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# Nanostructures and their application in antimicrobial drug delivery system

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## ABSTRACT

As multiresistant and pan-resistant infections continue to emerge, and because the development of novel antimicrobial drugs is a slow process, nanotechnology offers valuable alternatives for fighting resistant bugs, mainly by improving the therapeutic effect of current antimicrobials. Antibiotic resistance is one of the greatest global health threats of the 21st century, but nanotechnology is offering new solutions to the problem. Nanostructured biomaterials, nanoparticles in particular, have unique physicochemical properties such as ultra small and controllable size, large surface area to mass ratio, high reactivity, and functionalizable structure. These properties can be applied to facilitate the administration of antimicrobial drugs, thereby overcoming some of the limitations in traditional antimicrobial therapeutics. Carbon-based nanomaterials such as fullerenes, carbon nanotubes (CNTs) (especially single-walled carbon nanotubes (SWCNTs)) and graphene oxide (GO) nanoparticles) show potent antimicrobial properties. Nanocoating and shuttle systems have shown great promise in vitro and animal models. Noble metals nanostructures, particularly silver, have attracted much attention in the fields of medicine due to their unique properties which are strongly dependent on the size and shape of metal nanomaterials. Recent development of nanocarriers, improved the drug therapy of different diseases, together with the mechanisms of microbial inhibition.

**Keywords:** *Antimicrobial drugs; Nanotechnology; Antibiotic resistance; Nanomaterials; Microbial inhibition.*

## 1. INTRODUCTION

Infectious microorganisms when invade the epithelial surfaces try to spread throughout the human body mainly through the circulatory system. Macrophages then remove them from the blood by phagocytosis. Macrophages are present in all major organs such as spleen, liver and bone marrow (Bakker-Woudenberg, 1995). After infectious microorganisms are phagocytosed by the macrophages, they are trapped inside phagosomes, which are then fused with lysosomal granules inside the cytoplasm of the cell leading to formation of phagolysosomes. Thereafter, oxygen reliant or oxygen non-reliant microorganisms killing mechanisms induced by enzymes occur inside the phagolysosomesin order to digest the trapped microorganisms. However, this macrophage digestion is evaded by many of the microorganisms via inhibition of the phagosome-lysosome fusion, escaping from the phagosomes formation, withstanding oxidative and non-oxidative killing mechanisms, or resistance from the lysosomal enzymes. These mechanisms of defense by the microorganisms make intracellular infections tricky to eliminate resulting in infectious diseases that vary from staph infections to HIV (Bakker-Woudenberg, 1995).

An antimicrobial agent is defined as a substance which kills or inhibits the growth of microorganisms. Antimicrobial drugs were discovered in 1960s and many infectious diseases have been overcome thereafter (Coates, 2002). Antimicrobials agents destroy microorganisms by binding with some of the vital compounds in bacterial metabolism, as a result inhibit the normal cellular functions and impeding the synthesis of functional biomolecules. For example, tetracyclines and clindamycin imped vital protein synthesis; lactams like penicillins and cephalosporins inhibit bacterial cell wall synthesis; sulphonamides and trimethoprim inhibit enzyme synthesis and quinolones and metronidazole inhibit nucleic acid synthesis. Some antimicrobial agents like penicillins are not effective against broad range of bacteria, whereas others like ampicillin, are effective against broad spectrum of Gram-positive as well as Gram-negative bacteria (Walker, 2000). Although there has been great development in antimicrobial therapy many infectious microorganisms and intracellular infections remain difficult to treat. One most important cause is that many antimicrobial agents have low activity inside the cells and are not easy to transport through the cell membranes, thereby resulting in low or negligible bactericidal or inhibitory effects on the intracellular bacteria. Moreover toxicity due to antimicrobial agents caused to healthy tissues have a major limitation to their use.

For example Aminoglycosides toxicity involves nephrotoxicity and ototoxicity and have to be given in controlled dosages. Moreover, antimicrobial agents develop or acquire resistance against infectious microorganisms on prolonged usage. In 2002, more than seventy percent of the bacteria resulting in hospital-acquired infections developed resistance to at least one common antimicrobial agent in the United States. Alternative antimicrobial drug delivery systems and strategies have been developed in order to overcome these issues. For these reasons nanotechnology has found a widespread application in medicine and various other medical areas especially that in drug delivery. To come up to these subjects, in the present scenario nanoscale materials has emerged up as novel antimicrobial agents. “Nanotechnology” is the application of scientific discipline to manipulate matter at the molecular level. It is the most promising field for generating new applications in medicine. Materials have improved physical, chemical, and biological properties, phenomena, and functionality due to their nano scaled size.

Nanotechnology is concerned with the understanding and control of matters within 1- 100 nm of range, at which scale materials have unique physicochemical properties including large surface area to mass ratio, ultra small size, high reactivity and unique interactions with biological systems (Zhang *et al*., 2008). Drugs are loaded into nanoparticles through adsorption, physical encapsulation or chemical conjugation. This results in therapeutic index and pharmacokinetics of the drugs to increase significantly in comparison to their free drug counterparts. Nanoparticle-based drug delivery have various other advantages like prolonging the systemic circulation lifetime, enhancing serum solubility of the drugs, delivering of the drugs to the cells and tissues of interest, releasing of the drugs at a sustained and controlled manner, and concurrently delivering multiple therapeutic agents to the same cells for combination therapy. Nanoparticles carrying therapeutic agents enter the host cells through endocytosis and then release the therapeutic agent to treat intracellular infections caused by microbes. As a result, a number of nanoparticle-based drug delivery systems have been approved for clinical uses to treat a variety of diseases and many other therapeutic nanoparticle formulations are currently under various stages of clinical tests. Nanoparticles like polymeric nanoparticles, liposomes, dendrimers and solid lipid nanoparticles have been widely used as antimicrobial drug delivery systems and of which several products have been commercialized into pharmaceutical market (Peer *et al.*, 2007).

