Research & Reviews in Biotechnology & Biosciences
Website: www.biotechjournal.in
Volume-8, Issue No: 1, Year: 2021 (January-June)
DOI: http://doi.org/10.5281/zenodo.5118458

ISSN No: 2321-8681
Research Paper
PP: 5-21
Peer Reviewed Journal

Antimicrobial Activities of Single Nano particle (SNPs) & Core-shell Nanomaterials (CSNMs) with Special Reference to Binding Mechanisms

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Article History

Received: 25/01/2021
Accepted: 27/02/2021

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ABSTRACT

Recently single Nanoparticles (SNPs) /Nanomaterials (NMs) are gaining much attention due to their peculiar characteristic properties even in biomedical field is one of it. The scope of nanoparticles/ NMs in future is vast and promising yet it is facing some challenges due to toxicity and scalability. However, the anti-microbial activity of some metal nanoparticles shows a deep impact and can act as an alternative to pharma drugs amidst of their drug resistance, dosage, side effects, half-life, cytotoxicity & inventory issues.

Scientists, over the years are solving the puzzles behind the mechanisms of binding of different nanoparticles to the microbial surface and their mode of action. Core-shell nanomaterials (CSNMs) are proved to be better when compared to single nanoparticles in terms of zone of inhibition. This may be due to different algorithms of their properties like composition, size & shape with additional moiety.

This review article explains detail synthesis, characterization of SNPs/ CSNMs different types along with the functional group moieties present in the microbial surface which can bind specifically or non- specifically to these nanoparticles. This article also explains binding mechanism of different single nanoparticles as well as coreshell nanomaterials. Finally, the comparison of single & core-shell nanoparticles as an anti-microbial agent has been analyzed along with their future prospectus.

Keywords: Nanomaterials; Core-shell nanomaterials; Microorganisms; Antimicrobial agents; Binding sites.

Research & Reviews in Biotechnology & Biosciences ISSN No: 2321-8681
Website: www.biotechjournal.in **Research Paper**
Volume-8, Issue No: 1, Year: 2021 (January-June) PP: 5-21
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1. INTRODUCTION

Nanotechnology is a field of Science known from last century. Nanomaterial is matter/ material having at least one dimension sized from 1 to 100 nanometres (Vert et al., 2012). Nanotechnology is manipulation of matter at the level of atomic, molecular, supramolecular scale (Narayan et al., 2004). Nanoparticles are one of the types of nanomaterials which consist usually of agglomeration of particles with sizes in particular distribution, ranging in nanometers (Ealia and Saravanakumar, 2017). 'Nano' should be considered as different state of aggregation of matter because nanoparticles have quite different physical & chemical properties than bulk material (Ealia and Saravanakumar, 2017). This strange behavior of nanoparticles can make them useful for biomedical application.

Microorganisms or microbes are microscopic organisms which exist as single cell or in a colony of cells (Lim, 2001). Unicellular prokaryotes & eukaryotes are taken into consideration as microbes. Microbes can be found anywhere on earth including air, water, soil, rocks & earth's crust & in body of organisms (Szewzyk et al., 1994). Some microorganisms are beneficial or non pathogenic to host while some are harmful or pathogenic because of their characteristic structures. They range in size from less than 100 nm to almost a millimetre (Batt, 2016). Cell wall is important structural component of microbial cell which help for protecting cell against environment. Cell membrane present inner to cell wall is also involved in chemical interactions with substances foreign to microbial cell.

2. CLASSIFICATION OF MICROORGANISMS

Bacteria & Archea are prokaryotic while most protozoa, some fungi & algae are eukaryotic microbes. Viruses are also included in microorganisms according to some definitions (Batt, 2016).

2.1 Bacteria

Bacteria represent most wide group of microorganisms actively involved in various ecological & environmental reactions (Szewzyk et al., 1994). Bacteria typically have size of few microns with number of shapes & morphologies. They may exist as single cell or associate with characteristic patterns. Bacterial cells are surrounded by cell membrane & cell wall. Being prokaryotes, they lack membrane bound well organized organelles (Lodish et al., 2013). On the basis of cell wall & membrane associated with it; they are classified as Gram positive & Gram negative bacteria. Gram positive bacteria have thick layer of peptidoglycan while that of Gram negative bacteria have thin layer with an additional outer membrane of lipopolysaccharide. Bacteria possess extracellular structures such as flagella, capsule, fimbriae & pili (Beachy, 1981). These structures are often essential in virulence of pathogenic bacteria.

