Intracellular Bacterial Infection

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ABSTRACT

Intracellular pathogenic bacteria cause chronic, persistent and latent infections. The treatment for intracellular infection faces many challenges, such as targeting bacteria with antimicrobial drugs and resistance to multidrug (MDR). The present study delivers a possible elucidation to this problem via Nano Drug Delivery. Nanomaterials are relatively small as compared to ordinary antibiotics. The nanoparticle has unique physical and chemical properties, a large surface area, and an electric charge. Additionally, its antibacterial drug encapsulation can make it easier to enter the cell and improve the concentration in the target tissue. In the current review, the purpose is to find a more effective nanometer system to fight against intracellular bacterial infection. Current observation describes the drug resistance mechanism of bacteria, the bactericidal mechanism of antibiotics, the way of nanoparticles overpasses the cell membrane and approaching the cell, the strategies, prospects and challenges of nanoparticles treating intracellular bacteria.

INTRODUCTION

Intracellular bacteria evade the host's innate and adaptive immune system. Intracellular bacteria have great harm to the animal and human health, mainly divided into facultative and obligate intracellular bacteria, including *Staphylococcus aureus, Mycobacterium tuberculosis, Bacillus leprae, Brucella, Salmonella typhimurium, Legionella pneumophila, Rickettsia intracellular* and *Chlamydia*, *etc*. Intracellular bacteria are considered difficult to eliminate, which can lead to troublesome typhoid fever, tuberculosis and leprosy (Reece and Kaufmann, 2019). Intracellular bacteria are mainly located in host cells and often leads to a chronic persistent infection, which has a great impact on the development of animal husbandry. *Staph. aureus* is a typical zoonotic intracellular bacterium, which can not only cause upper respiratory tract, skin infections (including blisters and abscesses), mucosal damage, post-operative wound, bone, and even a breast infection, osteomyelitis, bacteremia, meningitis, endocarditis and pneumonia. In addition, breast cancer in cattle caused by *Staph. aureus* can increase the mortality rate of cattle, reduce milk quality, and hinder the development of the livestock economy. Methicillin-resistant *Staph. aureus* (MRSA) is a multi-drug resistant to antibiotics that can cause skin, blood, lung and other infections. Intracellular Laursenia causes an equine proliferative enteropathy (EPE) and porcine proliferative enteropathy (PPE) (Pereira *et al*., 2020), which leads to diarrhea, malnutrition, and even death can occur (Pereira *et al*., 2019). *Salmonella* mainly lives in the intestines of humans and animals which can lead to persistent diarrhea. *Salmonella* can also cause typhoid fever, immune damage, gastrointestinal diseases, and raise some of the complications, including bacteremia and reactive arthritis. In addition to human infection, *Salmonella* can cause systemic infection in pigs, contaminate pork products (Alborali *et al*., 2017). There are many types of mycobacteria and the most common is *Mycobacterium*

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tuberculosis that causes tuberculosis, and some of them can cause leprosy. Additionally, the mycobacterium that didn't cause tuberculosis and leprosy are called the non-tuberculous mycobacteria (NTM). Mycobacteria leads to fever, decreased immunity and causes lung, and lymphadenitis in children.

Ansamycins, fluoroquinolones, tetracyclines, macrolides are traditionally considered antibiotics for the treatment of intracellular infection (Pantosti *et al*., 2007). Such as penicillin, methicillin, fluoroquinolones, tetracycline and daptomycin are considered previously to treat *Staph. aureus* which is a typical intracellular bacterium while linezolid and daptomycin are now using for the treatment (Pantosti *et al*., 2007). Though, it is challenging for the traditional antibacterial drugs to enter into the host cells and Permeate the cell membrane. In addition, the exocytosis of intracellular bacteria results in the reduction and loss of antibiotic-active ingredients in the cells. Hence, treatment of intracellular bacteria is a great challenge because of parasitizing into the host cells. Presently, the treatment of intracellular bacteria is based on the long-term and extensive use of antimicrobial drugs. Conversely, it not only causes the resistance to stealthy bacteria but also origins in unnecessary economical losses due to the excessive consumption of antimicrobial drugs. So, the treatment of intracellular bacterial infection and bacterial resistance have been paid more and more attention by researchers. Nanomaterials have a unique property, which makes them more effective in *in vitro* experiments. Nanoparticle encapsulation antimicrobial drugs can be flexibly sized to pass more easily through airways and mucosa, can change the properties of the surface, allowing them to pass more easily through cytomembrane. Nanoparticle encapsulation antimicrobial drugs can target the infected cells more efficiently, resulting in a greater release of the active ingredient. At the same time, nanoparticle encapsulation antimicrobial drugs can also fight against the defense mechanism of bacteria. In addition, if we choose the appropriate nanomaterials to encapsulate a drug, the antimicrobial agents can be better targeted to the site of infection for improving the bioavailability of drugs and appropriately alleviate the resistance of antibacterial drugs. Therefore, the use of nanoparticle encapsulation antimicrobial drugs in the treatment of intracellular bacterial infections has a wide range of prospects.