## 2. NANOMATERIALS USED AS ANTIMICROBIALS: MECHANISMS OF ACTION

An antimicrobial agent should have the capacity to reach the vital molecular sites and targets which are involved in bacterial metabolism like cell wall synthesis. Moreover, it needs molecule alteration by enzymes and prevents expulsion by efflux bombs. There are many natural as well as engineered nanomaterials or nanostructures that have confirmed strong antimicrobial properties through various mechanisms which include generation of reactive oxygen species through photocatalytic action that damage viruses and vital cell components of bacteria, disruption of energy transduction, compromising the bacterial cell capsule, impedes the DNA synthesis and enzyme activity. Some of the mechanisms are discussed (Azam *et al.*, 2012).

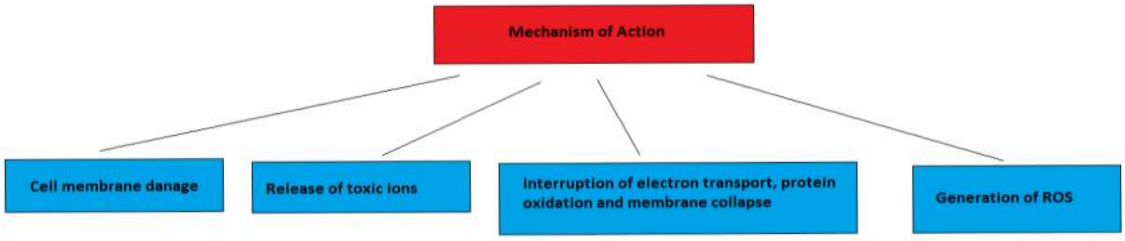


**Figure 1. Various mechanism of action of Antibiotics**

### 2.1. Cell membrane damage.

The mechanism of action of nanoparticles on cell membranes is recognized as non-specific and it is not known if polymixins has any involvement on this process. Polymixin antibiotics interact with cell membranes breaching crucial barriers of the microorganisms (Aruguete *et al.*, 2013). Cell permeability of the bacterial cell is altered when they get in touch with the nanoparticles. Experiments have demonstrated that there is a formation of "pore" or "hole" in the living bacterial cell membranes as a probable mechanistic hypothesis. Although the meaning of the term "pore" or "hole" still requires clarification, various images of cellular damage have given clear proof of this effect. In extreme cases there is a formation of a literal pore in the bilayer cellular membrane which leads to total loss of the cell membrane (Leroueil *et al.*, 2007).

### 2.2. Release of toxic ions.

It has been confirmed that $Ag^{+}$, $Zn^{2+}$, $Cd^{2+}$ ions have the tendency to react in bacteria with various groups of proteins. $Ag^{+}$ has the ability to form sparingly soluble salts. This is also considered as one of its vital mechanistic step in destroying bacterial cells. It is also seen that cell respiration is impeded when the chloride ions are precipitated in the form of silver chloride in the cell cytoplasm. Silver nanoparticles have a well-known antimicrobial efficiency especially against Gram-negative bacteria like *E. coli*. Therefore, the silver nanoparticles are used as antimicrobial agents which release silver ions and also penetrate the cells and interfere with their metabolic activities. Although silver has established much interest, $Zn^{2+}$ and $Cd^{2+}$ ions are also of great use. They can bind to sulfur-containing proteins of the cell membrane and alter its cell permeability. There are also sufficient proofs that Ag+ ions can also inhibit DNA replication thereby preventing bacterial cells multiplication. Silver nanoparticles show their bactericidal activity at a low concentration in the range of 9-10 mol/L (Niskanen*et al.*, 2010).

### 2.3. Interruption of electron transport chain, protein oxidation and cellular membrane collapse.

There is strong confirmation that nanoparticles are positively charged and cell membrane is negatively charged which is critical for antimicrobial activity of nanoparticles. Although the exact mechanism is still not clear, it has been clear that ions like silver can affect molecule alteration by altering membrane-bound respiratory enzymes as well as affect efflux bombs of ions that can cause in cell death. When nanoparticles come in contact with bacteria cascade of events can start which may lead to oxidation of respiratory enzymes and may aid the production of Reactive Oxygen Species that may lead to DNA degradation (Xia *et al.*, 2008).

### 2.4. Generation of ROS (Reactive Oxygen Species).

Oxygen is a powerful oxidizing agent and it is considered as the best acceptor of electrons during respiration it can be deadly for some bacteria. Ground state for oxygen molecule is Triplet oxygen ($3O_2$) which is toxic for cells. Not only can this singlet oxygen ($1O_2$) also be lethal for bacterial cell.

The production of singlet oxygen causes peroxidation of constituents of the cells like proteins and lipids. Singlet oxygen is not only a potent reagent but also promotes unwanted and continuous oxidations in the cell. $H_2O_2$, which is formed by the respiratory burst consumes $O_2$ and leads to the production of free radicals. Thus the basis of hydrogen peroxide action is the production of free hydroxyl radicals. This further leads to oxidation of proteins, DNA and membrane lipids (Bronshteint *et al.*, 2006). Reactive Oxygen Species cause the bacteria to lose the integrity of their cell membrane gradually, leading them to lose their ability to adhere to the surfaces, communication with other bacteria is hindered and so are the other vital functions. Out of many hypotheses for explanation of the mechanisms of action of nanoparticles one involves the generation of Reactive oxygen species and inhibition of the cellular adhesion. Whereas many microorganisms can defend themselves against the generation of Reactive oxygen species by the production of enzymes like superoxide dismutase, as a result oxidative stress is neutralized. Moreover bacteria fight back through two significant systems like the OxyR in response to hydrogen peroxide and SoxRS in response to superoxide.

These systems not only regulate reducing conditions but also repair damaged cell components (Seil, 2012). Finally it is well recognized that nanoparticles cause oxidative stress related to ROS when are exposed to bacteria.

