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Biomolecule stabilized/functionalized nanomaterials: Advanced synthesis strategies, characterization and unique properties as antimicroorganism agent

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Abstract

Nano sized materials has been considered as emerging materials in the field of nanotechnology due to its unique physiochemical and biological properties. Metal nanoparticles and biomolecule stabilized/functionalized nanoparticles are being explored in every part of science and engineering along with medical fields due to its less toxicity and high surface area to volume ratio. Up to present, several kinds simple and green approaches have been investigated for the synthesis of nanoparticles for a wide range of application. However, biomolecule stabilized/functionalized nanoparticles gained much interest in the nano-medicine division as antibiotics due to its broad bioactivity, and large surface area to volume ratio. This feature chapter accounts for advanced synthesis approaches, characterization technique for biomolecule stabilized/functionalized nanoparticles with its activity and mechanism as antimicrobial agent. Initially, we tried to summarize advanced synthesis strategies, and problem associated with the synthesis of biomolecule stabilized/functionalized nanoparticles and advanced characterization technique for biomolecule stabilized/functionalized nanoparticles. Furthermore, we explore the recent progress of antimicrobial performance of biomolecule stabilized/functionalized nanoparticles. In addition, we tried to introduce responsible properties and parameters of biomolecule stabilized/functionalized nanoparticles and their possible mechanism as antimicrobial agent. In the end, we discussed an outlook of biomolecule stabilized/functionalized nanoparticles as antimicrobial agent.

Introduction

Nanoparticles generally referred as particles with a dimension up to 100 nm [1]. Nanoparticles demonstrate completely new properties based on unique characteristics such as size, distribution, surface area and morphology, if compared with larger particles of the bulk materials [2]. Nanoparticles pass with a higher surface to volume ratio with decreasing dimensions of nanoparticles. As unique surface-to- volume ratio of nanoparticles is increased, their effectiveness can increase due to the increase in surface energy [2, 3]. Due to unique properties and application, nanoparticles used in various fields such as catalysis, diagnosis and therapy, drug delivery, antimicrobial, nano device, bio sensor, in soil to agricultural productivity, food safety, solar cells etc. [4-6]. However, the toxic nature, aggregation and less active sites of nanoparticles has limited in application, especially in the field of biomedical [7]. From this point of view, biomolecule capping/functionalizing of nanomaterials can suppress the toxic nature, aggregation of nanoparticles and enhance the biocompatibility and biological activity.

Due to the unique Physiochemical properties, biomolecule stabilized/functionalized nanoparticles played moderate toxicity, though they are relatively inert in its bulk form which can directly interact with protein and enzyme within mammalian cells. Nanomaterials with biomolecules capping/functionalizing have drawn considerable interest in now a days due to their unique properties such as large surface-to-volume ratio, high active edges, high positive charged potentials, the growing microbial resistance against metal ions, and other unique application [8-11]. For the few past decades, nanoparticles/biomolecule conjugated nanoparticles has attracted much attention because of its promising applications such as having unique electrical, optical and catalytic properties, sensors, the most important antibacterial, anti-viral, antifungal activity [4, 12-16].

To synthesis of nanoparticles, biomolecule stabilized/functionalized nanoparticles, there are various methods developed such as spray pyrolysis [17], electrochemical techniques [18], hydrothermal treatments [19], biosynthesis [20], sonochemical method [21], wet chemical methods [22] and chemical reduction method [23] with different morphologies. Among these processes, biomolecule stabilization is usually preferred, because this method is easy, cost-effective and efficient, and it can apprehend improved size and shaped by optimizing the experimental factors [24, 25]. Up to now, various green methods have applied to synthesis of nanoparticles/biomolecule stabilized/functionalized nanoparticles to improve the antimicrobial property.

In this chapter, we have extensively tried to introduce the recent progress of biomolecule stabilized/functionalized nanoparticles for the antimicrobial activity. In the subsequent section, we discussed the synthesis method and characterization of biomolecule stabilized/functionalized nanoparticles. A briefly, we discussed some issues regarding biomolecule stabilized/functionalized nanoparticles synthesis. Furthermore, a brief presentation of case study of antimicrobial activity. A special attention has been given to the parameters and mechanism of biomolecule stabilized/functionalized nanoparticles towards antimicrobial activity. Finally, a summary and outlook included to an essential application biomolecule stabilized/functionalized nanoparticles as antimicrobial agent.