Intracellular bacterial infection has become one of the inflexible diseases that jeopardize human and animal health. These bacteria can cause many life-threatening diseases that are tough to treat, such as tuberculosis, AIDS, and liver disease. Traditional antibiotics treatment has become increasingly hard to treat intracellular infections. For example, bacterial biofilm formation and the difficulty of antibiotics to enter and maintain intracellular concentration for SA and MRSA (Zhou *et al*., 2018). To explore a more effective treatment of intracellular bacteria nanometer system including metal nanoparticles and metal-oxide nanoparticles, polymer micelles, chitosan nanomaterials, gelatin nanomaterials, nano-suspension, poly (lactic-co-glycolic acid (PLGA)), solid lipid nanoparticles (SLN) and so on, the mechanism of intracellular bacterial resistance and the treatment of intracellular bacterial infection by nanomaterials and comparison of strategies of various nanoparticles in the treatment of intracellular bacterial infection are analyzed and summarized in the current study.

MECHANISMS OF BACTERIAL RESISTANCE TO ANTIBIOTICS

Abuse of antibiotics might be considered as one of the possible reasons for the resistance development against antibiotics. Bacteria have resistance genes and even become multi-drug resistant bacterium, progressively. Drug-resistant bacteria, especially super bacteria, have seriously threatened human and animal health. Exploration of the genetic and biochemical mechanisms of drug-resistant bacteria has great significance for reducing the spread of resistant bacteria and developing new methods to solve the problems of antibiotic resistance (Munita and Arias, 2018).

Genetic basis of bacterial resistance

From a genetic point of view, the major approach for bacteria to escape from the attack of antibiotics is mutations in the gene and horizontal gene transfer (HGT) of resistance-related DNA (Lin *et al*., 2015). The genetic mechanism of bacterial resistance to long-term heavy antibiotic use can be divided into inherent resistance, acquired resistance and multi-drug resistance (Lin *et al*., 2015). Intrinsic resistance refers to the natural resistance of a bacterium to an antimicrobial drug, which is determined by its intrinsic resistance genes, such as gram-negative bacteria. Thus, it is possible to restore the antimicrobial function by destroying the intrinsic resistance genes. Acquired resistance is controlled by acquired drug resistance genes of bacterial, which means that bacteria change their DNA through gene mutation and transfer to develop drug resistance. Some acquired drug resistance may result from intrinsic resistance. Multi-drug resistance (MDR) refers to the simultaneous resistance of bacteria to antimicrobial drugs with different mechanisms of action or structures. Superbug which can result in urinary tract infections, pneumonia, and skin infections, is typically

multi-drug resistant bacteria that is resistant to almost all antimicrobics. At present, combined therapy is the main method to treat multi-drug resistant bacteria, but nano-drugs will become a new way to treat multi-drug resistant bacteria in the future (Tuon *et al*., 2015).

Biochemical basis of bacterial resistance

The production of inactivating enzyme

Bacteria develop resistance against antibiotics by producing enzymes that inactivate the antimicrobial drugs, triggering them to become ineffectual earlier they target the infected cells. Antibiotics develop resistance through the action of inactivating enzymes including β-lactam, aminoglycosides, macrolides and rifamycins (Fig. 1). The most typical example is the production of β-lactam by bacteria, resulting in the amido bond of the active group of the β-lactam antibiotics breaking, thus losing its antimicrobial effect. In addition, MRSA is resistant to gentamicin due to the presence of a dual-functional enzyme, which consists of two aminoglycoside modifying enzymes (AMEs) (Lin *et al*., 2015).

Alteration in drug target

The alteration in drug target mainly refers to the fact that bacteria change the structure of proteins at the site of antibiotics action, hinders to bind to the bacterial surface, thus producing the antibacterial effect. Antibiotics that develop resistance through alteration in drug target include β-lactam, fluoroquinolones, rifamycins, vancomycin, macrolides, aminoglycosides (Fig. 1). For example, alterations in penicillin-binding proteins lead to the development of resistance against β-lactam antibiotics. Such as; MRSA possesses *mecA* exogenous gene which encodes PBP2a to develop the resistance against β-lactam drugs (Lin *et al*., 2015). Due to PBP2a, it is hard to bind with β-lactam drugs, making the drug unable to block the synthesis of the cell wall.