## 3. SECTIONANTIMICROBIAL ACTIVITY OF CARBON-BASED NANOPARTICLES/NANOSTRUCTURES

CNTs are nano-sized hollow cylindrical form of carbon which has been synthesized by Lijima in 1991. Since then, CNTs have been applied in many fields of science and technology. Kang in 2007 provided the first document that showed single-walled carbon nanotubes (SWCNTs) had strong activity against microbes on Escherichia coli (E. coli). They demonstrated that SWCNTs could cause severe membrane damage and subsequent cell death (Mitchell, 2001). In other study they presented the first evidence that the size of carbon nanotubes was an important factor affecting their antibacterial activity. They prepared single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) and investigated their antibacterial effect against E. coli. It was seen that SWCNTs had much more effect and were more lethal to bacteria than MWCNTs.

It was also observed that, cell membrane integrity was influenced by CNTs when they are in contact with bacteria. In addition to this morphology and metabolism processes of E. coli was also altered. SWCNTs can penetrate into the cell wall better than MWCNTs due to their smaller nanotube diameter. Furthermore, the superior surface area of SWCNTs initiated better interaction with the cell surface (Kang *et al.*, 2008).

Arias and Yang (2009) investigated SWCNTs and MWCNTs activity against microbes with different surface groups towards rod-shaped or round-shaped gram negative bacteria and gram positive bacteria. According to their results, SWCNTs with surface groups of -OH and -COOH indicated improved antimicrobial activity to both gram positive bacteria and gram negative bacteria while MWCNTs having the same surface groups had no significant antimicrobial effect. Their results showed that, formation of cell-CNTs aggregates caused to damage the cell wall of bacteria and then release of their DNA content. Yang, in 2010, studied the efficiency of SWCNTs length on their antimicrobial activity was investigated. It was seen that longer the SWCNTs stronger the antimicrobial activity which was due to their increased bacterial cells aggregation (Arias, 2003).

### 3.1. Fullerenes.

Fullerenes are made up of carbon atoms. They are soccer ball shaped in shape. Fullerenes showed antimicrobial activity against various bacteria like E. coli, Streptococcus and Salmonella. The antibacterial effect was probably due to inhibition of energy metabolism after internalization of the nanoparticles into the bacteria. It is suggested that derivatives of fullerenes can imped the growth of bacteria by impairing the respiratory chain. In the beginning there is a decrease of oxygen uptake which occurs at low fullerene derivative concentration and then an increase of oxygen uptake followed by an enhancement of hydrogen peroxide production occurs.

Another bactericidal mechanism which has been proposed was the induction of cell membrane disruption (Cataldo, 2008). As stated by the literature, the hydrophobic surface of the fullerenes easily interacts with the lipids of the membrane and then intercalate into them. The discovery of fullerenes ability to interact with biological membranes has encouraged many researchers to evaluate their antimicrobials applications. Among positively charged, neutral, and negatively charged fullerene compounds, cationic derivatives had the maximum antibacterial activity on *Shewanellaoneidensis* and *E. coli*; while the anionic derivatives were almost ineffective. This could be owing to the strong interactions of negatively charged bacteria with the cationic fullerenes (Nakamura, 2009).

Antibacterial effects of two water soluble fullerene derivatives were compared by Deryabin *et al.* in 2014. In their work, organic linkers were used to add protonated amine groups (AF) and deprotonated carboxylic (CF) groups to the fullerene cage. The former positively charged derivative showed antibacterial effects by binding to the cells of E. coli, whereas the later negatively charged one showed no significant antibacterial effects (Holister, 2003). They concluded that the protonated water-soluble fullerene derivative could be used in the synthesis of various chemical disinfectants. Fullerenes can also be applied in photodynamic therapy where they can be used as photosensitizers when their solubility is increased via functionalizing with hydrophilic groups. In fact, water soluble fullerenes produce superoxide when biological reducing agents are present and this process is relatively more cytotoxic towards microbial cells.

Tegos *et al.,* in 2005 tested antibacterial activity of fulleropyrrolidinium salts after photoirradiation and their results showed that bacterial cells and fungal cells were destroyed nearly up to 100% (Tegos, 2005).

**3.2. Graphene oxide.**

A single layer of carbon atoms which are tightly jam-packed into a two-dimensional crystal is normally called Graphene. Graphene oxide nanosheets are easily dispersed in water and produced by chemically modification of the graphene with hydroxyl, epoxy, and carboxyl groups. It is known that, membrane stress in GO, that result from direct contact with jagged nanosheets is the major reason for their antimicrobial mechanism. Both graphene and GO were shown to impede the growth of microbes.

Akhavan and Ghaderi (2010) validated the antibacterial activity of graphene sheets and proved thir antimicrobial potential against *E. Coli* & *S. aureus.* Antimicrobial potential of GO is due to direct contact of their sharp edges with bacteria caused RNA effluxes through the injured cell membranes microbes. On similar lines, Hu *et al.* (2010), Gurunathan *et al.* (2012) & Azimi *et al.* **(2014),** also studied the antimicrobial possessions of GO and reduced GO nanowalls. The antibacterial effect of the reduced graphene nanowires was comparable with single-walled carbon nanotubes. nanosheets. Furthermore, these studies signified that the surface chemistry and metal toxicity had a vital role in antibacterial activity of graphene oxide and its derivatives. The physical interaction of Graphene oxide with the cellular membrane, hydrogen bonding with a outer cellular component and production of intercellular free radicals like OH radicals were recommended as the possible antibacterial mechanisms.

Carbon nanocomposites made up of carbon nanostructures and metallic nanoparticles like carbon nanotubes-Ag and grapheme oxide-Ag nanocomposites, have been revealed the antibacterial potential against various microbes. However, the antibacterial activity of CNT-Ag was superior to GO-Ag nanocomposites which could be due to good distribution of the Ag nanoparticles into the carbon nanotubes.