Synthesis strategies and characterization of biomolecule stabilized/functionalized nanoparticles

Nanoparticles gained much popularity among the scientific society from the last few decades due to the wide range of unique application. The properties of nanoparticles largely depend on synthesis method. The synthesis of nanoparticles has been carried out using several kinds of procedure such as chemical, biological, and physical method [22, 26-30]. Basically, two approaches have been considered in all methods for the synthesis of nanoparticles, one is top down synthesis approach where bulk materials broken down to small nanoparticles and another synthesis method is bottom up synthesis approach where the nanoparticles prepared from atom, molecule or cluster. Physical synthesis method is considered in a top down approach. The fundamental scheme for the synthesis of nanoparticles presented in Table 1. Till now, several kinds of strategies has been developed which are considered in physical synthesis method such as laser ablation [31], vapor deposition [32], ball mill [33]. For example, Nine, Md J., et al. 2013 [34] synthesized well dispersed Cu_2O and Cu/Cu_2O nanoparticles by the implement of wet ball mill method, where the Cu particles were hydrolyzed in ball mill equipment with the presence of water. This method can produce different kind of morphology and state of nanoparticles under the ball mill condition.

Table 1: Different useful method for nanoparticles synthesis

Synthesis of nanoparticles		
➢ Top down approach	➢ Bottom up approach	
❖ Physical Method	❖ Chemical Method	❖ Biological method
• Mechanical ball mill	• Microwave reduction	• Using plant extracts
• Vacuum vapor deposition	• Hydrothermal	• Using agriculture waste
• Laser ablation	• Solvothermal	• Using biomolecules
• Ion implement	• Electrochemical method	• Using microorganism
• Spray pyrolysis	• Sol-gel method	• Using algae

On the other hand, the chemical and biological synthesis methods are considered in bottom-up approach. In chemical synthesis method, chemical reduction [35], sol-gel [36], microwave reduction [37], hydrothermal [38], electrochemical [38] methods are gaining much interest among the researcher for the synthesis of nanoparticles. For example, Kruk, Tomasz, et al. 2015 [39] prepared monodispersed Cu nanoparticles by chemical reduction method for antimicrobial activity. In real time experiment, colloidal Cu nanoparticles were synthesized by the reduction of $CuSO_4.5H_2O$ source in sodium dodecyl sulfate-SDS micellar solution in the presence of hydrazine monohydrate as reducing agent. The synthesis reaction was performed at room temperature condition. This method is very effective for the synthesis of uniform particles size and dispersion of nanoparticles.

From the few past decades, the biological synthesis method becomes a popular synthesis method due to the simple preparation method, low toxic, eco-friendly, low cost and most importantly, this method provides several kinds of functional group for the functionalization of nanoparticles. This unique synthesis strategy has been applied to the synthesis of nanoparticles for the application in the biomedical field. Generally, the biological synthesis method utilizes the plant extract [40,41], microorganism [42,43] and organic molecule [44] to stabilize the nanoparticles. By the using of protein, amino acid from plant extract or microorganism as reducing agent, the nanoparticles can be formed with uniform controlled size, shape and homogeneous dispersion. This method also offered the less synthesis time, stability and ready dispersion of nanoparticles in water [27]. In biological synthesis method, various extracts of plant such as

Eclipta prostrata [45], Citrus medica Linn [46], Azadirachta indica [47], Emblica officinalis [48], Aloe vera [49], several microorganisms, including Neoscytalidium dimidiatum [50], Bacillus brevis [51], Phenerochaete chrysosporium [52], Pseudomonas sp. [53]. Not only the plant extracts and microorganism but also the biomolecule such protein [54], amino acid [55], starch [56], polymers [57] can be stabilized and capped the nanoparticles with uniform dispersion, controlled size and shape. The main advantage of the biological synthesis method is the presence of proteins, amino acids, or secondary metabolites in the biological sources, which act as the stabilizing, capping agent and functionalizing of nanoparticles with uniform size and shape by avoiding the particle aggregation.