Osmotic barrier of antibacterial drugs

It mainly refers to the mutation of bacteria and the loss of a specific porin that cause the changes of the permeability of bacterial cell wall and membrane, resulting in the antimicrobial drugs can not enter the body. Antibiotics that develop resistance through reducing the permeability of biofilms to drugs include β-lactam, tetracyclines and fluoroquinolones (Fig. 1). According to the membrane properties mediated by bacterial plasmids, bacteria can limit or remove antibacterial drugs, such as β-lactam and tetracyclines. In addition, the decreased membrane permeability caused by chromosomal mutations is the main mechanism of bacterial resistance to aminoglycoside drugs (McManus, 1997).

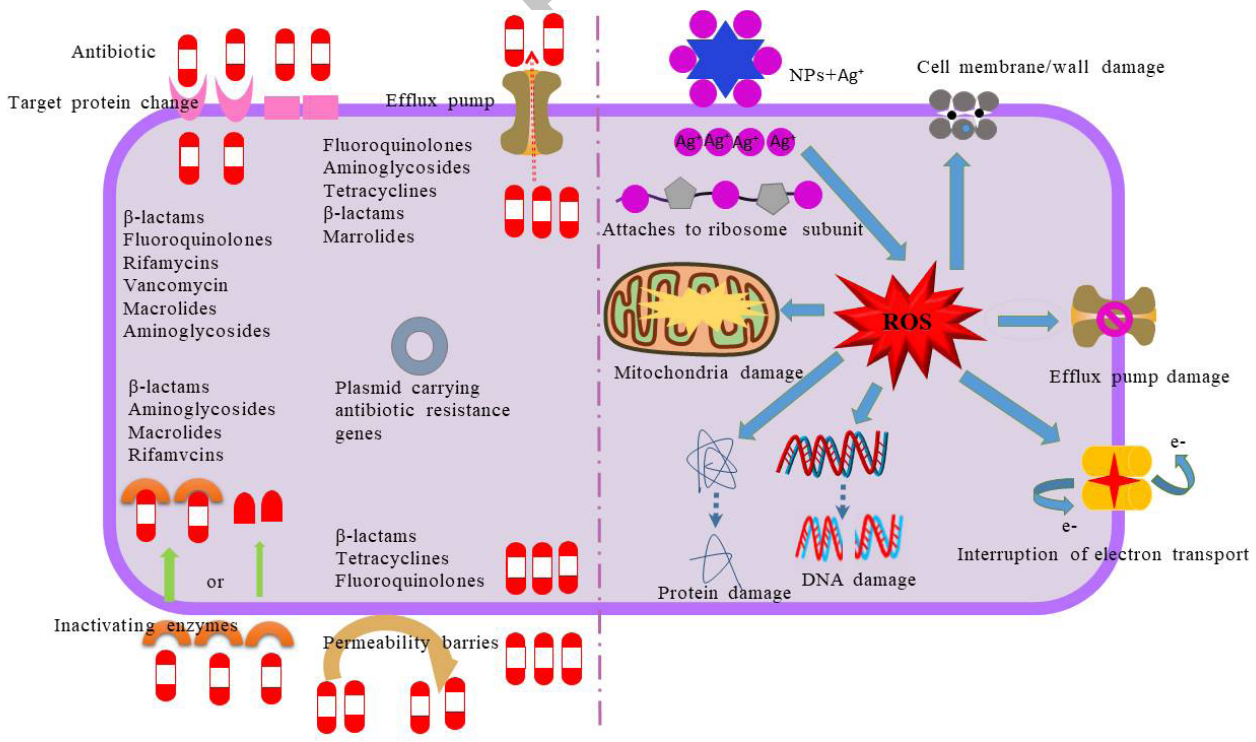

Fig. 1. Biochemical mechanisms of bacterial resistance (left) and antibacterial mechanism of mental nanoparticles (right).

Active efflux mechanism

The efflux pump is a protein on the cell membrane of bacteria. Dozens of bacteria have efflux pumps on their surfaces, which are part of the cell's secretory system and can reduce the concentration of drugs in the bacteria, leading to drug resistance. Antibiotics that develop resistance through efflux mechanism to drugs include fluoroquinolones, aminoglycosides, tetracyclines, β-lactam and macrolides (Fig. 1). In addition to resistance genes such as mecA, the resistance strategy for *Staph. aureus* also has the effect of an efflux pump (Zhou *et al*., 2018), which can pump antibacterial drugs out of bacteria.

CELLULARIZATION AND ANTIBACTERIAL EFFECT OF NANOPARTICLES

Nanoparticles can inhibit the drug resistance of bacteria whether they are used as an active agent or as drug carriers. As agents, nanoparticles can reduce bacterial by physical means, such as light. As drug carriers, nanoparticles can mainly protect antibiotics, reduce the degradation and loss of antibiotics, bypass the drug resistance mechanism of bacteria (Gao *et al*., 2021), and promote the concentration of drugs *in vivo*.

At present, the most popular nanoparticles for the treatment of intracellular bacteria are metal nanoparticles. Understanding the mechanism of metal nanoparticles' cell entry can enable us to design a metal nanoparticle from structure, size, and material aspects to eradicate intracellular bacteria.