**3.3. Nanostructured Hydrogel Webs Containing Silver.**

Newly synthesized nanobiomaterials not only prevent biofilm formation in microbes, but also related infections. Such a thermoplastic biomaterial hydrogel nanofibrous webs containing silver has shown antimicrobial properties. They were prepared from multiblock poly ethylene glycol and polyhedral oligosilsesquioxane (PEG-POSS) polyurethanes and electrically spun into nanofibrous webs (150nm) with or without silver nitrate ($AgNO_3$). The nanofibrous hydrogels (NHs) displayed eccentric contraction during uptake of water, leading to formation of an exclusively dense structure in comparison to hydrogels synthesized from cast films. Antimicrobial efficiency of nanofibrous hydrogels was tested on microbes like *E. coli*. NHs which did not have silver incorporated in it, showed quick and most widespread formation of biofilm, whereas those containing silver showed exceptional biofilm resistance. These hydrogels had a distinctive antimicrobial activity and find their application in wound dressings in accidental or burn case, which merges sustained bactericidal property and lack of swelling during uptake of water (Cataldo, 2008).

The nanostructured hydrogel webs with and without silver nitrate ($AgNO_3$) were sterilized by using UV illumination (λ: 254 nm) in a closed cabinet before testing of any antimicrobial activity, to prevent the contamination prior to testing. These sterilized hydrogels was tested against *E. coli* for their antimicrobial potential. Microscopically evaluation showed that hydrogel with $AgNo_3$ has excellent antimicrobial potential as compare to hydrogel webs without silver.

Electro spunnano fibrous mat with 1% $AgNO_3$, has showed extended antimicrobial properties against *E. Coli* as compare to cast film, when their antimicrobial activity has been evaluated for 14 day test (Jain, 2009). This extended antimicrobial potential was due to its compact internal structure that may control release rate of silver ions on the surface of hydrogel. These hydrogels are used for wound dressings, bandages and reconstructive bone and oral surgery (Cataldo, 2008) due to their non swelling nature and lateral wicking effect.

**3.4. Antimicrobial nanospheres thin coatings prepared by advanced pulsed laser technique.**

It involves the production of thin films made from polymathic acid-chitosan-magnetite eugenol (PLA-CS-$Fe_3O_4$@EUG) nanospheres by matrix assisted pulsed laser evaporation (MAPLE). Scanning electron microscopy and transmission electron microscopy examination proved that the homogenous magnetite eugenol nanoparticles have an average diameter of about 7 nm, where as the polylactic acid-chitosan-magnetite eugenol nanospheres diameter size range from 20 to 80 nm. This matrix assisted pulsed laser evaporation deposited coatings behaved as bioactive nanosystems and showed an extensive antimicrobial property by inhibiting the adherence as well as formation of biofilm of bacteria like *Pseudomonas aeruginosa* and *Staphylococcus aureus* bacteria strains. Not only this, synthesized nano-coatings displayed a good biocompatibility and helped the normal growth and development of the human endothelial cells. These nanostructures provide application as an efficient alternative for preventing and treating infections caused due to various microorganisms. Matrix assisted pulsed laser evaporation deposited thin coatings made from polylactic acid-chitosan-magnetite eugenol imped the formation of biofilm not only in the initial phase but also during maturation of biofilm. When examined for antimicrobial activity, biofilms of Staphylococusaureus were significantly inhibited during entire tested points of time, whereas biofilms of Pseudomonas aeruginosa were especially affected after 24 to 48 hour of incubation. Although these nanostructures showed a great antimicrobial activity, many tests stated that these nanostructures may cause toxicity to host cells not only in higher concentrations but even at therapeutically active doses.

The results clearly prove that manufactured polylactic acid-chitosan-magnetite eugenol nanosystems merge the eugenol and confirmed efficacy of $Fe_3O_4$ with the biodegradability and biocompatibility of polylactic acid and chitosan polymers resulting in a safe nano-biocomposite. Because of these properties polylactic acid-chitosan-magnetite eugenol thin film coatings

provide their application for the manufacture of novel devices with high efficiency and low costs or biomedical surfaces.

**3.5. Polymeric Nanoparticles for Antimicrobial Drug Delivery.**

Polymeric nanoparticles (PNP) have been synthesized to deliver a diversity of antimicrobial agents to treat infectious diseases and delivered these agents at target site. PNPs have shown great therapeutic efficacy and potency. The antimicrobial activity of arjunglucoside-loaded poly (D,L-lactide) (PLA) nanospheres have shown greater therapeutic efficacy against *Leishmania donovani* has as compared to the free drug counterparts and lesser side effects (Tyagi, 2005). Saquinavir, an antiviral agent loaded with Poly(ethylene oxide) (PEO)-modified poly(epsilon-carprolactone) (PCL) & phosphorothioate antisense oligonucleotide encapsulated in poly lactic-co-glycolic acid (PLGA) nanoparticles have shown greater therapeutic efficacy against HIV has compared to the free drug. As these polymers protects phosphorothioate antisense oligonucleotide from enzymatic degradation and cytochrome c metabolism in systemic circulation (Berton, 2006; Ahmad, 2006). Rifampicin-loaded polybutylcyanoacrylate and beta lactam loaded glycosylated polyacrylate nanoparticles have shown enhanced antibacterial activity against Staphylococcus aureus and Mycobacterium avium due to an enhanced bioavailability of these drugs in systemic circulation and targeted delivery of drugs to macrophages (Ahmad, 2006). In another example, ampicillin and primaquine shown enhanced antimicrobial potential when encapsulated in polyisohexylcyanoacrylate nanoparticles have been studied against *Salmonella typhymurium & Listeria monocytogenesin* (Pandey, 2006).

## 4. NANOBIOMATERIALS STRATEGIES BASED ON NATURAL PRODUCTS: RECENT PROGRESS IN BIO AND NANOTECHNOLOGY

Even in this era of antibiotics, the major causes of mortality and morbidity worldwide are microbial infections. This factual reality is caused mainly by the acquisition of antimicrobials resistance, which is supported by irrational usage of antibiotics that humans developed during time. Recent focus of research is to develop novel therapeutic approaches to diminish and remove pathogenic resistant bacteria. Recent findings state that microbial, plant or animal-derived products can be directed as useful tools in modulating pathogenic bacteria virulence and resistance, with minimum effects against superior hosts. Novel approaches, using bio and nanotechnology were developed for stabilization and efficient delivery of natural antimicrobial compounds.