Recently, Jayarambabu, N., et al. 2020 [58] synthesized Cu nanoparticles using Curcuma longa extract as stabilizing and capping agent. In synthesis method, the mixed copper acetate dehydrates and C. longa extract solution treated under the microwave irradiation for 180s at 200W. The formation of copper nanoparticles was confirmed by the change of color from yellow to brick brown. This C. longa extract solution stabilized the nanoparticles with uniform morphology and particles size. Ulaeto, Sarah B, et al. 2019 [59] accounted Ag nanoparticles using neem extract as stabilizing agent for antimicrobial activity. In synthesis method, initially, the Ethanolic Extract of Neem Leaves (EENL) was synthesized by stirring of dried neem leaves in ethanol/water solution and followed by filtration. After that the EENL and silver nitrate solutions stirred for 24hr at dark condition to obtain the Ag nanoparticles. Finally, the mixture was centrifuged, washed and freeze dried to obtain the silver nanoparticles as powder form. The O-C=O, C-O, -C=O, C-NH_2, H-N-C=O functional groups in neem having high affinity towards silver ion to reduce, and stabilize the Ag nanoparticles. This neem leaves extract can be stabilized nanoparticles with smaller size, controlled shape and crystalline structure. In another investigation, Cu nanoparticles was synthesized using Syzygium aromaticum bud extract as capping and stabilizing agent [60]. First, the Syzygium aromaticum bud extract was prepared by boiling at 80 ^{0}C in the water and followed by filtration. Later, the solution of Syzygium aromaticum bud extract and cupric acetate stirred at 30 ^{0}C, yielding Cu nanoparticles. Syzygium aromaticum bud extract stabilized Cu nanoparticles with high crystalline structure and uniform morphology. The protein molecules inSyzygium aromaticum bud extract act as the surface coating agent to stabilize and avoid the agglomeration of nanoparticles. The protein molecules inSyzygium aromaticum bud extract containing the lots of functional groups -C-H, -C-N, secondary amines, -C=C-, C=O in amide linkage, and Amide-I. This kind of functional group having high affinity to a metal ion, which are the responsible sites for nanoparticles formation.

In short, the biomolecules from natural sources containing a lot of functional groups which having high interaction to metal ion and act as reducing, capping and stabilizing agent for nanoparticles. The functional groups in natural biomolecules can be stabilized nanoparticles with small in size, controlled shape and uniform dispersion, which are highly active for biological activity. The bio molecule stabilized can be characterized by using different kind of techniques. Table 2 summarized the characterization technique for nanoparticles.

Table 2: Some useful characterization technique for nanomaterials characterization

Technique for characterization	Characterization properties
Scanning electron microscopy	Particle size, shape and morphology
Transmission electron microscopy	Particle size, shape, materials phase, morphology
X-ray diffraction	Solid state properties such as structure, composition and physical properties of materials
UV-visible spectroscopy	Quantitative determination of analysts, such as metal ion, organic compound, biological macromolecules
Laser light scattering	Sample homogeneity, particle size, aggregation
X-ray photoelectron spectroscopy	Elemental composition, empirical formula, chemical state, electronic state

Antimicrobial activity and mechanism

For the few past decades, biomolecule stabilized/capped/functionalizednanoparticles have been extensively explored as an antimicrobial agent against a wide range of microorganism such as gram-positive and gram-negative bacteria, virus, fungi and so on. The antimicrobial property of nanoparticles largely depends on different kind of properties such as capping agent, the dose of nanoparticles, particle size, morphology, etc. At this point of view, biomolecules serve as a stabilizing/capping/functionalizing agent and produce nanoparticles with uniform size, morphology and controlled shaped, which greatly influence the antimicrobial property.

