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Review Article

APPLICATION OF NANOSTRUCTURES IN ANTIMICROBIAL THERAPY

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ABSTRACT

There are many infectious diseases that may be biofilm mediated, medical device-mediated or from some other agent, are now becoming life-threatening. Despite of availability of many antimicrobial agents, new drugs or therapeutics, these infections have continued to be a global health challenge. Nowadays, conventional antimicrobial agents have failed against many infections due to the emergence of multiple drug-resistant strains. Even, if there is a therapeutic efficacy of these drugs, there inappropriate amounts are resulting in an adequate therapeutic index, local and systematic side effects, including irritation, reduction in gut flora and other manifestations. To overcome such situations, nanostructures have exclusive physicochemical properties as they are ultra small, their size can be controlled, greater surface area to mass ratio, high reactivity and functionalizable structure. Encapsulation of antimicrobial drugs in these nanoparticle systems helps in reducing many side effects. It also helps in the sustained release of drug for a larger time period. Several metal and metal oxide nanoparticles such as silver, gold, zinc, etc. have shown a promising antimicrobial activity. Liposomes, polymeric nanoparticles, dendrimers, and solid lipid nanoparticles have achieved great success as efficient antimicrobial drug delivery systems. These nanoparticles use multiple biological pathways to exert their antimicrobial mechanism such as cell wall disruption, inhibition of RNA synthesis, protein synthesis, etc. Moreover,these preparations of nanoparticles are more cost-effective than that of antibiotic synthesis with lesser or no side effects.

Keywords: Infectious diseases, Antimicrobial resistant, Nanoparticles, *Liposome*s, Polymeric nanoparticles, Dendrimers, and solid lipid nanoparticles

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INTRODUCTION

The present review is focused on various classes of nanoparticles, along with their mechanism of action and various nanostructured delivery systems. For the same literature on nanotechnology and nanoparticles of last twenty years were studied. The various literature is studied for, how bacteria start developing resistance against traditionally used antibiotics and what are the various nanoparticles used as treating bacterial infection, to write this review. The present study also includes various drug delivery systems are used to deliver the drugs and flashed the light on the usage of nanoparticles as a potent antimicrobial agent. History of existence of microorganism dates back to more than 3.8 billion years. For ages, they have survived with greatest genetic and metabolic diversity. Some of them are causing many infectious diseases, whereas others are contributing in maintaining environmental factors. These bacteria usually live in microbial communities that are composed of various species. Microorganisms invade the epithelial surface and spread throughout the body via the circulatory system. The cells of the immune system like macrophages, phagocytes these invading microorganisms resulting in formation of phagolysosomes. Thereafter, by utilizing oxygen dependent or oxygen independent bacterial killing pathways these microorganisms are killed. But, some of them evade the macrophage digestion and thereby resulting in intracellular infection and thus in diseases [1]. For such microorganisms antimicrobial drugs are the answer that kills or inhibit the growth of invading microorganism. Use of antimicrobial drugs dates back to 1928, when Alexander Fleming began the antimicrobial drug era with Penicillium [2]. Afterward, many other antimicrobial drugs like chloramphenicol, streptomycin and many others came into light. Antimicrobial kills the microorganism by binding with the some of the important components required for their metabolism and hence inhibiting their synthesis of functional biomolecules. Like, penicillin and cephalosporins inhibit the bacterial cell wall synthesis whereby the tetracycline inhibits protein synthesis.

But with the advent of biofilm, conditions have become worse. Biofilm-associated infectious diseases have been estimated as one of the major causes of many microbial infections in the body, resulting in morbidity and mortality. Biofilms are actually the agglomerates of microorganisms that affix themselves to the surfaces by secreting various binding molecules like adhesion proteins. After settling down on the surface, they start proliferating and forms colonies inside the peptidiglycan envelop, ultimately resulting in the formation of mature biofilm. At this mature stage, it becomes difficult for even the body's immune system to recognize them, as a result, it changes to chronic infection. Available antibiotics are not sufficient to fight back with these biofilms. Moreover, many microorganisms have become resistant to available antibiotics [3].

The concept of evolution by natural selection is one of the major grounds behind this. As all the living organisms are continually adjusting to cope better with the environment. Microbes are no exception to this phenomenon. They are defending themselves against these antimicrobials by diverse mechanisms. They may alter the drug target, inactivate the enzyme, inhibit efflux transport or develop alternate metabolic pathways for their growth. There are more than 70% infections causing bacteria that have developed resistance for one of the commonly used drug for their treatment. Now, it has been established fact that continuous usage of these antimicrobials is the most important factor that has brought evolutionary changes in these microorganisms, that appears in the form of resistance against microorganism [4]. In addition to this there are certain other factors which include;

a) Hospitalization for a longer period of time

b) Usage of suboptimal dosage of particular antibiotics

c) Increased rate of usage of enveloping devices and catheters

d) Usage of antibiotics in other fields like that of agriculture and household.

Mechanism of antimicrobial agents

For the better understanding of resistance against antimicrobial agents and to find out the solution of this resistance, it's necessary to understand the action of antimicrobial agents. Broadly antimicrobial agents are either bacteriostatic or bactericidal [5]. Bacteriostatic agents inhibits or stops the further proliferation and growth of invading microorganism and also takes a help of the host immune system for the killing of invading microorganism. While bactericidal agents with or without the support of the host immune system kills the invaded microorganism [6]. These antimicrobial agents actually

affect the metabolism of invading microorganism by the any of the following methods

a) They inhibit nucleic acid synthesis

b) They inhibit cell membrane function

c) They inhibit cell wall synthesis

d) They inhibit ribose synthesis

Various antibiotics follow various paths for their antimicrobial activity. The mechanism of penicillin is bactericidal in which they inhibit the cell wall synthesis, tetracyclines are bacteriostatic in nature and inhibits the protein synthesis, carbapenems are bactericidal in nature and inhibits the cell wall synthesis; quinolones are bactericidal and inhibits DNA synthesis; sulfonamides and macrolides are bacteriostatic and follows competitive inhibition and inhibition of protein synthesis [7].

Resistance against antimicrobial agents

Before 1990's no case of antibiotic resistance was observed much, but gradually their number started increasing. Microorganism was becoming resistant to ensure their survival and they achieved it by developing many mechanisms based on the chemical structure of antimicrobial agents. Their mechanism includes:

a) Reduction in uptake of antimicrobial agent

b) New system for efflux of antimicrobial agent

c) Overexpression of the target for the antimicrobial agent

d) Secretion of alternative enzyme rather than the enzyme for which antimicrobial drug was targeted

e) Through mutation changes in the binding site of the antimicrobial agent which reduces the binding of antimicrobial agent with that of the microorganism.

There are various categories of these antibiotics and microorganism develops various mechanisms for different antibiotics.

Resistance for β-lactum antibiotics

β-lactum antibiotics includes penicillins, cephalosporins, carbapenems and cephamycin. In these antibiotics, β-lactum ring plays an important role which inactivates the set of transpeptidases required for peptidoglycan synthesis in bacteria [8]. With the passage of time microorganism developed resistance and develop mechanism either by an alteration in drug target enzyme or alteration in permeability, so that drug cannot reach the target [9].

Glycopeptide resistance

These comprise of vancomycin and teicoplanin. Their antimicrobial activity is due to binding of D-alanyl-D-alanine side chain of peptidoglycan and thus inhibits the synthesis. Now, Microorganisms has developed a mechanism through which they started secreting D-ala-D-ala ligase resulting in the rebuilding of peptidoglycan side chain to express D-alanyl-D-lactate type that has less affinity towards the glycopeptide drugs [10].

Tetracycline resistance

Tetracycline is another important class of antibiotics that are commonly used against a wide range of Gram negative and Gram positive bacteria, and another class included is mycoplasmas, chlamydiae, rickettsiae, and protozoan parasites. There common use is for both of humans and in veterinary are attributed to their easy availability, low cost, low toxicity and effectiveness against wide spectrum. These were discovered in 1940's, to exert their effect against microorganism, they prevent the attachment of aminoacyl-tRNA to the ribosomal acceptor (A) site and thereby inhibiting the protein synthesis. Most commonly used drugs of this class include tetracycline, doxycycline, minocycline [11]. For this particular antibiotic, they utilize the export protein, which is from the major facilitator superfamily. These proteins are membrane-associated and are coded by tet efflux genes. These genes transport the tetracycline from the cell. As a result, there is a reduction in the intracellular drug concentration due to which the ribosomes are protected within the cell. Theses tetracycline efflux proteins have similar protein and amino acid structure like that of other efflux proteins that are involved in multi-drug resistance quaternary ammonium resistance, and chloramphenicol and quinolone resistance [12].