2.2 Fungi

Fungi belong to the group of microorganisms that include eukaryotic yeasts & moulds & placed in separate kingdom in classification. Fungi include symbionts of plants, animals & also parasites. Fungal cells contain membrane

ISSN No: 2321-8681
Research Paper
PP: 5-21
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bound nuclei & cell organelles. Fungal cell wall is made up of mannose, glucans & chitin. Fungal cell is characterized by presence of mycelium, microconidia & macroconidia at various stages of lifecycle (Warnock, 2012).

2.3 Algae

Microalgae are microscopic organisms; having size ranging from few microns to few hundred microns (Pepper and Gentry, 2015). They belong to Kingdom algae which are eukaryotic organisms & hence have well developed cell organelles. Cell wall is composed of several layers of chemical groups different for different species of algae. Major components of cell wall are cellulose, glycoproteins, polysaccharide along with some other material forms rigid network (Palmer et al., 2004).

2.4 Protozoa

Protozoa are single celled eukaryotes having size 1 micrometers to several millimeters. Unlike fungi & algae, they do not possess rigid cell wall (Pepper and Gentry, 2015). They are enveloped by elastic structures of membrane. In some protozoa, cell is supported by membranous envelope called pellicle. They have well organized cell organelles such as cilia, vacuoles, oral groove, mitochondria, anal pore & nucleus. Most of the protozoa go through stage of life cycle called oocyst; which is hard thick walled & resistant to environmental conditions & antimicrobial agents (Panno, 2014; Bertrand, 2015).

2.5 Viruses

Viruses are the type of microorganisms which require living cells for their own replication (Pepper and Gentry, 2015). The viral particle is composed of- Genetic material (RNA/DNA) & Capsid, which is a coating around the genetic material. The capsid is made up of proteins. In some viruses there is another additional outer membrane made of lipids called as envelope (Pepper and Gentry, 2015). Generally, the envelope is host derived. The virus can be of any shape ranging from simple helical, icosahedral structure to the complex structure.

CLASSIFICATION OF NANOPARTICLES

Nanoparticles are present as simple structure or composite structure & hence named as single nanoparticles & core-shell nanoparticles respectively.

2.2 Single nanoparticles

Based on dimensions, nanoparticles are classified as into three: One dimension, two dimension & three dimension nanoparticles (Bhatia, 2016, Preeti Nigam, 2015). Based on chemical nature, they can be classified as organic, inorganic & carbon based nanoparticles (Refer Figure 2). Dendrimers, micelles & liposomes, etc are called as organic nanoparticles & have properties of biodegradable & nontoxic. Metal & metal oxides are categorized into inorganic nanoparticles. Almost all metals can be synthesized into their nanomaterials (Ealia and Saravana kumar, 2017). Most commonly used metals for nanoparticles synthesis are Aluminium (Al), Cobalt (Co), Gold (Au), Copper (Cu), Iron (Fe), Lead (Pb), Silver (Ag), Zinc (Zn). Metal oxide nanoparticles are synthesized to modify the properties of metal nanoparticles (Waghmode et al.,2013).

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3.2 Core-shell nanoparticles

Core-shell nanoparticles are composite structures formed by using particular Nanomaterial to coat another Nanomaterial (Shi, 2015). They are defined as comprising a core (inner material) & shell (outer material) (Ghosh-Chaudhuri and Paria, 2011). Because of surface modifications these nanoparticles provide new properties beyond the approach of single nanoparticles (Shi, 2015). Core-shell nanoparticles are favored by researchers because of unique properties such as stability, control, self assembly, enhanced biological properties. The choice of shell mainly depends on application of nanoparticles (Ghosh-Chaudhuri and Paria, 2011).