Jaiswal, Swarna, et al. 2010 [61] investigated the role of biomolecule capping agent of nanoparticles as an antibacterial agent. To investigate the role of capping agent, silver nanoparticles were capped using β-cyclodextrin (β-CD) biomolecule. β-CD capped silver nanoparticles exhibited significantly higher antimicrobial activity than uncapped silver nanoparticles. The β-CD capping agent forming smaller particles, which have a high surface-to-volume ratio and provide higher interaction with the bacterial cell surface. The β-CD also promote the release of silver ions, which increase the intimate contact between silver nanoparticles and bacteria, resulting the bacterial degradation.

In another study, Ahmad, Aftab, et al. 2017 [62] demonstrated the impact of surface modification of silver nanoparticles using biomolecule for antimicrobial activity. Chitosan biomolecule was used to modify the silver nanoparticles surface. Chitosan modified silver nanoparticles exhibited higher activity against gram-positive and gram-negative bacteria than non-modified silver nanoparticles due to the presence of high positive surface potential of chitosan modified silver nanoparticles. The positively charged chitosan modified nanoparticles having the better surface for bacterial attachment. The positive charge in a chitosan modified silver nanoparticleselectrostatically binds negatively charged lipopolysaccharide in gram-negative bacteria cell and teichoic acid in gram-positive bacteria cell surface, resulting the cell permeability, cytoplasmic leakage and cell death of bacteria.

Recently, Du, Juan et al. 2019 [63] synthesized green silver nanoparticles using Forsythia suspensa fruit extract as stabilizing and capping agent and investigated the activity and mechanism of green silver nanoparticles against food-borne pathogen. The antibacterial results revealed that silver nanoparticles exhibited very strong activity against various food-borne pathogens including S.aureus (Gram-positive) and V.parahaemolyticus (Gram-negative). The S.aureus (Gram-positive) and V.parahaemolyticus (Gram-negative) can be inhibited by incorporating the different dose of silver nanoparticles. The antibacterial activity was increased with the increasing amount of the silver nanoparticles dose. During the antibacterial action using silver nanoparticles, the nanoparticles interact with bacterial cell and release the nucleic acid from the cell, resulting the disturb of normal function of cells and leading to death. In case of cell thickness of bacteria, gram-positive bacteria having a thick layer of peptidoglycan, which provide more stability of gram-positive bacteria than gram-negative bacteria.

Tailoring size and shape of nanoparticles greatly influence the activity of nanoparticles against microbial. Kumari, Madhuree, et al. 2017 [64] investigated the shape and size effect of biosynthesized silver nanoparticles against MDR bacteria. The cell free extract of T.viride was used to synthesis of silver nanoparticles for antimicrobial activity. The cell free extract of T.viride can produce different size and shape of nanoaprticles by varying the pH, reaction time and temperature. 2-5nm spherical shaped nanoparticles exhibited maximum antimicrobial activity against different microbial among all synthesized sizes and shaped controlled silver nanoparticles. 2-5nm spherical shaped nanoparticles were synthesized at 30^0C, pH 7 for 24hr incubation in the presence of cell free extract of T.viride as capping agent. The smallest sized nanoparticles showed best activity may be due to the smallest particles having large surface-to-volume ratio, which expose the high area to interact the microbial cell [27].

Copper based nanoparticles has been extensively studied for bio catalytic application, especially as an antimicrobial agent due to its strong toxicity against microorganism [65]. Shewanella loihica PV-4 culture medium was used for synthesis of copper nanoparticles as antimicrobial agent [65]. This biosynthesized copper nanoparticle exhibited high activities for bacterial inactivation. Copper nanoparticles attract both outside and inside of E. coli cell and damaged the function of cell membrane and cytoplasm by chemically or physically. During antimicrobial process, the organic matter in E. coli damaged by chemical oxidation. The nanoparticles bind thiols to generate reactive oxygen species, which is the responsible for chemical oxidative stress and cell damage of bacteria through the penetration of nanoparticles into the cell.