Chloramphenicol resistance

Chloramphenicol is an antibiotic that also attacks on the ribosomal unit of microorganism. It binds itself to the 50s ribosomal unit and inhibits the peptidyl transferase of protein synthesis. Microorganism develops resistance against this antibiotic by developing such a mechanism that inhibits the binding of antibiotic with its ribosomal unit. It inhibits the activity of antibiotic by the release of enzyme chloramphenicol acetyltransferase. In Gram negative bacteria and in gram a positive bacterium this enzyme is released with the support of cat gene. Sometimes the resistance is developed due to the active drug efflux system or may be decreased outer membrane permeability [13].

Aminoglycoside resistance

Aminoglycosides are a class of antibiotics that are distinguished by the presence of an aminocyclitol ring which is linked to the amino sugars in their structure. They have potency against a broad range of bacteria. This class includes streptomycin, kanamycin, gentamycin, tobramycin, and amikacin that are commonly used against the infection caused by many gram positive and gram negative bacteria. The antimicrobial potential of this drug is due to the irreversible binding of the drug with the ribosome, this blocks the protein synthesis in the bacteria. To resist against this antibiotic bacteria has developed such a mechanism that they modify aminoglycoside by generating more than 50 aminoglycoside-modifying enzymes [14]. There are many modifying enzymes and depending upon their type of modification they are classified as: aminoglycoside nucleotidyltransferases, aminoglycoside phosphotransferases and aminoglycoside acetyltransferases. The aminoglycoside acetyltransferases induce modification of aminoglycoside at the amino group, while aminoglycoside phosphotransferases and aminoglycoside nucleotidyltransferases induces modification at a hydroxyl group of aminoglycoside [15]. Due to these modifications aminoglycoside losses, its ability to bind with the ribosome and hence could not modify the protein synthesis of bacteria. In addition to this, bacteria follows efflux mechanism and rRNA mutations [16].

Quinolone resistance

Quinolone is another class of antibiotics that was discovered in 1962 during the synthesis of chloroquine and antimalarial drug. During that time its nalidixic salt was discovered. Thereafter its many derivatives were introduced in the market against many bacterial cells. Of all the salts discovered fluoroquinolones are the most important one with the subsititution of fluorine atom at 6 position of quinolone molecule. By the substitution of fluorine atom at 6 position the potential of quinolone was enhanced to a greater extent. These agents exert their effect by inhibition of certain important enzymes that are required in DNA synthesis. These agents attack bacterial topoisomerase enzymes viz., DNA topoisomerase IV and DNA topoisomerase II (DNA gyrase). After being altered by the drug theses enzymes alter the topology of double-stranded DNA, thereby inhibiting its synthesis. To overcome this drug attack bacterial generation has developed resistance against this drug by one of the mechanism: they either develop a mechanism for alteration in drug target enzyme or they alter the permeability mechanism for the drug [17]. In Gram negative bacteria topoisomerase-II is the target enzyme for the drug (quinolones) while in gram positive bacteria it is topoisomerase-IV. Whatever were the targets, but the bacteria followed changes in the amino acid sequence by substitution within the quinolone resistance-determining region where, they replaced the hydroxyl group with bulky hydrophobic residue. This conformational change has brought a change in the binding site of the drug. As a result, drug was unable to bind with the specific enzymes and thus was not able to kill the bacteria [18].

Macrolide, lincosamide, and streptogramin (MLS)

Macrolide, lincosamide, and streptogramin are special classes that inhibit the bacterial protein synthesis. In gram, negative bacteria

mechanism for resistance against this class includes low permeability of the cell membrane towards these hydrophobic compounds. But the Gram positive bacteria have developed various mechanisms that are as follows:

- By utilizing adenine-N6-methyltransferase they induce a post-transcriptional modification in 23 S rRNA that alters a binding site of MSLB antibiotics.
- An activated drug efflux mechanism that pumps out the drug, and thus there is low concentration inside the cell which saves the ribosome.
- Release of hydrolytic enzymes that modify the antibiotic by the addition of acetyl group.

Sulfonamides and trimethoprim resistance

Sulfonamides are bacteriostatic and to inhibit the bacterial growth they follow the competitive inhibition pathway. Gram negative bacteria have developed resistance for sulfonamides by the attainment of either of the two gene sul1 and sul2 that secrets dihydropteroate synthase enzyme, which is not repressed by the drug [19]. Trimethoprim also follows the pathway of competitive inhibition. Enzyme dihydrofolate reductase is required in the bacterial metabolism for the synthesis of amino acid and nucleotides. Trimethoprim is an analogue to dihydrofolic acid and with the help of dihydrofolate reductase, it helps in the synthesis of amino acid and nucleotides. This drug actually binds with the enzyme in a competitive manner and inhibits the normal metabolism of the bacterial cell. To sustain bacterial population now evolved a mechanism that deactivates this drug by any one of the mechanism; they started overproduction of the dihydrofolate reductase enzyme; adopted mutation in the dihydrofolate reductase enzyme gene for the attainment of another gene (dfr) that encodes resistant dihydrofolate reductase enzyme gene. So far 15 dihydrofolate reductase enzyme type has been recognized [20].

Multidrug resistance

With the passage of time and in the urge to survive these organisms has developed multidrug resistance, which has become a gigantic challenge in infectious disease management. It is believed that this multidrug resistance is mediated by the genetic mobile element like integrons, transposons and plasmids [21]. These organisms most commonly are resistant to chloramphenicol, penicillin, erythromycin, tetracycline, trimethoprim and tetracycline.

One of the most resistant mutants is NDM-1, first came in to view in New Delhi and now has spread worldwide. NDM-1 is a New Delhi metallo beta-lactammase enzyme produced by *Klebsiella* that confers bacterial multiple drug resistance. It was identified in a patient that is not responding to any antibiotic [23]. These microbes replicate so fast that if the very small number of them become resistant, the resistant strain replicates, increase their number and spread throughout the population. One of the major limitations in using higher doses of antimicrobials is that they cause toxicity like ototoxicity or nephrotoxicity to healthy tissues.

These days due to lack of other alternatives most of the biofilm-mediated infections are treated with antibiotics only. Although attaching mature biofilm with these antibiotics does not work and much higher dosage is required. Because these drugs cannot penetrate the extracellular polysaccharide sheath which covers the biofilm. Theses biofilm-associated bacteria's are less susceptible to the antibiotics than that of the planktonic bacteria [24]. Moreover; it is not possible for the host to tolerate very high dosage of available antimicrobials [25].

Nanotechnology has brought some hopes to act as new alternative antimicrobial agent. Nanostructures have large surface area relative to their size and provide high activity, even at a very low dose. The mode of action of the nanoparticle is mainly by direct contact with bacterial cell wall and it does not need to penetrate the cells. This brings the hope that nanoparticles would be less prone to resistance like that of antibiotics.

Nanoparticles over conventional drugs

Over the last few decades, in the search of new antimicrobials application of nanotechnology has shown promising applications to overcome the problem of antibiotic resistance [26, 27].

Nanostructures are very small (nanoscale) in size, and the structure is formulated in such a way that there are a large number of atoms at the surface level relative to the total number of atoms. This factor actually imparts unique characteristics to the nanostructures, their surface to volume ratio becomes large and their electronic energy states become discrete, and this leads to exceptional electronic, optical, magnetic and mechanical properties. Antimicrobial nanostructures are usually made from the organic and inorganic materials and thus are classified as organic nanoparticles and inorganic nanoparticles. After bringing the organic and inorganic material to nanoscale their property changes to a greater extent, their easily operatable (as per the requirement) crystallographic surface structure and a large surface to volume ratio makes them attractive tool to solve the various problems. Due to their small size like that of other biological molecules and structures, they are extensively explored in the field of medicine. In medicine, they are mainly utilized in targeted drug delivery, imaging, sensing, artificial implants and as antimicrobials. The drug is entrapped, encapsulated, dissolved or attached to the matrix. Depending upon the preparation method nanospheres, nanoparticles or nanocapsules are formed.

Physical encapsulation, adsorption or chemical conjugation is the techniques used for loading of drugs in the nanoparticles. By utilizing drug loaded Nanoparticles pharmacokinetics and therapeutic index of the drugs can be improved significantly.

In nanocapsules, the drug is confined to the cavity surrounded by a unique polymer membrane. Nanosphere themselves is a matrix system in which drug is physically and uniformly dispersed.