The methods of metallic nanoparticles entering the cells include membrane depolarization, endocytosis, diffusion, and binding to the receptors on the cell membrane and so on. Furthermore, metal nanoparticles can pass through the oxidation pathway (to produce ROS), which can damage proteins, DNA, and bacterial membranes, as well as non-oxidative pathways, to kill bacteria while reducing cytotoxicity and damage to mitochondria (Fig. 2).

The exact antibacterial mechanism of nanoparticles has not been confirmed yet and there are few reports in the relevant literature, the currently known mechanisms of action can be divided into the following aspects:

Nanoparticles interact with bacterial cells through electrostatic interactions. Metal nanoparticles (MNPs) can contact bacteria to produce a reactive oxygen species (ROS), which damages the proteins, DNA, mitochondria and efflux pump. And metal nanoparticles play a bactericidal effect by ROS that can interrupt the electron transport and attaches to the ribosome subunit (Fig. 1). In addition, the nanoparticle can release metal ions directly to damage a bacterial cell.

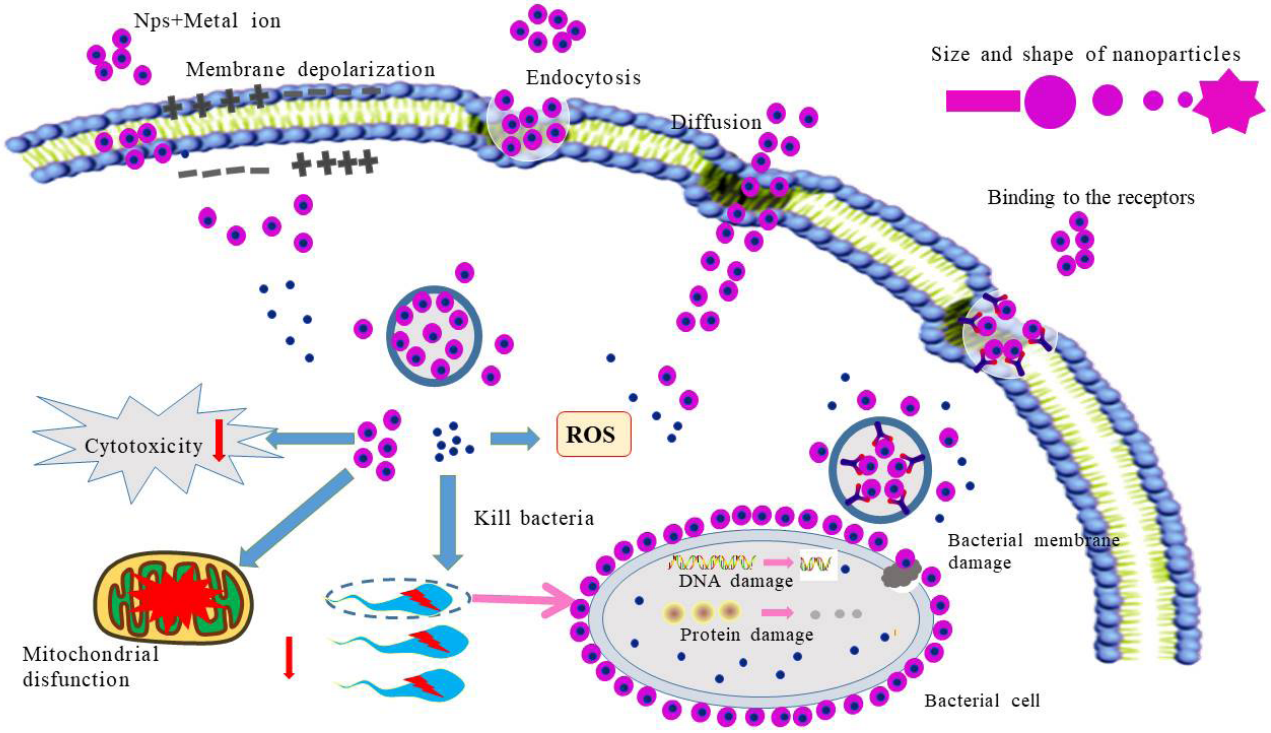

Fig. 2. Cellular way of metal nanoparticles.

The interaction of the nanoparticles with the bacterial cell membrane changes the membrane permeability and destroys the membrane, resulting in a depression on the membrane surface. Therefore, nanoparticle encapsulation antimicrobial drugs are more likely to enter cell membranes and produce bactericidal effects.

Nanoparticles can directly inhibit bacterial growth by interfering with intracellular components, flocculating intracellular components and binding to ribosomes, inhibiting the generation of proteins and cytoderm, and binding to the genetic material DNA and so on.