Nanostructured emulsions made up of bullfrog (Ranacatesbeiana Shaw), copaiba (Copaiferalangsdorffii) resin-oil and copaiba essential oil have the antimicrobial property against bacteria and fungi associated to skin ailments or diseases. The essential oil was extracted from copaiba resin oil and these oils, along with bullfrog oil, were characterized by gas chromatography combined with mass spectrometry. Then nanostructured emulsions were synthesized. The antimicrobial efficacy was determined by the property to inhibit biofilm formation (antibiofilm determination) and the Minimum Inhibitory Concentration or MIC determination. For this determination strains of the genera Candida, Pseudomonas and Staphylococcus were used. The Minimum Inhibitory Concentration assay in alliance with the bioautography proved that some esters of oleic and palmitic acids, a-curcumene, α –himachalene, α-fenchene and isothujol probably inhibited some strains. Copaiba resin oil and essential oil enhanced the antimicrobial property of the pure oils (particularly against Candida and Staphylococcus). The copaiba samples had greater antimicrobial effect in comparison to bullfrog oil nanostructured emulsion. Whereas, bullfrog oil based nanostructured emulsion had a significant antibiofilm activity. Finally, Copaiba and bullfrog oils are hopeful candidates for antimicrobial activity because of significant antimicrobial and antibiofilm activities.

Other nanobiomaterials strategies based on natural products include Poly-caprolactone (PCL) and Poly lactic acid(PLA) nanofibrous mats loaded with thymol. It was prepared by Karami *et al.* by solubilizing the polymers in a mixture of chloroform and dimethylformamide along with thymol. It was seen that thymol acted as plasticizing agent and rearranged the polymer chain layer resulting in decreased viscosity of the polymer solution and as a result the average diameter of nanofiber was decreased. Due to the limited solubility of thymol in the electrospun polymer solutions only 1.24% v/v of herbal drug could be loaded in the fibers.

A bimodal release profile was seen: there was a burst release in the first 12 hour, followed by a gradual drug release up to 48 hour. The antibacterial efficacy of the hybrid mat was carried out through the disc diffusion method. There were formation of inhibition zones of 7.8 mm and 10.4 mm against *E. coli* and *Staphylococus aureus* respectively (Curtis, 2004).

**4.1. Magnetite Nanostructures.**

In today scenario resistance to infectious agents has led development of novel routes for fighting infectious diseases. One of this is the use of magnetic nanoparticles or MNPs. Recently developed most important directions are: (i) development of antimicrobial compounds having severe decrease of the MIC of the drug used; (ii) inhibiting the development of biofilm and microbial adherence on coated medical surfaces. These new directions are potential alternative in developing newer strategies to impede and prevent infectious diseases that are resistant to drugs and other biofilm forming bacteria (Grancharov, 2003). Iron oxide MNPs are developed for the delivery of drugs for the treatment of various infectious diseases caused by yeasts, fungi and bacteria. Iron oxide MNPs have possible benefits due to their size and less amount of drug required to restrain microbial multiplication. The option to manipulate them using magnetic fields and image them is really advantageous (Chifiriuc, 2013).

$Fe_3O_4$ MNPs have also been utilized recently for delivery of vaccines. This has enabled them to be used for controlling of various infectious diseases such as malaria, AIDS and others. MNPs intracellular uptake in the viral size is high, whereas when antigen is incorporated on the NPs surface CD4 and CD8T cells may evoke. MNPs can also be used for the treatment of respiratory diseases. MNPs have various advantages like reducing the amount of the drug deposit into the lungs, avoid deposition in the liver thereby preventing biological metabolism, reach alveoli due to their minute size, have the option of imaging using various optical techniques to view nanoparticles (Morozov, 2014).

### 4.2. MNPs to Inhibit Yeasts.

*Candida albicans*

Magnetite nanoparticles having 20 nm maximum diameter, surfactant used was oleic acid and Rosmarinusofficinalis essential oil (NPs-EO) used as an antimicrobial agent (Chifiriuc, 2012).

*Candida tropicalis*

The MNPs with R. officinalis essential oil coated onto a catheter previously used in C. albicans section showed a distinct inhibition compared to uncoated catheter against C. tropicalis. After 24 hour of cell culture the countable viable cells observed were the lowest but at 48 and 72 hours the countable viable cells observed were higher which could be due to the early production of germ tubes and pseudohyphae (Chifiriuc, 2012).

*Candida krusei*

MNPs can be used in treatment of Candida krusei infections although at this point of time the mechanism of action is not clear. Essential oils have been stabilized with MNPs and used in the inhibition of fungal biofilms (Saviuc, 2011).

*Candida glabrata*

Studies show that inoculation of *C. glabrata* produced a monolayer biofilm which was homogenously spread over the uncoated as well as coated coverslip surface. In this there was a central large microbial colony which showed midpoint disruption. This midpoint disruption revealed an inhibition mechanism of the MNPs (Saviuc, 2011).

S. cerevisiae strain was used for the formation of in vitro microbial biofilms on different substrata and fatty acid-functionalized magnetite nanostructures were used to inhibit them.

The results showed great potential, showing the importance of magnetic nanoparticles in the inhibition of fungal biofilms (Saviuc, 2011).

Studies have reported the production of coated functionalized magnetite nanoparticles of Nystatin coated with chitosan acts as an antimicrobial agent showed a distinct *C. albicans* biofilm inhibitory effect by preventing the formation of fungal biofilms (Hussein, 2014). Nanocomposites of magnetite, silver NPs and chitosan proved to have significant antimicrobial effects against *C. albicans* (Markova, 2012). MNPs with dextran caoting have great potential in the treatment of *Candida krusei* infections by adhereing and preventing formation of biofilm (Markova, 2012).

### 4.3. MNPs to Inhibit Bacteria.

*Escherichia coli*

Studies have shown that magnetite NPs coated with chitosan and grafted with cephalosporins have antibacterial activity against E.coli. These microspheres were synthesized by wet chemical precipitation of ferric and ferrous ions in aqueous solution with chitosan and sodium hydroxide.