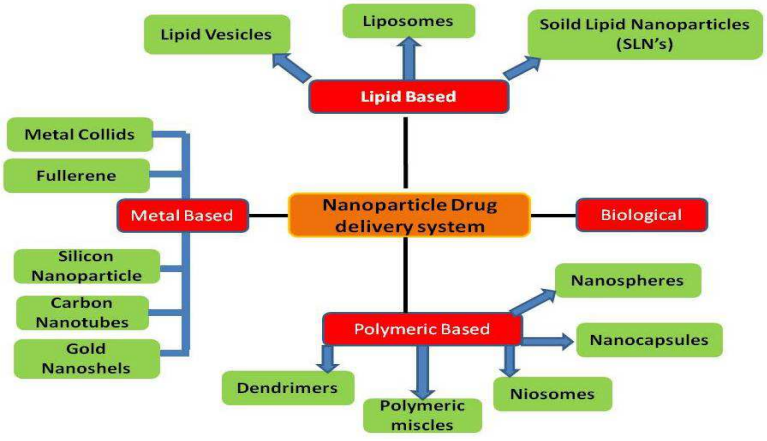

Fig. 1: Various types of drug delivery systems based upon the type of matrix used [22]

Inorganic and organic nanoparticles

Inorganic nanoparticles

There are many metals or metal oxides that have been widely studied for their antimicrobial potential. It includes silver, gold, titanium oxide, zinc oxide, etc., it is believed that most of the metal oxide nanoparticles exert an antimicrobial effect by following the mechanism of reactive oxygen species generation; some are effective by generations of their ions or sometimes due to their physical structure [28].

Silver nanoparticles

Silver nanoparticles have been widely used for various micro-organisms, due to the potency of silver particle being themselves as antimicrobial agents. Before the antibiotic era people used to take this metal to treat burns, open wounds and various infectious diseases. Its traditional use was clogged after the advent of antibiotics, but now again it has acquired the popularity to be used as silver nanoparticles. There are many mechanisms proposed for the efficacy of silver nanoparticles as an antimicrobial agent. It is implicated that silver has a high affinity for sulphur and phosphorous, due to which they exert adverse effects on bacteria. There is a sulphur containing proteins on the bacterial cell membrane, silver nanoparticles reacts with sulfur-containing amino acids and thus affects the cell viability.

Similarly, silver ions released from these nanoparticles get themselves attached to phosphorous moieties into DNA and inactivates DNA replication, resulting in the death of microbial population [28, 29]. It is also suggested that attachment of silver nanoparticles to sulfur-containing proteins of cell membrane increases cell permeability, leading to the death of bacterial population [30]. The concentration of Ag+released from these nanoparticles shows various effects. At the lower concentration, these ions (Ag+) uncouple respiratory electron transport from oxidative phosphorylation, thus inhibiting respiratory chain enzymes or disturb the membrane permeability [31]. Whereas, at higher concentration, it interacts with cytoplasmic components and nucleic acid resulting in their damage and thus cell death [32]. It has also been postulated that their small size less than 10 nm makes pores in the cell wall, releasing the cytoplasmic content into the medium, which leads to cell death [34]. Later, to enhance the bioactivity composites of these silver nanoparticles with various polymers was introduced [33-35]. Of all other polymers, chitosan (cationic polysaccharide) gained the maximum success, it is composed of (1,4)-linked 2-amino-2-deoxy-β-D-glucose units. The composite was made by directly attaching the silver particles to glucose units, and it has shown more enhanced antimicrobial efficacy than its individual components. Polycationic chitosan binds to the negatively charged cell membrane, which decreases the osmotic stability of cell membrane, simultaneous silver particles create pores in the membrane, causing rapid disintegration of cell wall [36]. It has been found that silver nanoparticles showed promising results against many resistant bacteria. Methicillin-resistant Staphylococcus aureus showed the maximum sensitivity for silver nanoparticles followed by Methicillin-resistant Staphylococcus epidermidis and Streptococcus [37]. These particles were found to be effective against Salmonella typhi and Klebsiella pneumonia [38]. In addition to concentration, various shapes also affected the antimicrobial activity. As it has been found that triangular silver nanoparticles were more effective against E. coli than that of spherical or rod-shaped [39].

Gold nanoparticles

The use of gold particles in rejuvenation has been very well documented in the Indian Ayrvedic system (Swarna Bhasam), in Chinese medical history and also in the western world (nervine). Its first bacteriostatic effect was studied by Robert Koch in 1920 against *M. tuberculosis* [40]. Gold nanoparticles are biologically inert but can be engineered to possess chemical or photothermal functionality. They can be made active on near infrared radiation. At this range, they can annihilate bacterial cells by photothermal heating. By utilizing photothermal radiation in combination with photo-sensitizers they have been found to be effective against Methicillin-resistant *Staphylococcus aureus*. Toluidine blue O which is a photosensitizer has been conjugated on the surface of Gold nanorods, working as both photodynamic and photothermal agent and utilized for photodynamic antimicrobial chemotherapy [41]. This combination actually inactivates Methicillin-resistant *Staphylococcus aureus*. Recently, these light-absorbing nanorods conjugated with specific antibodies and found to be effective against *Staphylococcus aureus* by using laser [42]. Theses gold nanoparticles later made conjugated with antibiotics like vancomycin, which killed vancomycin-resistant *enterococci* [43]. Likewise, cefaclor (antibiotic) in combination with gold nanoparticles have strong antimicrobial activity on both Gram-positive (*S. aureus*) and Gram-negative bacteria (*E. coli*) compared to cefaclor and gold nanoparticles alone. Theses antibiotics actually inhibit the formation of peptidoglycan layer and simultaneously gold particles make the cell wall porous and generate holes in the cell wall which leads to leakage of cellular component and thus, cell death. Gold particles also found to get bind with DNA, inhibiting its transcription [44].

Zinc oxide nanoparticles

Zinc oxide nanoparticles are found to be highly toxic to pathogenic strain while the minimal effect on human cells [45]. They have the ability to sustain the harsh conditions, which favours their application as a potent antimicrobial agent [46]. Furthermore, their potential to act as an antimicrobial agent depends upon concentration and particle size. They are also found to completely lyse the foodborne bacteria *Salmonella typhimurium* and *Staphylococcus aureus* [47].

They work by disintegrating the cell membrane and increases membrane permeability resulting in leakage of cellular components and leads to death. They can be used against food-related bacteria *Bacillus subtilis, Escherichia coli* and *Pseudomonas fluorescens*, *Listeria monocytogenes, Salmonella enteritidis* [48]. Another mechanism that zinc nanoparticles follows is the generation of hydrogen peroxide from the surface of zinc oxide nanoparticles, which due to oxidative stress inhibits the bacterial growth [49]. Also, the release of Zn2+ions damages the cell membrane and interacts with the intracellular contents and thereby disturbing their functionality [46]. In addition to this, they have a white color, UV blocking ability and also prevent biofilm formation which makes them more suitable to be used as a coating material designed for the medical field [50].

Titanium dioxide nanoparticles

These nanoparticles showed their activity due to the photocatalytic generation of strong oxidizing power when illuminated with UV light. Titanium dioxide nanoparticles are first illuminated with UV light, Photoexcitation TiO_2 generates active hydroxyl radicals, which catalyze the bacterial killing [51, 52]. These nanoparticles are found to be effective on *E. coli* biofilm, against bacteria and fungi. These are also helpful in disinfecting contaminated surfaces and can be proven to be helpful for preventing biofilm formation. Their mechanism mainly based upon the generation of reactive oxygen species.

Magnesium oxide nanoparticles

Magnesium oxide nanoparticles are highly ionic with the greater surface area and crystal morphology, providing it numerous surface reactive sites. Aerogel magnesium oxide nanoparticles (prepared by aerogel procedure) are square and polyhedral in shape with a diameter of 4 nm. These have the unique property that they can adsorb a significant amount of chlorine and bromine and can retain them for a longer time period, as a result, can work as potent disinfectant [53]. These nanoparticles have greater surface area and enhanced surface reactivity, due to which they can adsorb higher amount of halogen. As their size is also very small so they can cover a larger number of bacteria and can bring halogen in greater proximity to the cells [54].

These types of nanoparticles have shown excellent activity against *E. coli¸ Bacillus megaterium* and *Bacillus subtilis*. Halogenated magnesium oxide nanoparticles show positive charge in water suspension, which is opposite to that on the bacterial and spore cells. As a result, in water suspension due to opposite charges these two makes aggregates composed of AP-MgO nanoparticles and bacteria. Thereafter they affect the cell wall and leads to the death of the microorganism [55].