The glycocalyx is the main component of *Staph. aureus* biofilm, which is anionic (Zhou *et al*., 2018). Therefore, some cationic nanoparticles loading antibacterial drugs can be combined with them, causing the cell membrane of *Staph. aureus* to be destroyed, create cavities, and outflow of bacterial contents, such as proteins and genetic material.

NANOPARTICLE ENCAPSULATION: ANTIMICROBIAL DRUGS TO INTRACELLULAR BACTERIAL INFECTION

Nano-drugs for staphylococcal infections

Staph. aureus is a typical zoonotic bacterium, especially one that can cause mastitis in cattle (Algharib *et al*., 2020). Nowadays, liposomes are the most commonly used nano-drugs to treat the *Staph. aureus* infection. Liposomes can be used to encapsulate antibiotics in the treatment of bacterial infections because of their good affinity between the lipid bilayer and biofilm. In the treatment of *Staph. aureus* infection, liposomal lutein has an excellent anti-biofilm and antibacterial effects (Zhou *et al*., 2018). PLGA nanoparticle encapsulated with ciprofloxacin has good efficacy in inhibiting the cell membrane formation of *Staph. aureus*. In addition, some MNPs, such as gold nanoparticles (AuNPs), can effectively inhibit the biofilm formation of *Staph. aureus* (Zhou *et al*., 2018). AgNPS also can prevent vancomycin-resistant *Staph. aureus* (VRSA) colonization in the cell by preventing the biofilm formation of VRSA (Ghahremani *et al*., 2020). MRSA is a serious threat to human health which has a variety of drug resistance mechanisms that make many antibiotics ineffective. At present, scientists have discovered that a variety of nano strategies can treat MRSA including metal and metal oxide nanomaterials, carbon-based nanomaterials, liposomes, polymeric nanomaterials and so on (Gao *et al*., 2021).

Nano-DrugNano-Drugs for Mycobacterium *infection*

Intracellular mycobacteria grow more slowly. *Mycobacterium tuberculosis* causes such as; tuberculosis, while non-tuberculous mycobacteria are less harmful but can still cause lung and bronchial infections. Nanoparticles to treat mycobacterium include MNPs, magnetic nanoparticles and so on. Compared to the other MNPS, nano-Se has lower toxicity, anti-biofilm ability, and can treat *Mycobacterium tuberculosis* and non-*Mycobacterium tuberculosis* infection (Estevez *et al*., 2020). The potential of silver nanoparticles (AgNPs) and other MNPs to treat mycobacterium remains to be explored (Simoes *et al*., 2020). Magnetic nanoparticles modified on the surface of isoniazid can be used to fight *Mycobacterium tuberculosis* (Zargarnezhad *et al*., 2020). PLGA nanoparticle encapsulated with argF antigen (argF-NPs) is helpful to *Mycobacterium bovis* infection.

Nano-drugs for Salmonella *infection*

Salmonella can cause a systemic infection including typhoid form and non-typhoid form (Khan *et al*., 2021). *Salmonella* is resistant through multiple mechanisms, and traditional antibiotics are hard to treat. The mortality and morbidity of humans and animals caused by it are serious globally (Khan *et al*., 2021). There are few examples of nanoparticles being used to treat *Salmonella*. AgNPs can be used to treat multi-drug resistant salmonella isolates (Farouk *et al*., 2020). Multiple thorn-like AuNPs can also be used for the detection of *Salmonella typhimurium*, and edible vaccines encapsulated with chitosan nanoparticles can also be used for *Salmonella* enteritidis. And ampicillin nanocarriers mainly work by destroying the cell wall of *Salmonella*. In addition, *Salmonella* can also be killed by ciprofloxacin liposomes and core-shell nanomaterials encapsulating gentamicin (Khan *et al*., 2021).

NANOMEDICINE FOR INTRACELLULAR THERAPY

Nanoparticles have diameter ranges from 1nm~100nm. Nanomaterials are composed of several nanoparticles, so the specific surface area of nanomaterials is large. Nanomaterials have unique physical and chemical properties with a surface charge and easy functionalization. So, nanoparticle encapsulation antimicrobials can be better target the sites, allowing antibiotics to enter into the cell through the biofilm and play a better antibacterial role. Many scientists have explored nanomaterials to treat different intracellular bacterial infections.

At present, nanomaterials mainly used to improve the effect of antimicrobial drugs include metal nanoparticles and metal-oxide nanoparticles, polymer micelles, chitosan nanomaterials, gelatin nanomaterials, nanosuspension, PLGA, SLN and so on (Table I).

Table I.- Nanomedicine for intracellular therapy.