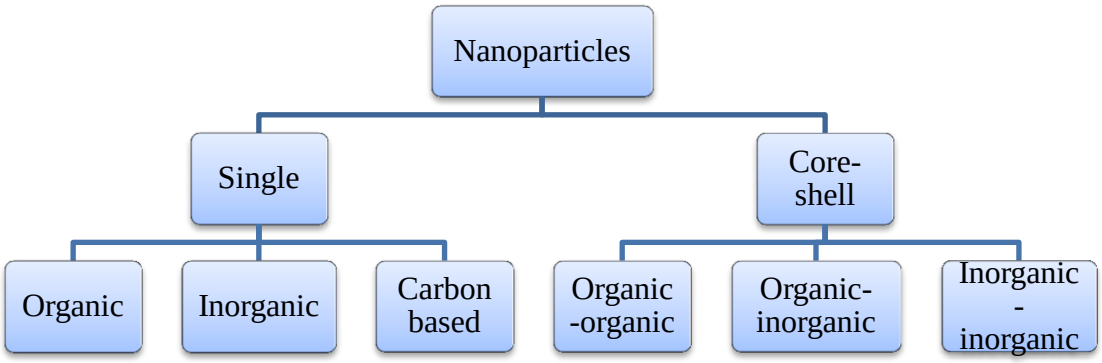

Figure 2: Classification of Nanoparticles

Depending upon reaction occurring between core & shell, they are classified as chemical coated & physical coated nanoparticles. Depending upon components involved in them, classified as organic-inorganic, inorganic-organic & inorganic-inorganic (Refer Figure 2) (Shi, 2015). Examples of core-shell nanoparticles include $Ag@ZrO_2$, Pd@Ir, Pd@Ir, $AgCl@SiO_2$, ZnO@APTES, ZnO@Polyacrylamide, Chitosan-Ag, Cu/Cu_2O@TEG, Cu_2O@Tween, AIS@ZnS, $Fe_3O_4@C_{18}$, $Ag@Fe_3O_4$, γ-Fe_3O_4@Ag, ZnO@PAH, Au@glucose & many more. Complexity & diversity of core-shell nanoparticles give us broader sense of their applications.

3. NANOPARTICLES AS ANTIMICROBIALS

Synthesis of nanoparticles using biological agents such as bacteria, fungi, algae, protozoa is well known field (Buzea and Pacheco, 2016). Metals & metal oxide nanoparticles can act as alternative approach to conventional drugs such as antibiotics in case of various infectious diseases caused by bacteria, fungi, parasites & viruses. Nanoparticles because of ultra- small dimensions & wide surface area provide opportunity to interact with components of pathogens such as cell wall, cell membrane & internal cell organelles like DNA & enzymes (Allahverdiyev et al., 2013). Large numbers of single & core- shell nanoparticles can be used with great

potential & suitability (Khezerlou et al., 2018). Microbes generate antibiotic resistance rapidly; but in case of nanoparticles microbes need to acquire multiple mutations to become resistant. Multidrug resistance in case of antibiotics is very pronounced; while in case of nanoparticles drugs resistance has been reported very rarely.

Scope of this review is to summarize the different types of nanoparticles, their antimicrobial action & binding mechanism to microorganisms.

4. MATERIALS AND METHODS

5.1 Synthesis of Nanoparticles

The synthesis of nanoparticles can be achieved by employing various methods according to our need. Various studies have showed high number of bottom-up (where atoms get assembled on their own to form a nanoparticles) and top-down (where the large molecules are broken down to get the particle of nano size) methods of nanoparticles synthesis (Khatami et al., 2018). Despite of practicality of top-bottom method, widely preferred bottom-up method achieves the synthesis of nanoparticles, especially core shell nanoparticles in following ways (Khatami et al., 2018).

a] One-step synthesis: - Core and shell are synthesized simultaneously.

b] Two-step: - First, core is synthesized and then shell is coated around the core.

c] Multiple-step: - First, core is synthesized and then the first coat of shell is applied. After that the second coat of shell is applied on the surface of first shell.

In case of single nanoparticles synthesis also these two approaches are used. The few ways of bottom-up method of nanoparticles synthesis are mentioned below (Pareek et al., 2017).

a] Chemical Reduction Methods: In this method, nanoparticles are synthesized by the reduction of ionic salts by reducing agents (E.g. hydrogen, hydrazine etc.) in suitable medium. The medium conditions are maintained with stabilizing agents (Pareek et al., 2017).

b] Microemulsion Methods: In this method, salts and reducing molecules are dispersed in water-in-oil or oil-in-water emulsion and are then mixed where surfactant is present in the medium (Pareek et al., 2017).

c] Electrochemical Methods: In this method, the anodically dissolved metal sheet forms an intermediate compound which is then reduced at cathode and nanoparticles are obtained (Pareek et al., 2017).

d] Laser Ablation: In this method, the high flux laser radiations are used to convert the material into plasma in order to obtain the nanoparticles (Pareek et al., 2017).

e] Microwave Methods: In this method, the reactants- metal salts and polymer surfactants forming a solution are irradiated with microwaves and nanoparticles are synthesized (Pareek et al., 2017).