Various antibiotics encapsulated into the chitosan magnetite microspheres retained their properties. Moreover the minimal inhibitory concentration was decreased to a great extent, ranging from 2 to 7.8 times for the E. coli tested strains. The tested antibiotics were ceftriaxone, cefepime, cephalosporins and cefoperazone. (Chifiriuc, 2012).

*Staphylococcus aureus*

Magnetite nanoparticles synthesized by co-precipitation method, cross-linked with chitosan and grafted with two selected aminoglycoside antibiotics namely kanamycin and neomycin were tested against S.aureus. These chitosan-magnetite nanoparticles containing the antibiotics possessed excellent antibiotic activity against S.aureus strains.

Moreover, the amount for both kanamycin and neomycin used was less to inhibit the growth of S.aureus. As an approximation the amount of kanamycin or neomycin used with chitosan-magnetite nanoparticles needed to inhibit the growth of S.aureus was half the amount of these antibiotics required without MNPs.

This outstanding antimicrobial activity was because of the higher surface area to volume ratio of the MNPs and hence more surface of the antibiotic was in contact with the microorganisms but also the control release ratio (Grumezescu, 2013).

*Pseudomonas aeruginosa*

Magnetite NPs cross-linked and grafted with chitosan and aminoglycoside respectively also inhibited the growth of P.aeruginosa. The incorporation of the nanoparticles with the antibiotics evidently improved the antimicrobial action due to the higher surface area to volume ratio and to the controlled release of the aminoglycosides. Magnetite NPs functionalized with eugenol also had the inhibitory activity against P.aeruginosa strains and showed similar anti-adherence property which enable them to be the perfect candidates for developing of new antibacterial agents (Grumezescu, 2014).

*Enterococcus faecalis* E. faecalis is a planktonic and biofilm-growing bacteria. Chifiriuc*et al.* study reported the antibacterial ability of magnetite nanoparticles against E. faecalis using current antibiotics. The antibiotics incorporated with MNPs were penicillin, vancomycin and streptomycin. The tests suggested that these acted by inhibiting E. faecalis biofilms. It was found that MNPs improved the antimicrobial efficacy of streptomycin because of the binding effect of MNPs to bacteria and the consequent membrane disruption. They can be used to develop improved strategies or biomedical devices (Grumezescu, 2014).

Magnetite nanoparticles doped with polyacrylamide (10–20 nm), MNPs stabilised with thioglycerol (Ramteke, 2010), FeO MNPs (66 nm) (Behera, 2012), Sucrose and Dextran coated MNPs (Ramteke, 2010) have showed effective inhibition against *E. coli*.

All these MNPs have prospective applications in the biomedical field, essentially as antimicrobial agents. MNPs showed their bactericidal potential due to presence of free radical groups like hydroxyl compounds, active oxygen generated species; reduced size and presence of specific sugars for the microbial enzymatic conversion.

## 5. LIPOSOMES FOR ANTIMICROBIAL DRUG DELIVERY

Liposomes are lipid vesicles, spherical in shape. These are bilayered membrane vesicles amphiphilic in nature and comprising of lipid molecules.

In 1965 liposome structure was first described, and in 1970s they were anticipated as new drug delivery systems.

Various studies and tests were carried out on their fundamental properties like lipid polymorphisms, lipid-protein and lipid-drug interactions leading to recognition of the potential of liposomes as a drug delivery system and started being transferred to practice (Vemuri, 1995). Dior in 1986 initially introduced the liposomes to the cosmetic. In 1995, the first liposomal drug delivery system accepted by the Food and Drug Administration was doxorubicin liposomes or Doxil to treat AIDS associated Kaposi sarcoma (Zhou, 2014). Liposomes can be made from either natural or synthetic lipids. Phosphotidylcholine is the commonly used lipids in the preparation of liposome. Phosphotidylcholine is an electrically neutral phospholipid containing fatty acyl chains of varying degrees of saturation and length. In order to adjust the membrane rigidity and stability cholesterol is used in the formulation. On the basis of structure liposomes can be dividedinto multileveled vesicles (MLVs), consisting of multiple phospholipid bilayer membranes and unilamellar vesicles(ULVs), consisting of a single lipid bilayer. Unilamellar vesicles can be further classified into smaller unilamellar vesicles(SUVs) and larger unilamellar vesicles(LUVs) depending on the size (Vemuri, 1995).

For synthesizing liposomes parameters to be taken into consideration are the physicochemical characters of the liposomal ingredient, various materials present within the liposomes, particle size, surface zeta potential, shelf time, polydispersity, batch-to-batch reproducibility and the possibility for large-scale production of safe and efficient products (Tan, 2016). Liposomes, mainly ULVs, are not formed spontaneously but they form when a sufficient amount of energy (e.g., via sonication, homogenization, shaking, or heating) is supplied to phospholipids placed in water. Typical methods for generating liposomes include sonication method like low shear rates which can result in MLVs and high shear rates which can generate ULVs, heading method and extrusion method (Olson, 1999).

## 6. POLYMERIC NANOPARTICLES FOR ANTIMICROBIAL DRUG DELIVERY

Various biocompatible and biodegradable polymers have been used extensively in the clinic for controlled drug release. The annual polymer-based controlled release systems are given to over 100 million patients each year. Langer and Folkman in 1976 developed the first polymer-based drug delivery system for macromolecule delivery. Earlier polymeric nanoparticles showed lesser therapeutic efficacy for the reason that they were rapidly cleared by the reticulo-endothelial system after intravenous administration. In 1994 this drawback was overcome after the finding of long-circulating stealth polymeric nanoparticles (Minamitake, 1994). These polymeric nanoparticles possessed quite a few unique properties for antimicrobial drug delivery. To begin with these polymeric nanoparticles can be produced with a sharper size variation and are usually structurally stable.