Nanomaterials	Antibacterial drugs	Bacteria	Mechanism	Effect	Disadvantages	References
Lipidosome	Rifampicin	*Mycobacterium bovis*	Binding to AMS	Reducing MIC	Cytotoxicity	Changsan *et al*., 2009
AgNPs	Amphetamine / Amoxicillin	*Staph. aureus*	Producing ROS	Increasing apoptosis and precise antibacterial	Membrane damage and environmental pollution	Vimbela *et al*., 2017; Mba and Nweze, 2021a
Polymeric micelle	Gentamicin	*Brucella*	Sustainable antibacterial surfaces and hydrogels	Rapid elimination of antimicrobial agents	Weak stability of micelles in vivo	Wen *et al*., 2018
Chitosan nanomaterials	Rifampicin	*M. tuberculosis*	Excellent adhesion and easy to be digested	Fine resistance to *M. tuberculosis*	Easy to degrade and need a carrier	Koli *et al*., 2019; Ji *et al*., 2014
AgNP/gelatin nanocomposites	Curcumin	*Staph. aureus*	Germicidal and antioxidant effects of gelatin	More stable drug	Controlling the amount of gelatin	Nam *et al*., 2015
Nano suspension	Clofazimine	Non *Tuberculous mycobacteria*	Inhibiting the synthesis of bacterial protein	Improving the clearance rate of *Mycobacterium*	Highly hydrophobic and limited in clinical use	Banaschewski *et al*., 2019
PLGA	Ciprofloxacin Hydrochloride	*Staph. aureus*	Electrostatic interaction	Prolonging antimicrobial drug release time	Difficulty in PLGA synthesis	Nootsuwan *et al*., 2018
SLN	Ciprofloxacin	*Staph. aureus*	Lipophilicity of SLN	Controllable release and enhanced antibacterial effect	Low drug loading and gelation	Shazly, 2017
Liposomes-encapsulated nucleic acid nanogel	Vancomycin	*Staph. aureus*	Mediated endocytosis	Reduction in intracellular and extracellular bacterial CFU	Low drug loading capacity, poor retention and delayed release	Obuobi *et al*., 2020
Nanogel	Vancomycin	*Staph. aureus*	PH sensitivity and adhesion	Good for transportation and targeted therapy	Unable to completely eliminate bacteria	Zhou *et al*., 2018
PBCA	Moxifloxacin	*M. tuberculosis*	Contacting with infected macrophages	Enhanced encapsulation efficiency and drug accumulation	Increasing in the time dependence of cytotoxicity	Kisich *et al*., 2007

Metal nanoparticles have non-specific targets with a strong antibacterial activity, which can resolve the problem of bacterial resistance. The production of ROS is the main antibacterial mechanism of metals and metal oxides. In addition, the antibacterial mechanisms of metal NPs are also composed of membrane interaction, cation release, free radical's formation, biomolecule damages and ATP depletion. AgNPs have an antibacterial effect on intracellular *Staph. aureus* and *Salmonella* species. The combination of AgNPs and antibiotics can enhance the antibacterial effect. For example, the combination of AgNPs and erythromycin shows a better bactericidal effect than the combination of vancomycin, penicillin G and amoxicillin against *Staph. aureus*. AgNPs produce ROS

by releasing Ag ions and trigger oxidative stress reaction, which affects the efferent pump and signal transduction pathway, thus increasing the intracellular concentration of antibiotics, but it is toxic to humans and the environment (Salleh *et al*., 2020). AuNPs have shown an antimicrobial effect. AuNPs destroy membrane integrity and activate the ROS related to cell death by electrostatic interactions, thus reducing the activity of metabolic enzymes. Nanometer zinc oxide (ZnONPs) has also been shown to an antibacterial effect on *Listeria monocytogenes, Salmonella enteritidis* (Jin *et al*., 2009) and MRSA. Zn^{2+} can destroy the cells through an interaction with intracellular components (Li *et al*., 2011) and produce ROS (Kumar *et al*., 2011). In the case of MRSA, Zn^{2+} inhibits the efflux pump, thus maintaining the intracellular concentration of the drug (Li *et al*., 2011). The combination of metal ion antimicrobial and photothermal sterilization is more effective.

AgNPs/gelatin nanocomposites are effective to pseudomonas aeruginosa and *Staph. aureus* and have been shown to have more than 99.99% inhibition against *Staph. aureus* (Galdopórpora *et al*., 2019). Gelatin showed a stable property, and its antibacterial and antioxidant properties can be enhanced by stabilizing the AgNPs and encapsulating drugs with it.

Polymer micelles abolish a cell membrane mainly through the electrostatic interaction between its surface and the surface of the cell membrane, making it easier for antibacterial drugs to enter the cell, so as to eliminate the intracellular bacteria. Polymer micelles can be used to form an antibacterial surface by co-grafting, anchoring, and bulk mixing. Sustainable antibacterial surfaces and hydrogels of polymer micelles are the focus of current research. Polymer micelles promote the rapid elimination of antimicrobial drugs *in vivo* and maintain a drug concentration, but their stability *in vivo* is weak (Wen *et al*., 2018).