Copper oxide nanoparticles

Copper oxide is a semiconducting compound having superconductivity, spin dynamics and electron correlation effects. Its functionalities like photocatalytic or photovoltaic properties and photoconductive makes it more accessible for application as an antimicrobial agent [56]. Copper oxide nanoparticles are found to be more effective against bacterial pathogens involved in hospital-acquired infections [57]. Copper ion release from copper oxide nanoparticles has a great affinity towards the amines and carboxyl groups that are abundantly present on the surface of *B. subtilis*, due to which it has been found to be quite effective against *B. subtilis* Moreover, the copper ion released interacts with bacterial DNA and intercalate with the nucleic acid. These copper ions disrupt the biochemical process and thus help in the killing of bacteria [58].

Aluminium nanoparticles

Aluminium oxide nanoparticles are found to be effective at higher concentrations. At a near neutral pH alumina nanoparticles possess a positive charge on its surface. Whereas, the surface of *E. coli* cells are negatively charged. Due to this, there is an electrostatic interaction between the negatively charged *E. coli* cells and nanoparticles, resulting in adhesion.

As the concentration increases, adhesion also increases. But alumina nanoparticles are free radical scavengers, so here the bacterial killing is due to the structure of the particle rather than reactive oxygen species [59].

Nitric oxide

The antibacterial potential of the Nitric oxide nanoparticle is dependent upon the size and shape. Being very reactive its clinical use is limited. Its mechanism to act on microorganism is through the release of reactive nitrogen species. It has shown promising result against methicillin-resistant *S. aureus* [61].

In table 1 many metal and metal oxide has been briefly described.

Table 1: Effective metal and metal oxides as nanoparticles

Metal/Metal oxide	Effective against	Proposed mechanism	Reference
Silver	*Pseudomonas aeruginosa* Ampicillin resistant *E. coli* Erythromycin-resistant *Streptococcus pyogenes*	Shows bacteriostatic mechanism by -Inhibits cell wall synthesis -Inhibits Protein and Nucleic acid synthesis	18
Gold	*E. coli* *Staphylococcus aureus*	Inhibition of peptidoglycan layer and generation of pores in the cell wall resulting in leakage of intracellular material and thus cell death	19
Zinc oxide	*Enterotoxigenic E. coli* *Botrytis cinerea* *Penicillium expansum*	Causes deformation in fungal hyphae and thus inhibits the growth Prevent development of Conidiophores and conidia leading to the death of fungal hyphae Disruption of cell membrane, lipids and proteins, leading to leakage of the intracellular component and thus death	20
Aluminium oxide	*E. coli*	At higher concentration disrupts cell wall	21
Iron oxide	*S. aureus*	Generates reactive oxygen species and thereby inducing lipid peroxidation and DNA damage	22

Organic nanoparticles

These are polymeric nanoparticles having polycationic property and believe to kill microorganism by either of the mechanism, in some cases, they release antibiotics or another antimicrobial agent, or by contact killing through cationic surfaces which includes quaternary ammonium compounds, alkyl pyridiniums, or quaternary phosphonium. Largely it has been stated that theses cationic groups are able to disrupt the bacterial cell membrane, while some utilize hydrophobic chains of a certain length with which they penetrate inside the bacterial wall and burst the bacterial membrane. As they have sufficient amount of positive charge so by an ion exchange mechanism between the bacterial membrane and charged surface, they have shown the efficient potential against many micro-organisms. To kill the bacteria they mainly follow the contact killing mechanism where theses polycationic entities possess multiple charges and get attached to the bacterial membrane [62]. Organic nanoparticles are less stable at a higher temperature, and cannot withstand the harsh conditions, due to which they are less preferable as compared to inorganic nanoparticles.

Quaternary ammonium compounds

Most commonly used quaternary ammonium chloride stearalkonium chloride, and cetrimonium chloride that acts as a disinfectant and their antimicrobial property is due to N-alkyl chain length. The interaction between positively charged moieties of the compound interacts electrostatically with the negatively charged bacterial membrane, thereby long hydrophobic tail of the compound integrates with hydrophobic membrane core where, they denature proteins and enzymes of the bacteria, resulting in the disruption of their mechanism and hence the death of the bacteria. Compound with a chain length of 12-14 alkyl groups has shown moderate activity against gram negative bacterial, whereas alkyl groups with a chain length of the carbon chains showed good activity against gram negative bacteria [63]. Quaternary pyridiniums compound has a heterocyclic ring having a nitrogen atom, and they have pyridinium group in the polymer chain which is responsible for the antimicrobial activity.

Polyethyleneimine is a synthetic nonbiodegradable, cationic polymer that has a primary, secondary, and tertiary amino functions. It has been made attached to many organic and inorganic, natural and synthetic, macroscopic and nanoscaled, monolithic and porous surface material. They have been found to be active against waterborne and airborne bacteria and fungi. These have been found to be effective against pathogenic and antibiotic-resistant strains without any report of the emergence of resistance. They basically rupture the cell membrane and results in the death of the microorganism. They are even friendly to mammalian cells. Imidazole derivatives have also been found to be a very effective antimicrobial polymer. They have the ability to form a hydrogen bond with drugs and proteins while its alkylated form known as imidazolium has the ability to aggregate electrostatically. They are quite stable and biocompatible to mammalian cells [64].

Organometallic polymers

Organometallic polymers have metals either in the backbone or present in the pendant group. Organotin polyamine ethers are one such derived group that has cyclovir in their backbone. They have shown promising results against herpes simplex virus-1 and varicella-zoster virus. They function by inhibiting RND and DNA of viruses [64].

Cationic quaternary polyelectrolytes

Acrylic or methacrylic derivatives are most commonly known as cationic quaternary polyelectrolytes. Their main mechanism for antimicrobial activity involves the membrane disruption of bacteria. These compounds provide wide flexibility by the alteration of hydrophobicity, molecular weight, surface charge, and other parameters [65].

Triclosan

Triclosan is one of the common antimicrobial agents used in emulsion form against the microorganism. Triclosan mixed with water-based styrene-acrylate emulsion found to be very effective against *Enterococcus faecalis*. The release of triclosan is based upon the solvent system used. Studies have shown that its release is very slow when water alone used as the solvent system while its release speeds up in n-heptane [66]. Triclosan, when incorporated in water-dispersible polyvinyl alcohol nanoparticles, showed greater antibacterial activity against *Corynebacterium* [67].

N-halamine compounds

In N-halamine compounds there is the presence of a reactive free halogen, which is responsible for its antimicrobial property, as with the release of this reactive halogen, the microbial cells get deactivated. Structurally, these compounds contain one or more nitrogen-halogen covalent bonds that are usually formed by halogenation of imide, amide, or amine groups. This provides them stability and also facilitates the slow release of active halogen species into the environment. These oxidizing halogens promote the direct transfer of an active element of the biological target site or through dissociation to free halogen in aqueous media [68].

Polysiloxanes

Polysiloxanes are linear polymers of silicon oxide. Their statistical block type copolymers containing quaternary ammonium salt groups as a lateral substituent found to be effective against *Escherichia coli* and *Staphylococcus aureus* [68]. Upon coming in contact with bacterial membrane they burst the membrane and results in the death of the microorganism.

Peptides

These are synthesized by the ring opening polymerization of amino acid monomer using various hydrophobic and hydrophilic amino acid. Most commonly peptide synthesized by ring opening polymerization of α-amino acid N-carboxy anhydride, which is a monomer using lysine as a hydrophilic amino acid and alanine (A), phenylalanine (F), and leucine (L) as hydrophobic amino acid. By varying the content of hydrophobic from 0-100 % five different kinds of co-peptides were synthesized (i.e., P(KA), P(KL), P(KF), P(KAL), P(KFL)). It was observed that polymer synthesized using phenylalanine showed better antimicrobial activity than that synthesized by leucine or alanine. Theses polymers have shown potential against *Escherichia coli, Pseudomonas aeruginosa, Serratia marcescens, and Candida albicans* [69].

5-Chloro-8-hydroxyquinoline

Another important antimicrobial polymer includes the polymers of acrylate containing 5-chloro-8-hydroxyquinoline. For their hydrolytic behaviour they were studied at physiological, acidic, and basic pH. It was observed that in theses polymers hydrolysis occurs by autocatalysis and is potentiated by pH, temperature and content of hydrophilic polymers. Their mechanism involves the disruption of cell membrane and there, by disturbing the membrane integrity [70].