Moreover, various particle properties such as zeta potentials, size and drug release profiles can be accurately tuned by selecting different surfactants, polymer lengths and organic solvents during their synthesis. The surface of polymeric nanoparticles characteristically contain functional groups that can be easily modified chemically with either drug moieties or targeting ligands. Polymeric nanoparticles have been regularly coated with lectin for targeted antimicrobial delivery. Lectin is a protein that binds to simple or complex carbohydrates present on most bacterial cell walls. For example, lectin-conjugated gliadin nanoparticles were studied in treatment of Helicobacter pylori associated infectious diseases (Mehnert, 1998). This lectin conjugated nanoparticles in particularly go and attach to carbohydrate receptors present on cell walls of H. pylori bacteria and discharge antimicrobial agents into the bacteria.

Other polymeric nanoparticles usually contain linear polymers such as polymethyl methacrylate and polyalkyl acrylates that through an emulsion polymerization process form nanocapsules. In emulsion polymerization process, firstly the monomers are solubilized in a polymerization media in the presence of surfactants. In order to activate polymerization, to the solution the polymerization initiators are added resulting in the formation of nanocapsules. Antimicrobial drugs are incorporated to the nanocapsules either by absorption to the nanocapsules during the polymerization process or covalently conjugated to the nanoparticles surface after they are formed. Hydrophobic drugs are favored by the absorption process as it requires solubilizing the drugs to an oil phase. Through covalent conjugations hydrophilic drugs are attached to the particle. It should be noted that in the case of covalent linkage the antimicrobial agents can be inactivated and should be verified of their activity before use. For example, in the treatment of staphylococcal infections it was observed that upon covalent attachment to nanoparticles the lactam and ciprofloxacin retained their potency whereas penicillin was inactivated (Abeylath, 2008).

### 6.1. Nanoparticulate drug delivery platforms for advancing bone infection therapies.

There has been an imminent increase in the frequency of chronic bone infections. Nanotechnological developed various nanostructures hold the maximum possibility for providing minimally invasive and maximally regenerative therapies for this rare but persistent condition. Biodegradable polymeric carriers, for example, pose themselves as a natural alternative to the use of poly (methyl methacrylate) PMMA beads. To this end, the favorite choice has fallen on poly(α-hydroxyl esters), including poly(L-lactic acid) (PLLA), poly(glycolide) (PGA) and poly-(D,L-lactide-co-glycolide) (PLGA), all of which have been experimentally applied as antibiotic carriers for the treatment of osteomyelitis (Koort *et al.* 2008). An immediate upside of their application comes from their ability to encapsulate comparatively large amounts of both hydrophobic and hydrophilic drugs and exhibit extended drug release profiles. The latter can be furthermore made tunable via control over composition and processing conditions. In the case of PLGA, for example, the lactide-to-co-glycolide ratio could be used as a control parameter in setting the degradation and release time scales under physiological conditions to range from a year or more for PLLA to around 6 months for PLGA with the lactide-to-co-glycolide ratio of 1:1 to about a month for pure PGA. A detrimental effect that entails the use of poly (α-hydroxy esters) and that was observed in the past is that their acidic degradation

products may favor the bacterial growth and promote hard tissue resorption and bone mass loss . Chronic inflammation is, in fact, known to often result as a response to implantation of PLLA-based polymers in bone tissue engineering (Plachokova*et al.*., 2007). Since the antimicrobial effectiveness of antibiotics is found only within a relatively narrow window of pH values and could decrease by as much as 16-fold following a pH drop from 7.4 to 5.5, this acidification entailing partial or complete degradation of poly (α-hydroxy esters) can negatively interfere with the antimicrobial action. Despite their superior release properties, this has suggested the necessity of combining poly(α-hydroxy esters) with alkaline inorganics, such as calcium phosphates. An immediate upside of their application comes from their ability to encapsulate comparatively large amounts of both hydrophobic and hydrophilic drugs and exhibit extended drug release profiles. The latter can be furthermore made tunable via control over composition and processing conditions (Mistry, 2005). In the case of PLGA, for example, the lactide-to-co-glycolide ratio could be used as a control parameter in setting the degradation and release time scales under physiological conditions to range from a year or more for PLLA to around 6 months for PLGA with the lactide-to-co-glycolide ratio of 1:1 to about a month for pure PGA. A detrimental effect that entails the use of poly (α-hydroxy esters) and that was observed in the past is that their acidic degradation products may favor the bacterial growth and promote hard tissue resorption and bone mass loss. Chronic inflammation is, in fact, known to often result as a response to implantation of PLLA-based polymers in bone tissue engineering (Plachokova, 2007). Since the antimicrobial effectiveness of antibiotics is found only within a relatively narrow window of pH values and could decrease by as much as 16-fold following a pH drop from 7.4 to 5.5, this acidification entailing partial or complete degradation of poly (α-hydroxy esters) can negatively interfere with the antimicrobial action. Despite their superior release properties, this has suggested the necessity of combining poly (α hydroxy esters) with alkaline inorganics, such as calcium phosphates (Uskoković *et al.*., 2011).