The approaches of top-down synthesis of nanoparticles are as follows (Pareek et al., 2017).

a] Micropatterning: The method involves, bombarding the precursor material with beams of electrons or UV/X-ray to remove

the particles of nano size (Pareek et al., 2017).

b] Pyrolysis: In this technique, the precursor material is forced through an orifice with high pressure. The outcome material is allowed to burn and the nanoparticles are recovered from the ash (Pareek et al., 2017).

c] Attrition: In this method simply, the precursor materials are crushed to produce nanoparticles. The process is also called as milling (Pareek et al., 2017).

Biological synthesis of nanoparticles mainly using plants and microorganisms is another developing approach these days. Microorganisms like bacteria, yeast and fungi are being explored for the nanoparticles synthesis due to their capacity to reduce metal salts with the help of reductase like enzymes (Singh et al., 2016). Due to low cost, eco-friendly nature, biocompatibility and medical applicability Phyto nanotechnology has given a new aspect to the nanoparticle's synthesis from plants (Singh et al., 2016).

5.2 Characterization of Nanoparticles

The characterization of nanoparticles SNPs/NMs/CSNMs is done by using two types of techniques i.e. spectroscopic and imaging with respect to the physical parameters like size & shape; their interaction with other biological & non-biological molecules etc. Usually employed techniques for the characterization Scanning Electron Microscopy (SEM), High Resolution Transmission Electron Microscopy (HRTEM), powder X-Ray Diffraction (XRD), Dispersive spectroscopy X-ray (EDX), X-ray Photoelectron spectroscopy (XPS) and for functional group detection, Fourier Transform Infrared spectroscopy (FTIR) etc (Waghmode et al.2014, Arruda et al., 2014). With these techniques characteristics like morphology, shell coating, complexity of nanoparticles, dimensions are determined (Arruda et al., 2014).

5.3 Isolation of microorganisms

5.3.1 Bacteria: *E. coli*, a Gram negative organism and *Staphylococcus aureus*, a Gram positive organism are mostly used as representative bacteria for the study. Generally these are strain specific organisms procured from various culture collection centres and maintained on the Muller-Hinton agar/broth (Nagaonkar and Rai, 2015; Mirahmadi-Zare et al., 2018).

5.3.2 Fungi: In the class of fungi, *Candida sp, Aspergillus sp*. are the representative organisms. Usually the procured strains of the fungi are maintained on Sabouraud's agar/broth (Dobrucka and Dlugaszewska, 2018).

5.3.3 Algae: Unlike bacteria and fungi, the need of culture media differs from species to species in case of algae group. The strains procured from culture collection centres are maintained accordingly. E.g., *Chlamydomonas sp*. are reported to be cultured on Tris-acetate-phosphate (TAP) medium (Halbus et al., 2020) while *Chlorella sp*. like fresh water algae are cultured and maintained on Algo-Gro freshwater media (Chen et al., 2012).

5.3.4 Protozoa: Like algae the medium used for culture maintenance differs according to the need. Organisms like *Leishmania tropica* are cultured on medium 199 with 10% inactivated FBS (Ahmed et al., 2015) while

some other reports stated the use of $RPMI_{1640}$ medium for *Leishmania major* (Jebali and Kazemi, 2013).

5.3.5 Viruses: Though the living or non-living categorization of viruses is a topic of debate, they do need living cells for their survival. Kidney cell lines (Mori et al., 2013; Gaikwad et al., 2013) are some of the popular cell lines used to culture the viruses. Embryonated eggs & animal inoculation techniques can also be used.

5.4 Inhibition of Microorganisms

5.4.1 Bacteria: To check the antibacterial effect of synthesized nanoparticles the Kirby-Bauer disc diffusion method is preferred (Nagaonkar and Rai, 2015; Mirahmadi-Zare et al., 2018). In this method the organism is uniformly spread on the agar and then the discs dipped in nanoparticles solution are placed and plates are incubated at appropriate conditions. After 24-48 hours plates are checked for zone of inhibition and potential of nanoparticles as antibacterial is decided (Nagaonkar and Rai, 2015; Mirahmadi-Zare et al., 2018).