Chitosan (CS) is a functional polymer with biocompatibility and degradability (Ke *et al*., 2021). Chitosan nanoparticles (CsNPs) include a silver nanoparticle, which can resist drug-resistant bacteria, even multi-drug resistant bacteria, through the interaction of chitosan and silver. In addition, chitosan nanomaterials can be refined anti-mycobacterial due to their excellent adhesion and easy digestion by colonic flora. However, chitosan is hydrophobic and is prone to aggregation under biological conditions. It can be modified on its surface to improve its hydrophobic properties and promote its application as a drug carrier (Hassani Najafabadi *et al*., 2014).

Clofazimine is aerosolized and delivered into the lungs to treat the non-tuberculous mycobacteria (Banaschewski *et al*., 2019). Its mechanism is to inhibit the production of bacterial protein, which can improve the clearance rate of mycobacterium *in vivo* and enhance its efficacy. However, its shortcoming is that it is highly hydrophobic, which limits its clinical application.

The fluidity of the liposome membrane and its physical and chemical properties make it have a good target specificity. Using liposomes to encapsulate the drugs has a long-term efficacy, which can resolve the low concentration of intracellular drugs in bacteria to improve the therapeutic effect. Liposome-encapsulated rifampicin binds to AMS through electrostatic interactions and reduces the MIC of the drug, for treating *Mycobacterium bovis*.

Liposome-encapsulated nucleic acid nanogel enhances the drug loading and has a synergistic anti-inflammatory effect on the intracellular infection of *Staph. aureus* (Obuobi *et al*., 2020). Nanogel drug delivery system showed a good antibacterial effect on methicillin-resistant *Staph. aureus*. Its adhesion and pH sensitivity helps the target drugs to the site of infection (Zhou *et al*., 2018).

Solid lipid nanoparticles (SLN) are drug carriers with the advantages of polymer nanoparticles and liposomes. It allows active or passive targeting of antimicrobial drugs to the target site, thereby increasing the concentration of the drugs. Through the fusion with the cell membrane, so that the drug achieves a better bactericidal effect. SLN can be used to encapsulate more drugs and has a good encapsulation rate. SLN is reported to enhance the antibacterial activity of tilmicosin against St *Staph. aureus* and *Streptococcus* (Zhu *et al*., 2018).

Poly lactic-co-glycolic acid (PLGA) and polybutylcyanoacrylate (PBCA) are degradable nanomaterials. PLGA is a composite polymer, and metal oxides combined with the formation of nanocomposite have an antibacterial effect. It can deliver multiple molecules simultaneously as a carrier and has been approved by the European Medicines Agency and the US Food and Drug Administration as a drug delivery system (Swartzwelter *et al*., 2020). Its mechanism is to destroy the integrity of the cell membrane, and it has good antibacterial activity against both Gram-positive bacteria (*Staph. aureus*) and Gram-negative bacteria (*Escherichia coli*). PLGA can prolong the efficacy of antimicrobial drugs. In addition, the superhydrophobic surface of PLGA also has antibacterial activity, and the antibacterial effect can be enhanced by the encapsulation of ciprofloxacin in a certain amount.

PBCA has good encapsulation with moxifloxacin and can release antimicrobial agents by contact with infected macrophages, which has good efficacy against Mycobacterium tuberculosis. However, the cytotoxicity increased with time (Kisich *et al*., 2007).

At present, new antimicrobial hybrid materials have also become a research hotspot. For example, liposome-

encapsulated nucleic acid nanogel can solve the problem of drug resistance of *Staph. aureus* (Obuobi *et al*., 2020). Polylactic acid and AgNPs-encapsulated chitosan can improve the antimicrobial effect of chitosan (Nootsuwan *et al*., 2018). In addition, the nanogels can be combined with other nanocarriers to achieve better results in Table I.

CHALLENGES AND PROSPECTS OF NANOMATERIALS FOR INTRACELLULAR BACTERIAL INFECTIONS

Traditional antibiotics and antimicrobial drugs have been unable to solve bacterial resistance or even multi-drug resistance, so it is essential to develop a new nano-drugs system for the treatment of bacterial infections, particularly intracellular bacterial infections. The basic mechanism research of nanomaterials for the treatment of intracellular bacteria is not yet settled, and from the preparation, characterization, stability study of nanomaterials to the way of nanomaterials entering into the cell, the drug encapsulation rate and the mechanism of action are all facing a huge challenge.