## 7. SOLID LIPID NANOPARTICLES FOR ANTIMICROBIAL DRUG DELIVERY

Solid lipid nanoparticles (SLNs) as antimicrobial drug delivery system have attracted much attention since 1990s. SLNs have mean diameters ranging from 50 nm to 1000 nm for various drug delivery applications (Mühlen, 1998). SLNs are mainly comprised of lipids that are in solid phase at the room temperature and surfactants for emulsification. Solid lipids used in SLN formulations contain fatty acids (decanoic acid, palmitic acid and behenic acid), triglycerides (trimyristin, trilaurin and tripalmitin), partial glycerides (glycerylmonostearate and gylcerylbehenate), steroids (cholesterol) and waxes (cetylpalmitate). Various types of surfactants are commonly used in form of emulsifiers so as to stabilize lipid dispersion, including soybean lecithin, poloxamer 188, phosphatidylcholine, sodium glycocholate and sodium cholate. Various methods of preparing SLNs include high shear mixing, spray drying, ultra-sonication, and high pressure homogenization (Eldem, 1991). SLNs have exceptional properties like large surface area, small size, the interaction of phases at the interfaces, high drug loading and are attractive for their potential to enhance performance of antimicrobial agents, pharmaceuticals and other materials. Several unique properties of SLNs make them a promising antimicrobial drug delivery platform, leading to a few cosmetic and pharmaceutical products for skin care applications. To start with the application of SLNs on skin, readily form a thin film and reduces water evaporation and retain the moisture of the skin (Mehnert, 1998). The SLNs occlusive property promotes molecule penetrations into the skin. Moreover, SLNs encapsulated antimicrobial agents such as retinol and retinylpalmitate have shown slower drug expulsion than the free drug counterparts and better drug penetration rate (Muller, 2002). SLNs are stable in water and dermal cream and therefore can be readily included into cosmetic preparations. In addition to topical applications, SLNs in the forms of tablets, capsules and pellets can also be used for oral administration. Tobramycin is an orally administered antimicrobial drug for cystic fibrosis patients. P. aeruginosa infections commonly parasitize in the gastrointestinal tracts of the cystic fibrosis patients. Tobramycin mainly inhibit these P. aeruginosa infections (Yang, 1999). Another example of SLNs drug delivery system includes the inhibition of M. tuberculosis. It involves the pulmonary delivery of antimicrobials to treat tuberculosis which is a serious lung infection caused by M. tuberculosis. In some severe cases, tuberculosis infection spreads from the lungs and affects the lymphatic systems. SLNs can ease the delivery of anti-tuberculosis drugs such as rifampin, isoniazidand, pyrazinamide to the lungs as well as to the lymphatic systems (Pandey, 2005).

### 7.1. Antimicrobial nanotechnologies: what are the current possibilities?

The current challenges of multi-drug resistance development in human pathogenic microorganisms are engaging researchers in exploring the field of nanotechnology-derived approaches and products as new tools of key developments for manufacturing effective antimicrobials. But, the real contributions are still uncertain. Although there are several budding leads of nanotechnology and the growing trends in publications and patents, therapeutic microbiological applications have not yet made it to the market. Numerous reasons could explain the scarcity of commercial applications. These include high initial production investments, new nanotechnology regulation in the developed and developing countries, and public perception. The rapid progress of nanotechnology in other key areas may over time be transferred to therapeutic microbiological applications as well, and accelerate their development (Shingai, 2006).

These encouraging developments also concern the antimicrobial pharmaceutical sector, in which continuous breakthrough innovations are strongly needed because of the development of antibiotic resistant and virulent strains of the bacteria. Since the 20th century, infectious diseases have been treated using different technological innovations, including antibiotics and vaccines, and scientific community is now seeking in nanotechnology a new paradigm in developing antimicrobials, especially to overcome

antimicrobial drug resistance (Mahmoudi et al., 2011). Polymeric nanoparticles-based delivery devices are also being explored for a wide range of agents, including small molecules, peptides, proteins and nucleic acids. Researchers have now started using these nano-factories for developing novel antimicrobials that modulate the quorum sensing systems of bacteria rather than their viability. In addition, medical devices play a critical role in modern health-care practice, but their use may increase the risks of nosocomial infection. To solve this issue, numerous nanomaterial-coated or embedded medical devices including catheters, endotracheal tubes and wound dressings have been developed and approved for clinical use (Parisi, 2014). Multinational industries are searching the potential that nanotechnology interventions offer in the antimicrobial sector. The nanoscale could be used to one or more dimensions and the form of the particles can be in aggregate, agglomerates or nano-scaled materials. Additionally, nanomaterials are used in different pharmaceutical sectors, but the clinical approval process is structured to ensure that sponsors demonstrate adequate safety and efficacy before a product is released in the market (Zhang, 2010).

Several nations are now providing definitions and regulatory frameworks for the application of nanotechnology in medicine17. In the European Commission (EC), the main regulation covering nanotechnology applications is the REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) (Parisi, 2014). The EC has recently (2011) adopted a recommendation on the definition according to which 'nanomaterial' means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions are in the size range 1–100 nm. According the US Decision- Making Concerning Regulation (DMCR), some definitions apply the size range to two or more dimensions using either external or internal structures as units to be measured. Some definitions also include criteria related to physical or chemical characteristics (e.g. size distribution, shape, charge, or the ratio of surface area to volume), or to the display of unique or novel properties or 'nanoscale phenomena' (Kaparissides, 2006).

In addition to economic and technological benefits, the challenges associated with the safety of nanomaterials and nanotechnologies are global. The commercial benefits may be outweighed by the potentially unsafe characteristics of nanomaterials. Meanwhile, nanomaterials and nanotechnologies are assuring the ability of key pharmaceutical sectors to compete globally; their success also demands a guarantee of their safety. But, some survey-based studies on consumer preferences reveal that overall public opinion is not negative or neutral towards nanotechnology, and that it is particularly influenced by perceived benefits and usefulness of the technology (Satterfield, 2009).

In conclusion, antimicrobial-nanotech innovative products/drugs are facing complications in reaching the market, making antimicrobial drugs still a marginal sector for nanotechnology. This is due in specific to the high production costs, unclear technical benefits and judicial uncertainties, as well as public opinion. However, the research and development landscape is encouraging and the opportunities offered by nanotechnology in the development of nano-medicines for combating microbial infections are being actively explored. Moreover, nanotechnology is becoming the driving force behind a variety of developments in other fields as well. The knowledge gained in other emerging research areas, including diagnosis, targeted drug delivery, medical devices, energy and packaging, may over time be transferred, or provide spill-overs, to antimicrobial drug development applications as well. For example, surface modifications of antimicrobials using nanoparticles could also enhance their antimicrobial effect along with the range of combined therapy (antibiotics plus antimicrobial nanoparticles), and quorum sensing nano-inhibitors can provide real solutions for effectively combating infectious diseases.

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## 6. CONFLICTS OF INTEREST

The authors declare no conflict of interest.