5.4.2 Fungi: For fungi the aforementioned method can be used (Dobrucka and Dlugaszewska, 2018). Some studies have modified the method and instead of discs, wells are made and specific volume of nanoparticles solution is added in the wells and after sufficient incubation zone of inhibition is checked (Dobrucka and Dlugaszewska, 2018).

5.4.3 Algae: In case of algae, cell viability assays are preferred, in which the cell concentration before the addition of nanoparticles and cell concentration after the incubation with nanoparticles is compared (Halbus et al., 2020).

5.4.4 Protozoa: To check the antiprotozoal potential of nanoparticles, like algae, cell viability assays are first choice and by calculating % viability, effect of nanoparticles is interpreted (Ahmed et al., 2015).

5.4.5 Viruses: The antiviral activity of nanoparticles is checked in similar ways mentioned above. The nanoparticles and viral suspensions are allowed to interact with each other in a suspension and then the supernatant of the suspension is transferred into preferred cell lines to check the infectivity (Mori et al., 2013). If the number of cells infected is less it implies the good antiviral activity exhibited by nanoparticles. A viral plaque assay is another strategy used where plaques (zone of inhibition) are formed due to viral infection to the host cells (Gaikwad et al., 2013). If plaque number is reduced on treatment with nanoparticles, then it is said that nanoparticles can be used as efficient antiviral.

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ISSN No: 2321-8681
Research Paper
PP: 5-21
Peer Reviewed Journal

5. RESULTS & DISCUSSION

5.1 Antimicrobial activity of Single & Core-Shell Nanoparticles

Sr. No	Nano molecule	Type of nano-particle	Size of nano-particle (nm)	Name of microorganism	Action	Binding site	Ref.
1	Al_2O_3	Single	60	*E. coli, Bacillus subtilis, P. fluorescence*	Anti bacterial	Cell wall	(Jiang et al., 2009).
2	Au	Single	10	*Staphylococcus aureus*	Anti bacterial	Cell membrane	(Fang et al., 2011).
3	Au@Ag	Core-shell	30-34	*E. coli, P. aeruginosa Enterococcus faecalis, Pediococcus acidilactici*	Anti bacterial	Cell Wall/ LPS of outer membrane	(Banerjee et al., 2011).
4	Fe_3O_4@Au	Core-shell	10	*E. coli*	Anti bacterial	FimH protein of pili	(Park et al., 2016).
5	Ag@ ZrO_2	Core-shell	38-40	*E. coli, S. aureus*	Anti bacterial	Cell membrane	(Dhanalekshmi and Meena, 2015).
6	Ag	Single	30	*Aspergillus niger*	Anti fungal	Cell wall	(Zubaidi et al., 2019).
7	Chitosan-Ag	Core-shell	373	*Fusarium oxysporum*	Anti fungal	Cell Wall, Mycelium	(Dananjaya et al., 2017).
8	$NiFe_2O_4$@ Ag; $NiFe_2O_4$@ Mo	Core-shell	35	*Alternariasolani, Fusarium oxysporum*	Anti fungal	Cell Wall, Cell Membrane	(Golkhatmi et al., 2017).
9	TiO_2	Single	21	*Nitzschia closterium*	Antialgal	Cell wall	(Xia et al., 2015).
10	ZnO@PAH	Core-shell	135	*Chlamydomonas reinhardtii*	Antialgal	Cell wall, cell membrane, chloroplast	(Halbus et al., 2020).
11	ZnO@Ag	Core-shell	20-50	*Leishmania major*	Anti protozoal	Cell membrane	(Nadhman et al., 2014).
12	TiO2@ Ag	Core-shell	20-50	*Leishmania tropica*	Anti protozoal	Cell membrane, enzymes, DNA	(Allahverdiyev et al., 2013).
13	Ag	Single	50	Hepatitis B	Antiviral	Surface	(Lu et al., 2008).
14	TiO_2	Single	25	Ms2 Bacteriophage	Antiviral	Capsid coat	(Syngouna and Chrysikopoulos, 2017).