Quaternary phosphonium or sulfonium groups

These are the polycationic polymers that contain quaternary phosphonium or sulfonium groups. They are effective against the microorganism as they have positively charged moieties which electrostatically interacts with the negatively charged bacterial membrane. Their hydrophobic tail integrates with hydrophobic membrane core, where it damages the proteins, enzymes and other cofactors that are required for normal metabolism and thus kills the bacteria. Due to the presence of phosphonium theses, polycationic polymers are found to be more effective than quaternary ammonium salt polymers. It was well documented that with the increase in the length of alky chain and phosphonium units the antimicrobial potential of the compound was enhanced. Antimicrobial potential of N-isopropylacrylamide and methacryloyloxyethyl trialkyl phosphonium chlorides was increased to a greater extent with the increase in alkyl chain length and phosphonium [71].

Benzoic acid, phenol, and p-hydroxybenzoate esters

Esters of benzoic acid, phenol and p-hydroxy benzoate are most commonly used as disinfectants. In a monomeric form, they are well documented and established antimicrobial agents. For the synthesis of new and more efficient antimicrobial agents, e attempts have been made to incorporate polymeric backbone in their structure. Three components have been incorporated with them viz: p-hydroxyphenyl acrylate, allyl phydroxyphenyl acetate, and p-2-propen oxyphenol, and it was found that p-phydroxyphenyl acrylate showed the maximum antimicrobial potential against both the bacteria and fungi[72]. This activity is attributed due to the presence of phenyl group, which exerts stereoelectronic effect. Compounds with acryl or acryloxy groups bound to the phenyl moiety exhibit superior antimicrobial activities [73]. Benzaldehyde also belongs to this class which is known for its wide range of bactericidal, fungicidal, and algaecidal activities. When this benzaldehyde attached with the polymer of methyl methacrylate, it showed activity against *Bacillus macroides, Pseudomonas aeruginosa* and *Dunaliella tertiolecta* [74].

Polycationic nanoparticles

The polycationic nanoparticle is unique in its ability to kill the microorganisms. Unlike other organic nanoparticles, it induces programmed cell death in the target microorganism. Although, it is still debatable that whether the nanoparticles induces such signals or not, but if it is there it can be very helpful in case of biofilm as antibiotics have poor ability to penetrate inside the biofilm [21]. Yet there are growing evidence in this context.

Polymeric nanosized antimicrobials

Theses nanosized antimicrobial agents are nonvolatile and chemically stable. In addition to long-term antimicrobial activity, being polymeric in nature, they can bind to the surface of interest and also does not penetrate the biological membrane of skin [75]. As these are polycationic in nature so they have a high surface density of active groups which is responsible for their high efficacy against microbes. Quaternary ammonium compounds have been reported to express a broad range of antimicrobial activity against both Gram-positive and Gram-negative bacteria. Polyamines are quaternary ammonium polyethyleneimines that are proved to be highly effective antimicrobial nanoparticles. Theses polyamines after incorporation into various polymeric matrixes showed a wide range of bacterial targets [76]. Lipid nanoparticles also showed the higher extent of the biocompatibility wide range of microbes as a target.

Chitosan

Chitosan is very well known organic compound having wide range of antibacterial, antiviral, and antifungal activity. Its conjugated form with hydroxycinnamic acid showed the enhanced activity than that of chitosan alone [77]. Its widespread application is credited to its characteristics being biocompatible, nontoxic, low immunogenic, higher antibacterial property and capacity to act as an absorption enhancer. These nanoparticles are obtained by N-deacetylation of the N-acetyl glucosamine polymer chitin, which is commonly found in the exoskeleton of insects. Some reports have shown that when it gets attached with metal (Zn) it reduces its activity [66]. So for better activity, it should be preferably bound with the antibiotics [78]. Its mechanism of action is not much clear, but reports have shown that it affects drug efflux system, respiration and respiration nodulation cell division and also the drug transport system. It is believed that it may be due to the interaction of membrane lipopolysaccharide with chitosan. As this interaction, results in destabilization of membrane protein due to which the membrane of the organism lyse and hence leads to death [79].

Antiviral studies on nanoparticles

Much of the research is dedicated to the antibacterial potential of various nanoparticles, but the area of serious viral infection still needs to be explored. The disease caused by a viral infection is equally imperative. There are many viral infections that have affected throughout the world and need attention due to their health, social and economic impact. Targeting the virus and maintaining the host cell viability is a challenging task. Of so many

nanoparticles, metal nanoparticles have been tested for their antiviral potential due to their structure of core material or ligands shell [80]. Silver nanoparticles show a promising result against HIV-1 at non-cytootoxic concentrations. There are a number of *in vitro* assays that have demonstrated the mechanism of antiviral activity of silver nanoparticles. Silver nanoparticles exert its antiviral activity at an early stage of viral replication. It has been shown that it acts like a virucidal agent or inhibits the viral entry. To act as a virucidal agent in both cell-free virus and cell-associated virus, it inhibits CD4-dependent virion binding, fusion and stops its infection causing ability. Moreover, silver nanoparticles inhibit post-entry stages of the HIV-1 life cycle and thereby acting against a variety of circulating HIV-1 strains [80]. In addition to silver nanoparticles, gold nanoparticles have also exhibited antiviral properties [81]. The *in vitro* studies has shown tat gold nanoparticles that are coated with multiple copies of an amphiphilic sulfate-ended ligand binds itself to the HIV envelop glycoprotein gp120 and inhibits HIV infection of T-cells. Theses nanoparticles actually create a high concentration zone for the binding of glycoprotein gp120, and as a result the virus binds itself to the nanoparticle rather than the biological entity at a nanomolar concentration. To infect, theses viruses follows the adsorption or the fusion pathway and when they get multivalent gold nanoparticles with sulfated ligand they binds themselves to theses nanoparticles. Frequency of binding of gp120 glycoprotein with virus depends upon the ligand density [82].

Gold nanoparticles with various anionic groups exhibited the activity against influenza virus also [83]. These virus binds with the cell surface of the host, gold nanoparticles inhibits the binding of the virus to the host cell surface by blocking the binding sites. This antiviral property of anionic gold nanoparticles is attributed to the charge density and functional groups present 0n the surface of the gold nanoparticle. As these anionic gold functional group interacts with the viruses by multivalent bonds and the small size of the gold nanoparticles facilitate their entry through the endosome vesicle, possibly allowing them to hinder the fusion step of infection also [84]. Most important aspects of gold nanoparticles is this that in addition to the antiviral property they have exhibited no or very low toxicity [85]. Gold nanoparticle, composed of gold core bonded with mercaptoethanesulfonate on its surface through the thiol group exhibited no toxicity to normal cell and also inhibited the various influenza strains including the most recent pandemic swine influenza virus H1N1 strain. This study revealed that the effect of gold nanoparticle is not limited to some particular influenza strain.

Studies have also revealed the potential effect of silver nanoparticles coated with mercaptoethane sulfonate against Herpes simplex virus Type 1 [86]. Theses nanoparticles have sulfonate end groups that compete with viruses for the binding to cellular heparin sulfate. As theses nanoparticles bind with cellular heparin sulphate they block the entry of the virus into the cell and subsequently stop the infection. In addition to antiviral potential against Herpes simplex virus, they did not impart any cytotoxicity to host cells. Due to the heparin sulfate dependence for entry, these can be further used as the topical agent also. There are studies that have shown its effectiveness against HIV-1, monkeypox virus, respiratory syncytial virus and hepatitis [87, 89]. Thus, nanoparticles with a functionalized group ion their surface can be used as antiviral agents.

Antifungal activity of metal nanoparticles

There are several fungal infections that needed to be treated with multiple antibiotics in immune-compromised patients. Although there are a lesser number of studies showing fungistatic and fungicidal effects of nanoparticles but some of them is still explored. Silver nanoparticles have been explored for their fungistatic and fungicidal effects on the specific pathogenic yeasts causing life-threatening fungal infections in the patients that are in intensive care. Antifungal property of silver nanoparticle has been tested and proved to be effective against pathogenic *Candida sp.* [90]. There are reports showing the potential activity of silver nanoparticles against *Trichophyton mentagrophytes*, dermatophytes and *Candida species*. Their antifungal potential is due to its effect on mycelia [91]. Silver nanoparticles targets the cell membrane of yeast cells, it creates pits/pores in its membrane and thereby disturbing its membrane potential. The formation of pores in the membrane subsequently leads it to death [92].