The primary challenge confronted to the drug-loaded nanoparticles is how to deliver drugs into host cells for the treatment of intracellular infections such as; MRSA, *Mycobacterium tuberculosis*, and *Salmonella*. Initially, the drug-loaded nanoparticles may need to pass through the epithelial cells of the gastrointestinal tract infection, the stratum corneum of the skin infection, or the mucous membrane of the respiratory tract infection to reach the targeted bacteria (Kamaruzzaman *et al*., 2017). Nanoparticles containing antibacterial drugs need to enter into the host cell through diffusion, endocytosis, binding to cell-specific receptors, cell membrane depolarization, etc., to release the drug and to exert the antibacterial effects. Despite this, only a small part of antibacterial drugs can reach the target.

The second challenge faced by the drug-loaded nanoparticles is how to resolve a sequence of difficulties in the preparation of nanomaterials, as well as their stability, targeting, *etc*. Most antimicrobial nanocomposites are highly toxic, need to be a complicated modification, and have low encapsulation rate, production process defects and other problems. For example, metal nanoparticles and liposome nanoparticles are cytotoxic (Changsan *et al*., 2009). PLGA synthesis is difficult in the early stage. SLN has a low drug loading and gelation. The liposome-encapsulated nucleic acid nanogels have a low drug loading, poor retention and delayed release of hydrophilic substances (Obuobi *et al*., 2020). The antibacterial effect of chitosan is affected by the pH value of the solution (Li *et al*., 2016).

At present, the development of an efficient, targeted and degradable nano-drug delivery system for treating intracellular infection has become a hotspot. After bacterial infection, pathogenic factors, temperature and pH change in the micro-environment. Responsive polymer materials are designed to improve the efficacy of drugs on intracellular infectious diseases. The drug delivery system for intracellular infection has become a research hotspot. Nanoparticle encapsulation antimicrobial drugs with photothermal therapy or photodynamic therapy have also been helpful in the treatment of intracellular bacterial infections. In the future, reducing the toxic effects of nanoparticles and improving the stability (Mba and Nweze, 2021b) *in vivo* will become an important direction for research on the treatment of intracellular bacterial infections by nanoparticles.

DISCUSSION

The irrational use of antibiotics is considered one of the most important causes for the development of bacterial resistance against antibiotics. Currently, the resistance against antibiotics has become one of the leading threats to public health (WHO, 2014). Presently the nanometer systems deliver a novel perspective for the inhibition of drug-resistant bacteria. Nanomaterials are mainly divided into organic nanomaterials (Polymer micelles, liposomes) and inorganic nanomaterials (metals and metal oxides) (Khorsandi *et al*., 2021), while the inorganic nanomaterials have higher stability and wider applications. Nanoparticles show a high efficacy and treatment index against bacteria either to be used alone or in a combination with antibiotics (Mba and Nweze, 2021a). This era of technology showed a great contribution to solving the current problem of multi-drug resistant bacteria and superbugs that are threatening public health globally. For different intracellular bacteria, there are different nanomaterials coated with corresponding antibiotic therapy. The current study tries to introduce the strategies of treating an intracellular bacterial infection with various nanomaterials, such as metal nanoparticles (MNPs), AgNP/gelatin nanocomposites, PLGA, SLN, liposome-encapsulated nucleic acid nanogels, and nanogels encapsulated with the corresponding antibiotics for the treatment of *Staph. aureus* infection, liposome-encapsulated rifampicin for the treatment of *Mycobacterium bovis*, gentamicin encapsulated with polymer micelles for treating *Brucella* infection, Clofazimine encapsulated with nano-suspension for the treatment of non-tuberculous *Mycobacterium* infection, PBCA was encapsulated with moxifloxacin for the treatment of *Mycobacterium tuberculosis* infection. Additionally, there are many examples of nanomaterials that combine to produce better

antimicrobial effects. Though antibacterial nanomaterials show excellent biocompatibility, they are toxic to humans and animal health. Therefore, it is important to optimize the dosage regimen and to monitor the pharmacokinetic parameter. Nanoparticles have the potential to reduce toxicity by changing their size and structure to become biocompatible materials (Hong *et al*., 2019).

Presently, the mechanism of action of nanoparticles on intracellular bacteria has not yet been studied. Further studies should be carried out on the mechanism of antibacterial activity and to establish the specific nano-systems against different intracellular bacteria. Besides that it is also necessary to study the resistance mechanism of intracellular bacteria to better construct the nano-system to solve the problem of bacterial resistance from different perspectives. Moreover, the antibacterial effect can be enhanced by the combination of drug use, nanoparticle encapsulation, or multiple combinations of nanoparticle encapsulation.

Photodynamic antibacterial nanoparticles are the most popular subject of investigation by experts at present. It can respond to the microenvironment *in vivo*, leading to non-invasive treatment and helping to reduce bacterial resistance. Nanomaterials can not only attain the delivery of antibacterial drugs but can also effectively control the number and frequency of the delivered drugs (Abed and Couvreur, 2014). Briefly, nanomaterials are most promising drug for the treatment of intracellular bacteria.

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Statement of conflict of interests

The authors declare no competing financial interests.

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