Table 1: Examples of Nanoparticles showing diverse antimicrobial activity

Antimicrobial activity of Single & Core-Shell Nanoparticles of some metals and their oxides has been studied for many years (Khezerlou et al., 2018). It is observed that diverse numbers of nanoparticles Single & Core-Shell are able to bind microorganisms. These include Aluminium (Al), Cobalt (Co), Gold (Au), Copper (Cu), Iron (Fe), Lead (Pb), Silver (Ag), Zinc (Zn) & their oxides. Nanoparticles binding eventually result into death of microorganisms. On the basis of activity of nanoparticles against microorganisms can be divided as

ISSN No: 2321-8681
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PP: 5-21
Peer Reviewed Journal

antibacterial, antifungal, antialgal, antiprotozoal & antiviral (Refer Table 1).

The single and core shell nanoparticles of diverse size range are seem to be useful as an effective antimicrobial agents (Khezerlou et al., 2018). The binding sites for the NPs like components of cell wall, cell membrane are common (Chen et al., 2012; Slavin et al., 2017; Xia et al., 2016). But the pili proteins, mycelium & DNA are some specific binding sites reported for the potential antibacterial, antifungal & antiprotozoal nanoparticles (Lin et al., 2002; Lyden et al., 2017).

6.2 Sites involved in binding of Single & Core-Shell Nanoparticles to bacteria

The exact mechanism for antimicrobial activity of nanoparticles is still being researched. It is not surprising that nanoparticles could bind to surface & also could get internalized by microorganisms as they do for heavy metals (Diep et al., 2018). But it is surprising that nanoparticles show different level of binding to microorganisms than metals of the same (Lim, 2001; Ealia and Saravanakumar, 2017). Actively involved binding sites of bacteria & their composition is listed in Tables 2.

Table 2: Sites involved in binding of Single & Core-Shell Nanoparticles to bacteria

Sr. No	Binding site		Composition	Possible functional groups/ chemical moieties involved in binding	Ref.
1	Cell wall	Gram Positive	Peptidoglycan	Peptide port, N-acetylglucosamine & N-acetylmuramic acid glycan strands.	(Slavin et al., 2017; Webster and Seil, 2012).
		Gram Negative	lipopolysaccharide (LPS), lipoproteins, teichoic acid	Phosphorylated glucosamine group of Lipid A domain.	
2	Cell membrane		Phospholipids, glycolipids, sterols, glycoproteins, G proteins	Sulphur groups of sulphur containing proteins, fatty acid groups of lipids & carboxylic groups.	(Slavin et al., 2017; Fang et al., 2011; Morones et al., 2005).
3	Type I Pili		Fim A, Fim H pilin proteins	Peptide of protein Fim H; amino acid involved is not known.	(Lin et al., 2002).
4	Enzymes		Cystein & other amino acids	Thiol group of cystein.	(Slavin et al., 2017).
5	DNA		Nucleotide, deoxy-ribose sugar, nitrogen bases	Phosphorous moieties of nucleotide.	(Morones et al., 2005).

Functional groups of components of cell wall & cell membrane remain the primary binding sites for SNPs (Fang et al., 2011; Lin et al., 2002). Gram positive bacteria are found to be resistant to some nanoparticles due to presence of peptidoglycan in cell wall (Slavin et al., 2017; Webster and Seil, 2012). Comparatively less thick cell wall of Gram negative bacteria allows the penetration & binding of NPs to the components of cell membrane. Pili proteins, sulphur moieties of various respiratory enzymes & phosphorus moieties of DNA are some unique target sites for the NPs attachment (Fang et al., 2011; Xia et al., 2016; Rai et al., 2018).

Table 3: Sites involved in binding of Single & Core-Shell Nanoparticles to fungi

Sr. No	Binding site	Composition	Possible functional groups/ chemical moieties involved in binding	Ref.
1	Cell wall	Chitin, ß-1,3 glucan, glycoproteins, mannoproteins	Sulphates, amines, carboxylic, hydroxyls, aldehyde groups.	(Xia et al., 2016).
2	Mitochondria	Outer & inner membrane, cristae,	Membrane proteins; amino acids involved are not known.	(Zhang et al., 2013).
3	Mycelium, microconidia, macroconidia	Cell wall & Membrane	Surface proteins; amino acids involved are not known. Sulphates, amines, carboxylic, hydroxyls, aldehyde groups.	(Lyden et al., 2017).
4	Proteins	Sulphur containing & non-sulphur amino acids	Sulfahydryl group of sulphur containing amino acids.	(Xia et al., 2016; Rai et al., 2018).
5	DNA	Nucleotide, deoxy- ribose sugar, nitrogen bases	Phosphorous moieties of nucleotide.	(Osonga et al., 2020).