Methods of preparation of Nanoparticles

Nanoparticles can be formulated with a variety of materials including proteins, polysaccharides and synthetic polymers. Three common methods include

a) Dispersion of Preformed polymers

b) Polymerization of Monomers

c) Ionic gelation or Coacervation of hydrophilic polymers

Of these three, dispersion of preformed polymers is most common, and this technique can be used in various ways

a) Solvent evaporation method

b) Spontaneous emulsification or solvent diffusion method

c) Polymerization method

d) Coacervation/Ionic gelation Method

e) Supercritical fluid technology

a) Solvent evaporation method

In this method, polymer is first dissolved in organic solvent. Hydrophobic drug is also dissolved in the same solvent, which is used to dissolve the polymer. Both are then mixed in a solution containing a surfactant or emulsifying agent. As a result, oil in water emulsion is formed. After achieving the stability of this emulsion organic solvent is evaporated (by reducing pressure/continuous stirring). It is then subjected to high-speed homogenization or ultrasonication to achieve small, particle size [93].

b) Spontaneous emulsification/solvent diffusion method

In this technique mixture of water-miscible solvent and a small amount of water-immiscible organic solvent is used. This results in the spontaneous diffusion of solvents and leads to interfacial turbulence between two phases. This results in the formation of small particles [94].

c) Polymerization method

For the formation of nanoparticles, monomers are polymerized in aqueous solution. The drug is incorporated either by dissolving in polymerization medium or by adsorption onto the nanoparticles. Nanoparticle suspension is then subjected to ultracentrifugation to remove surfactants or stabilizers. After the completion of ultracentrifugation, particles are re-suspended in the isotonic surfactant free medium [95].

d) Coacervation/ionic gelation

In this method, biodegradable hydrophilic polymer like chitosan, gelatin or sodium alginate is used. Two aqueous phases one is polymer chitosan and another is polyanion sodium tripolyphosphate) is taken and mix well. Positively charged chitosan interacts with negatively charged triphosphate. Due to the electrostatic interaction between two phases, they got bound top each other, forming coacervates with a size in nanometers [96-97].

e) Supercritical fluid technology

This method is used to overcome the hazardous effect of organic solvent as it is harmful to the environment as well as to physiological systems. Supercritical fluids are those solvents that remain in their fluid state above its critical temperature regardless of pressure (Most widely used supercritical fluid is CO_2). But this technique is more expensive and can be achieved by Supercritical anti-solvent or rapid expansion of critical system method in special equipment [98].

Nanoparticles for antimicrobial drug delivery

With the passage of time and due to the limitations of conventional dosage or delivery system, studies have been conducted to find out

the alternatives. Most important aspects of drug delivery involve that it should possess

a) Controlled drug delivery

b) Targeted drug delivery

The nanoparticle has come into sight as an effective and potential drug delivery system. Drugs are effective only at a particular concentration. As discussed earlier in the section that nanoparticles have such surface properties that can help in controlled and targeted drug release. Due to their ultra-small and controllable size, they can easily penetrate body cells and can function well. Nanoparticle-mediated drug delivery offers many advantages over the conventional therapy that can be summarized as

a) Enhanced therapeutic efficacy of drugs due to the controlled and sustained release of drug at the site of action.

b) Better bioavailability and the right proportion of drug at the targeted site.

c) The drug can be incorporated in the system without any chemical reaction.

d) Multiple drugs can be delivered to the same cell for combined synergistic therapy.

e) Drug release and degradation profile can be modified, simply by modulating the size of the nanoparticle.

Lipid-based

Liposomes are spherical phospholipid bilayer vesicles with a structure consisting of amphiphilic lipid molecules shown in fig. 2.

They possess the efficient properties like lipid polymorphisms, lipid, protein and lipid drug interaction. In 1995, Doxil (Doxorubicin liposome) became the first liposomal drug delivery system to treat AIDS-associated Kaposi's sarcoma after the approval of food and drug administration [98-99]. Most commonly used lipid is phosphatidylcholine, which is an eclectically neutral phospholipid containing chain of fatty acyl of varying degree of saturation and length. Usually, cholesterol is incorporated to maintain the membrane rigidity and stability. Based upon the structure specification they are broadly classified into multilamellar vesicles (and unilamellar vesicles (fig. 3).

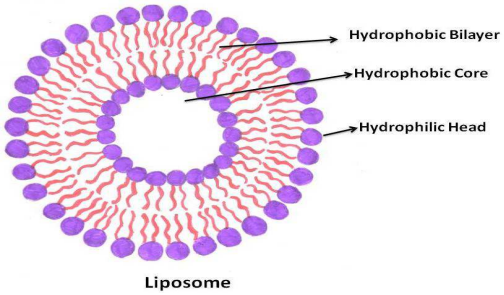

Fig. 2: Schematic illustration of liposome [150]

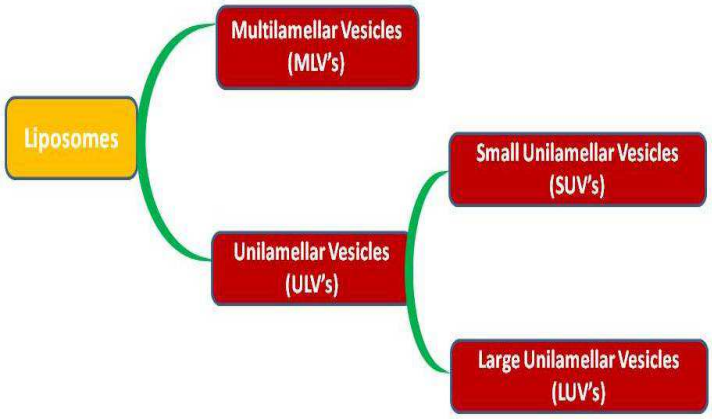

Fig. 3: Classification of liposomes [99]

Multilamellar vesicles possess multiple phospholipid layers while unilamellar vesicles have single phospholipid layer. While preparing liposome following parameters are taken into account

a) Physicochemical characteristics of liposomal ingredients

b) Materials to be contained within the liposome

c) Particle size

d) Shelf time

e) Polydispersity

f) Surface zeta potential

For the formulation of the liposome sufficient amount of energy via sonication, homogenization, shaking/heating is supplied to phospholipid placed in water. Sonication method is most preferable [100]. The *in vivo* stability of liposomes can be enhanced by providing "stealth" material on its surface. Like polyethene glycol is frequently conjugated with liposome that creates stealth layer and prolongs its circulation lifetime in the bloodstream. Polymyxin B-loaded nanoparticles are found to be quite effective against *P. aeruginosa* related infections. Liposomal encapsulation of polymuxin B has limited the side effect of the drug [101]. Another success story is of Am Bisome, which is a liposomal formulation of amphotericin B to treat *Candida spp*., *Aspergillums spp*. and other fungal infections in neutropenic, visceral leishmaniasis and methylmalonic academia patients. In table 2 some of the liposomes has been summarized.

Polymeric nanoparticles

These can be formed as nanospheres or nanocapsules fig. 4, is depicting its structure. Nanospheres are spherical matrix system in which drug is uniformly dispersed whereas nanocapsules are vesicular systems in which drug is confined to a cavity surrounded by a polymeric membrane.

In addition to being biodegradable and biocompatible they have following properties for antimicrobial drug delivery:-

a) There are structurally stable and can be synthesized with shape size distribution.

b) During their synthesis, by selecting different polymer length, surfactants and organic solvents their particle properties viz., size, Zeta potential and drug release can be precisely tuned.

Table 2: Various liposomes formulations and their effect as antimicrobial drug delivery system

Liposomal formulation	Drug	Targeted microorganism	Action	Reference
Dipalmitoylphosphatidylcholine (DPPC), cholesterol, and dimethylammonium ethane carbamoyl cholesterol (DC-chol)	Benzylpenicillin	*Staphylococcus aureus*	A small amount of drug, penetrates the membrane in a lesser amount and thus disrupts cell membrane integrality	102
Hydrogenated soy phosphatidylcholine, cholesterol, distearoylphosphatidylglycerol (DSPG)	Amikacin	Gram-negative bacteria	Bacteria is exposed for a longer duration	103
Egg phosphatidylcholine, Diacetylphosphate, cholesterol	Vancomycin or Teicoplanin	Methicillin-Resistant *Staphylococcus aureus* (MRSA)	-Increased drug uptake by Macrophages -amplified intracellular antimicrobial effect of each drug	104
Hydrogenated soy phosphatidylcholine, cholesterol, distearoylphosphatidylglycerol (DSPG)	Amphotericin B	*Aspergillus fumigatus*	-Drug delivery is targeted at the site of infection	105
Soybean phosphatidylcholine (PC) and cholesterol	Ampillicin	*Micrococcus Luteus* and *Salmonella typhimurium*	-augmented activity and stability of the drug	106
1,2-dipalmitoyl-sn-glycero-3-Phosphocholine (DPPC) and cholesterol	Polymyxin B	*Pseudomonas aeruginosa*	-decrease bacteria count in lung -Enhanced bioavailability -condensed lung injury caused by bacteria	107

c) Their surface contains functional groups that can be easily modified either with drug moieties or targeting ligands as per the requirement. Like, polymeric nanoparticles can be bound with lectin which is a carbohydrate that easily binds to most of the bacterial cell wall. Umamaheshwari *et al.*, 2003 studied lectin conjugated gliadin nanoparticle against the infection caused by *Helicobacter pylori* and found it to be quite helpful in overcoming the infection [108].

Initially, the use of polymeric nanoparticle did not attain the success due to the rapid clearance by the reticuloendothelial system after intravenous administration. But later the problem was resolved by using long-circulating stealth polymeric nanoparticle [109].

There are mainly two types of polymeric nanoparticles.

a) The one formed by spontaneous self-assembly of di-block copolymers consisting of hydrophobic and hydrophilic segments. The hydrophobic segment comprises the polymeric core that contains the drug while hydrophilic segment protects the core from opsonisation and degradation. Now, the rate at which drug release is required can be controlled by varying the length of hydrophobic chain, forming the core of polymer. Commonly used hydrophobic polymer core includes poly (Lactide-co-glycolide), poly (glycolic acid) PGA, Poly (cyano-acrylate) PCA, whereas commonly used hydrophilic segment is polyethene glycol. These are formed by the solvent evaporation method.

b) Like an amphotericin-B loaded poly (ε-caprolactone) nanospheres coated with non-ionic surfactant poloxamer 188 has shown great therapeutic efficacy against *Leismania donovani* as compared to free drug counterparts [110]. Some of the polymeric nanoparticles with their efficacy have been recapitulated in table 3.

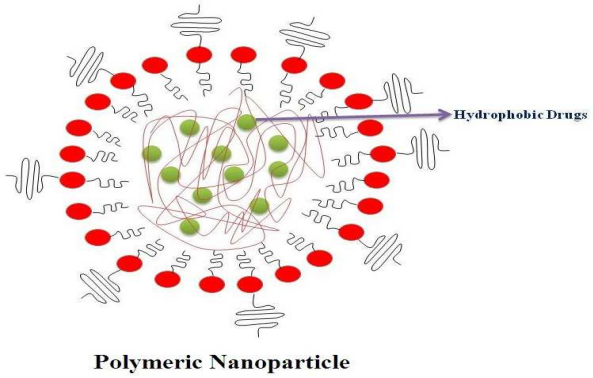

Fig. 4: Schematic illustration of polymeric nanoparticles [151]

Table 3: Various polymeric nanoparticle formulations and their effect as antimicrobial drug delivery system

Polymeric formulation	Drug	Targeted microorganism	Action	Reference
Poly-lactide-co-glycolide (PLG) nanoparticle	Rifampicin, isoniazid, Pyrazinamide, Ethambutol.	*Mycobacterium turberculosis*	-Augmented bioavailability	111
Polylactic-co-glycolic acid (PLGA) nanoparticles	Phosphorothioate Antisense oligonucleotide	HIV	-Protection of oligonucleotides from Degradation	112
Alginate nanoparticle	Rifampicin, isoniazid, Pyrazinamide, Ethambutol.	*Mycobacterium turberculosis*	-Improved pharmacokinetic Therapeutic efficacy increased	113
Glycosylated polyacrylate nanoparticle	Ciprofloxacin	Staphylococcus aureus and *Bacillus anthracis*	-Enhanced bioavailability -higher therapeutic efficacy	114
Polyethylene glycol (PEG)-PLA nanocapsule	Halofantrine	*Plasmodium berghe*	-Extended circulation half-life	115
Poly (D,L-lactide) (PLA) Nanospheres	Arjunglucoside	*Leishmania donovani*	-Abridged toxicity	116
Poloxamer 188 coated poly(epsiloncaprolactone) (PCL) nanosphere	Amphotericin B.	*Candida albicans*	-Lower *in vivo* toxicity due to reduced accumulation in kidney and liver	117

Dendrimers

Dendrimers are highly branched, regularly ordered globular macromolecular with 3-D structures, shown in fig. 5 which provide a high degree of surface functionality and versatility [118]. They mainly consist of 3 components: An initiator core, an interior layer composed of repetitive units and a terminal functionally layer attached to the outermost interior layer for the formulation of dendrimer nanoparticle dendrimer nanoparticles there are two approaches, one is divergent and another one is convergent. In the divergent approach, synthesis starts from the core and proceeds outwards by repetition of coupling and activation steps. Whereas, in the convergent approach, synthesis starts from the outer side (peripheral) and proceeds towards the core [119]. Dendrimers posses many exceptional properties that make them dexterous nanoparticles.

a) They have highly branched 3-D structure which gives them an adequate amount of surface area to size ratio and allows great relatively with microorganisms *in vivo*.

b) There is an accessibility of many controlled functional surface groups, poly disparity and their ability to imitate cell membrane adds to their potency as drug carriers.

c) Both hydrophobic and hydrophilic drugs can be uploaded, the hydrophobic drug can be uploaded inside the cavity and hydrophilic drugs can be attached to multivalent surfaces of dendrimers through covalent conjugation or electrostatic interaction [120].

d) Dendrimers with specific and high binding affinity for various viral and bacterial receptors can be synthesized [121]. For their prolonged stay in the circulatory system, they can be functionalized with Polyethylene glycol.

Moreover, by taking antimicrobial drugs as their building blocks they themselves become efficient antimicrobials. Dendrimers have greater antimicrobial efficacy than small drug molecules because of the high density of antimicrobials present on their surfaces. The polycationic structures facilitate the electrostatic adsorption to negatively charged bacteria. This adsorption increases membrane permeability and makes a passage for more dendrimers for entering in the bacteria leading to the leakage and eventually complete disintegration of the bacterial membrane [122].

A polyamidoamine dendrimer is the first dendrimer that becomes very popular but, become of toxicity due to terminal amine; its clinical use was limited. However, by masking these terminal amines, this limitation was resolved. Table 4 is showing commonly used dendrimers.

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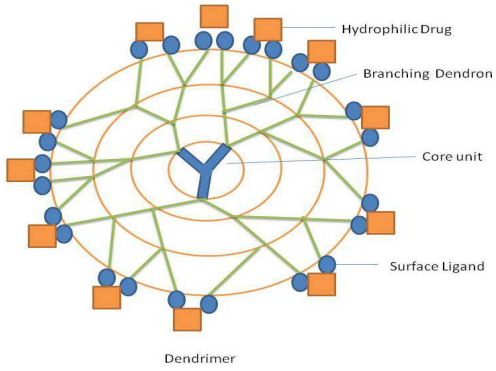

Fig. 5: Schematic illustration of dendrimers [118]

Table 4: Various dendrimers formulation and their effect as antimicrobial drug delivery system

Dendrimer formulation	Drug	Targeted microorganism	Action	Reference
Polyamidoamine (PAMAM) dendrimers	Nadifloxacin and Prulifloxacin	Various bacteria	-Enhanced water solubility	123
	Sulfamethoxazole	Streptococcus, staphylococcus aureus, Haemophilus influenza	-Steady drug release -superior antibacterial activity	124
	Silver salts	*(Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli)*	-Elevated payload -extended circulation half-life	125
Pegylated lysine based copolymeric dendrimer	Artemether	*Plasmodium falciparum*	-Augmented drug stability -augmented solubility -extended drug circulation half-life	126

Solid lipid nanoparticles

Solid lipid nanoparticles are colloidal drug carries shown in fig. 6 and attracted much attention since 1990's. Their size ranges from 52-100 nm and consist of physiologically biocompatible lipids, which remain solid at body and room temperature and disperse in aqueous solution. The lipids used to prepare SLNs include fatty acids (palmitic acid, decanoic acid), triglycerides (trilaurin, trimyristin), steroids (cholesterol) partial glycerides (glycerol monostearate) and waxes. Emulsifiers like soyabean lecithin, poloxamer 188, sodium cholate etc., are used to stabilize lipid dispersion.

To prepare Solid lipid nanoparticles (SLN's) high-pressure homo-genization emulsifier solvent diffusion and multiple emulsion solvent injections are commonly used.