Nanoparticles have shown their antifungal activity against various pathogenic & non pathogenic fungi. Actively involved binding sites of fungi & their composition is listed in Tables 3. They initiate lethal effect by first binding to surface of cells (Xia et al., 2016). The functional groups like amines, carboxyl, aldehyde etc. of fungal cell wall are some target sites for attachment of NPs (Zhang et al., 2013). Also nanoparticles can attach to mitochondrial proteins, regular functional proteins & nuclear proteins & can carry out the inhibitory function (Osonga et al., 2020; Li et al., 2015; Wang et al., 2019).

Table 4: Sites involved in binding of Single & Core-Shell Nanoparticles to algae

Sr. No	Binding site	Composition	Possible functional groups/ chemical moieties involved in binding	Ref.
1	Cell wall	Cellulose, glycoprotein, mannoproteins, xylans, sulphonated polysaccharides, phlorotannins	Cilicic acid, alginic acid of xylans, calcium ions, sulphonate group of polysaccharides. Glycoproteins.	(Li et al., 2015; Chen et al., 2012).
2	Pellicle	Glycoproteins	Amphiphilic proteins, charged groups on proteins such as glutamic acid, aspartic acid, histidine, tryptophan, unusual amino acids	(Li et al., 2015).
3	Cytoplasm	Cell organelles & cytoskeleton	Binds to sulphur present in biomolecules to form silver thiolates & ß-Ag_2S.	(Wang et al., 2019).
4	Extracellular products	Mono/ polysaccharides, fatty acids, hydrocarbons	Sulphate, amines, carboxylic, hydroxyls, aldehyde groups.	(Melegari et al., 2013; Zhao et al., 2016).
5	Cytosole & vacuoles	Organic, inorganic molecules, enzymes, proteins	Not known.	(Melegari et al., 2013).

The algal cell wall surrounding the algal cell acts as barrier for uptake of nanoparticles freely. Algal cell wall is semi-permeable having pore size of 5-20 nm. It has been hypothesized that only nanoparticles smaller than pore size can cross the barrier

ISSN No: 2321-8681
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& are internalized by endocytosis (Li X. et al., 2015). Actively involved binding sites of algae & their composition is listed in Tables 4. Acidic components present in the cell wall of algae, charged groups of the glycoproteins of pellicle are some of the primary binding sites for the antialgal NPs (Jebali and Kazemi, 2013; Melagari et al., 2013). Sulphur moieties present in the cytoplasm or functional groups of metabolites present may serve as binding sites for NPs (Zhao et al., 2016; Syngouna and Chrysikopoulos, 2017).

Table 5: Sites involved in binding of Single & Core-Shell Nanoparticles to protozoa

Sr. No	Binding site	Composition	Possible functional groups/ chemical moieties involved in binding	Ref.
1	Cell membrane	Phospholipids, glycolipids, sterols, glycoproteins, G proteins	Carboxylic & amine groups of weak fatty acids	(Ahmed et al., 2015).
2	Oocyst	Galactose, glucose, mannose	Not known	(Khezerlou et al., 2018).
3	DNA	Nucleotide, deoxy- ribose sugar, nitrogen bases	Phosphorous moieties of nucleotide.	(Ahmed et al., 2015).
4	Enzymes	Thiol containing amino acids	Thiol groups, cysteine residues	(Khezerlou et al., 2018).

Some nanoparticles & their ions can bind to various sites of protozoa. Actively involved binding sites of bacteria & their composition is listed in Tables 5. The major lipid composition of cell membrane provides carboxylic & amine groups of fatty acids as the ideal binding sites for NPs (Mori et al., 2013). Nanoparticles can bind to phosphorous moieties of DNA too (Mori et al., 2013).

Table 6: Sites involved in binding of Single & Core-Shell Nanoparticles to viruses

Sr. No	Binding site	Composition	Possible functional groups/ chemical moieties involved in binding	Ref.
1	Capsid coat	Protein	Not known	(Syngouna and Chrysikopoulos, 2017).
2	Envelope	Phospholipids, proteins, glycoproteins, lipids	Glycoproteins gB, gC, gD, gH, gL, gp120 & fatty acid groups of lipids.	(Antonie et al., 2012; Lara et al., 2010).
3	Viral attachment ligands	Lipids, proteins	Cell surface receptor proteins, amino acids involved such as lysine	(Cango et al., 2018).

Most of the nanoparticles bind irreversibly to target sites of viruses. Some investigations focus on synthesizing nanoparticles by targeting particular site of virus (Antonie et

ISSN No: 2321-8681
Research Paper
PP: 5-21
Peer Reviewed Journal

al., 2012). Actively involved binding sites of bacteria & their composition is listed in Tables 6. The viral capsid, envelope like structures are dominated by protein content followed by lipid molecules. Hence the amino acids & fatty acids are the usual binding sites for NPs (Lara et al., 2010; Diep et al., 2018).

6.3 Comparison of Single & Core-Shell Nanoparticles with respect to size, shape, binding site & inhibition

Antimicrobial efficiency & effectiveness depends on morphological characteristics, which has two important factors: material & size (Chen et al., 2012). Whether nanoparticles are single or cores-shell affects the binding strength of nanoparticles. When single & core-shell nanoparticles taken in same concentration are studied for their antimicrobial activity, it has been shown that shell adds extra benefit to core-shell (Halbus et al., 2020). Although the core & shell part of core-shell nanoparticles is made of different materials, the binding site remains same in most cases (Refer Table 7).

Table 7: Comparison of Single & Core-Shell Nanoparticles with respect to size, shape, binding site & inhibition.

Type	Nanoparticles		Shape, Size (nm)		Binding Site		Inhibition	
	Single	Core-shell	Single	Core-shell	Single	Core-shell	Single	Core-shell
Antibacterial	Ag	Ag@Au	Irregular 11-26	Spherical 20-25	Cell Wall	Cell Wall	10-12 mm	13-16 mm
Antifungal	Ag	Ag@Au	Irregular 11-26	Spherical 20-25	Cell Wall	Cell Wall	10 mm	13 mm
Antialgal	SiO_2	SiO_2@ TiO_2	92	144	Cell wall, cell membrane	Cell wall, cell membrane	76.6%	99.7%
Antiprotozoal	ZnO	ZnO@Ag	20-50, hexagonal	20-50, hexagonal	Cell membrane	Cell membrane	80%	90%
Antiviral	Au	AuNPs@ peptide triazoles	2 Spherical	13-123 Spherical & Hexagonal	Envelope	Envelope	IC50 value is 0.012 µm	IC50 value is 0.02 µm

Though the primary attachment site for the single NPs & CSNPs remain same, core shell nanoparticles show the enhanced antimicrobial activity (Dobrucka and Dlugaszewska, 2018). The size difference between single & core shell potential antiviral & antialgal nanoparticles may be one of the reasons for enhanced inhibitory effects. However material coating can be reason for antibacterial, antifungal & antiprotozoal nanoparticles (Dobrucka and Dlugaszewska, 2018).

CONCLUSION

Nanoparticles such as Au, Ag; oxides such as ZnO, MgO, CuO, TiO_2 are able to bind all types of microbes because of non specific nature of binding. Nanoparticles have

specific & non specific sites for binding because of structure of cell & functional groups present on cell of microorganisms. Nanoparticles get adsorbed on surface of cell or get internalized by cell or both. Binding & toxic effects of nanoparticles on microorganisms depend upon properties such as: cell wall thickness, cell volume, polysaccharide & other organic matter & ability to produce compounds that neutralize or reduce toxic effects & binding of nanoparticles. From the literature data one thing can be concluded that by synthesizing nanoparticles with specific route can have specific binding capacity. As from above tables binding sites composition of microbes is somewhat different and with this knowledge one can predict which nanomaterials are going to bind as well as penetrate. For example, if cysteine residue is with active site binding then gold NMs are very good diagnostics as well as an inhibitor.

Core-shell nanoparticles have extra benefits than single nanoparticles because of either size or additional moiety due to which they are more effective in antimicrobial activity than single nanoparticles. Core-shell nanoparticles can be used effectively for treatment of diseases caused by pathogenic microorganisms. Because of inherent antimicrobial property of nanoparticles, they can be used to deal with multiple drug resistant microorganisms. Therefore, nanoparticles can be considered as next generation antibiotics. If microbes with multiple epitopes then CSNMs are good solution to inhibit as well as diagnosis.

In future there is lot of scope for nanotechnology in the diagnostics of these microbial world and we must focus to is as NMs are economical and ecofriendly if synthesized by green approach.

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ISSN No: 2321-8681
Research Paper
PP: 5-21
Peer Reviewed Journal

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