These SLN's has many advantages as a drug carrier system that is summarized as follows.

a) Drug release can be controlled and targeted for immediate release or sustained release.

b) They are made up of physiological, biocompatible and tolerable lipids and thus non-toxic to the human body.

c) These formulations protect sensitive drugs from any photochemical or oxidative degradation as the drug is immobilized by solid lipids and drug leakage is reduced as compare to liposomes.

d) A slight modification in the SLN's formulation helps in the encapsulation of both lipophilic and hydrophilic drugs.

e) They possess property, which is unique in itself, as an application on skin, they readily forms a thin film to reduce water evaporation and retain skin moisture. This promotes their penetration into the skin. Like SLN's encapsulated antimicrobial agent (retinol and retinyl palmitate) have shown better drug penetration rate than that of their free drug [127]. Moreover, they are stable in water and chemical cream so can be incorporated in cosmetics and skin care products.

In addition to the topical application, they can be taken orally in variable conditions. Tobramycin is a drug used to treat *Pseudomonas aeruginosa* infections in fibrosis patient.[128] But, the rate of absorption of tobramycin is very low by the intestinal cells because P-glycoproteins on the brush border of the small intestine actively export the drugs from the cells. While tobramycin-loaded SLN's significantly suppresses the P-glycoprotein efflux pump and helps in better transport of tobramycin inside the cells. Table 5 shows some of the beneficial SLN's.

Besides the above-mentioned nanoparticles, there are some other organo metallic-based systems that are quite helpful as drug delivery carriers.

Many new drugs like paclitaxel have been found to have low aqueous solubility but high therapeutic efficiency. Nanoparticles are quite helpful in such conditions.

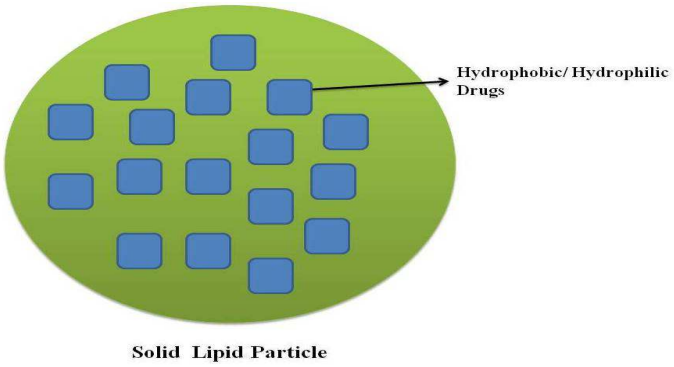

Fig. 6: Schematic illustration of solid lipid nanoparticle [127]

Table 5: Various solid lipid nanoparticles formulations and their effect as antimicrobial drug delivery system

SLN's formulation	Drug	Targeted microorganism	Action	Reference
Glyceryl tripalmitate and tyloxapol	Clotrimazole	Fungi	-Extended drug release -high physical stability High encapsulation efficiency	129
Stearic acid, soya phosphatidylcholine, and sodium taurocholate	Obramycin	*Pseudomonas aeruginosa*	-Increased drug availability	130
Stearic acid	Rifampicin, isoniazid, Pyrazinamide	*Mycobacterium tuberculosis*	-Advanced residence time -improved drug bioavailability -abridged administration frequency	131
Glycerol palmitostearate	Econazole nitrate	Fungi	-High encapsulation efficiency -controlled drug release profile -enhanced drug penetration through stratum corneum	132
Glyceryl behenate and sodium deoxycholate	Ketoconazol	Fungi	-Augmented physical stability	133
Stearic acid, soya phosphatidylcholine, and sodium taurocholate	Ciprofloxacin Hydrochloride	Gram-negative bacteria, Gram positive bacteria, and Gycoplasma	-Extended drug release	134

Nanoparticles and biocompatibility

Nanoparticles are in use for various biomedical applications viz., for the treatment of wounded infections, in drug delivery, as biosensors, as an antimicrobial agent. During their usage, they come in direct contact with various tissues and cells depending upon their mode of delivery, such as through oral ingestion, topical, inhaled or intravenous administration. The effect of various nanoparticles on a variety of tissues and cells is not very well understood, but their effect can be studied *in vivo* and *in vitro*. For the *in vitro* study, usually a cell culture method is used, where its carcinogenic potential, genotoxicity and cell proliferation pattern is studied. There are studies showing the cytotoxic [135], genotoxic [136] and carcinogenic [137] impact of metal-based nanoparticles, they have also shown the apoptosis-inducing potential [138] of metal-based nanoparticles. The activity of the nanoparticles is dependent upon their characteristics like electrical charges, their size, exposure concentration, shape, the surface structure of nanoparticles and on the individual characteristic of the metal and nonmetal used. Titatnium foils are covered with vertically aligned titanium dioxide nanoporous surface that facilitates its use. This vertical alignment enhances proliferation, mineralization of osteoblasts, vasodilation of endothelial cells and also the mobility [138]. It is also observed that the growth rate of osteoblasts increases three to four folds after exposure to titanium dioxide nanotubes [140]. The toxicity of nanoparticles also depends upon the size and charge of the nanomaterial used. Smaller size of negatively charged silicon dioxide has a strong blow on cell viability and genotoxicity, while their larger particles (100 nm) do not affect the cellular activity. Silver nanoparticles and gold nanoparticles are found to be more friendly and the biocompatible to the human cells [141]. Sodium oleate-coated nanoparticles have also shown a considerable amount of biocompatibility with human cells [142]. It is necessary that after the *in vitro* studies the nanoparticles should be assessed in animal models. Zinc oxide nanowires is tested in Hela and L929 culture cells, where it has responded well to Hela at all the concentration while it showed good result only at a lower concentration in L929 culture cells as it reduced the cell viability at higher concentrations [143]. The cytotoxic behaviour of nanoparticles varies from the higher animals to lower and also to the cell cultures. The biocompatibility of various nanoparticles can be modulated by slight variations in their surface structure. It has been observed that, nanoparticles affects directly to the major organs like heart, lungs or brain of the host. Studies have shown that Gold nanoparticles, when inhaled in rats at higher doses and at regular intervals, get accumulated in the lungs, which results in inflammation and increase in a number of macrophages [144]. The size-dependent (10, 50, 100, and 250 nm) intravenous administration studies in rats has shown the accumulation of 10 nm size in various organs including brain, kidneys, heart, testis and thymus. Further eye irritation, oral toxicity and other factors were studied in mice and guinea pig, and it was concluded that gold nanoparticles should be administered for a

shorter period of time [145]. The exact mechanism of the toxicity is still not clear, but it is mainly attributed to the generation of reactive oxygen species. On one hand, reactive oxygen species are essential but are fatal to eukaryotic cells also. It is a well-known fact that reactive oxygen species at lower concentration is required for various pathways, but when they exceed the limit they lead to oxidative stress, resulting in an alteration in DNA, proteins and oxidation of lipids and thus the death of the cells. Reactive oxygen species generated by titanium dioxide nanoparticles resulted in an inflammatory response in rats, mice and hamsters [146]. The effect of oxidative stress is not confined to one particular organ, but it affects the various body system including central nervous system (CNS), respiratory system and even the cardiac conduction [147]. Likewise, there are many organic nanoparticles that have not shown toxic effects. Polycationic nanoparticles quaternary ammonium polyethyleneimines did not alter the biocompatibility as tested by cell viability and secretion of TNF-α by the monocytes [148]. Polyethyl glycol coated Nanostructured lipid carriers found to have valuable perspective, safe and increased residence time to delivery of indomethacin to the ocular surface [149]. Although nanoparticles have shown promising results as an antimicrobial agent, but the shortcoming like oxidative stress-mediated DNA damage, protein damage etc., cannot be ruled out. Thus, their dose, exposure time and other factors need to be taken care of.

CONCLUSION

Since ages, many conventional antimicrobial drugs are in use. But, with the passage of time, microbes have developed resistance against these antibiotics or antimicrobials. As a result, it has become a serious problem to treat these infectious microorganisms, resulting in life-threatening adverse of diseases. Many new chemically modified drugs can be used at higher dosage leading to several side effects or even instigating microorganisms to develop a resistant mechanism for them also. Nanoparticles are offering a new era for new, efficient antimicrobial agents, offering effective control over the microorganism at lower dosage only. Their ultra-small size and biocompatible properties make them new lead as an antimicrobial agent.

AUTHORS CONTRIBUTIONS

All the author have contributed equally.

CONFLICT OF INTERESTS

Declared none

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