Not only the metallic nanoparticles but also the oxide based nanoparticles are highly active as antimicrobial agent. Nabila, Mohammed Ishaque, 2018 [66] prepared copper oxide nanoparticles as antimicrobial agent. Theactinomycetes mediated biosynthesis of copper oxide nanoparticles exhibited high activity against microbial. The inhibition of bacteria was increased with the increase of dose of copper oxide nanoparticles. Even cooper oxide nanoparticles exhibited higher activity than Ciprofloxacin antibiotics. The copper oxide nanoparticles highly reactive due to the large surface area of copper oxide nanoparticles. During antimicrobial process, the death of bacteria occurred due to the high surface-to-volume ratio of copper oxide nanoparticles, which permits the nanoparticles to interact with the cell membrane of the bacteria.

Furthermore, biosynthesized magnetic nanoparticle having a potential effect against microorganism due to its electromagnetic effect towards microorganism. In 2017, Patra, Jayanta 58 [67] prepared magnetic iron oxide nanoparticles using aqueous extracts of food processing wastes as capping and stabilizing agent. The aqueous extracts of food processing wastes synthesized magnetic iron oxide nanoparticles exhibited strong activity against foodborne pathogens. In antimicrobial mechanism, the magnetic iron oxide nanoparticles are having positive charges and microorganism having negative charges, which create the electromagnetic interaction between the microorganism and nanoparticles, resulting the death of the bacteria by the oxidation of microorganism.

Carbon based materials having a significant effect towards bio application [68], electrochemical [69], photochemical application [70], energy conversion and storage devices [71] and so on due to its high surface area, high mechanical strength, thermal and electrical conductivity, and optical properties. Carbon based materials supported metal composites gained much interest as antimicrobial agent. Linh, Ngo Thi Yen, 2017 [72] synthesized silver nanoparticles decorated porous reduced graphene oxide by green synthesis approach as an antimicrobial agent. The silver nanoparticles decorated porous reduced graphene oxide showed excellent activity against microorganisms. To find out the role of graphene oxide and reduced graphene oxide as an antimicrobial agent, Gurunathan, Sangiliyandi, et al. 2012 [73] demonstrated the antimicrobial activity of graphene oxide and reduced graphene oxide against Pseudomonas aeruginosa and proposed a possible antimicrobial mechanism. The graphene oxide and reduced graphene oxide exhibited strong antimicrobial activity. During antimicrobial activity, graphene oxide and reduced graphene oxide produce reactive oxygen species and the resulting cell death by altering the cellular redox status. In case of reduced graphene oxide, the reduced nanowalls are more sharpening of the edges which provide high interaction and charge transfer between the reduced graphene oxide and bacteria cell, resulting the more cell death of bacteria.

Summary and outlook

This chapter has introduced advanced synthesis strategies, characterization technique and properties of biomolecule capped/functionalized nanomaterials as antimicrobial agent. The useful biomolecules such as protein, lipid, peptides, glucose, sucrose, saccharides, carbohydrates, nucleic acids from natural sources have high interaction with metal ions and significantly improved the antimicrobial activity. By applying suitable preparation procedure for capping/functionalizing nanoparticles, it is very easy to synthesis of nanoparticles with controlled phase, size, shape, surface area, a potential functional group containing materials for antimicrobial agent. There are several kinds of parameter associated with antimicrobial agent to boost up the activity such as the smallest particle size which can be controlled by using biomolecules as stabilizing and capping agent and specific doses of nanomaterialsprovide high surface-to-volume ratio for direct contact of nanoparticles towards inside and outside of microorganism and damaged microorganism cell by chemical oxidation or physically.

The nanoparticles shapes, active edges and surface area, which increase the interaction between nanoparticles and microorganism, resulting the damage of microorganism membrane cells and DNA by reactive oxygen species production.

Increasing the positive potentials charged of nanoparticles with introducing biomolecules on the nanoparticle surface which increase the intimate contact between nanoparticles and microorganism by electrostatic bonding between positively charged nanoparticles and negatively charged molecules in microorganism, resulting damage of microorganism cell.

The high oxidation state of metal nanoparticles can be synthesized by using natural biomolecules as reducing agent which provides high interaction towards negatively charged molecules in microorganism and make oxidative stress and the production of reactive oxygen species, resulting the death of the microorganism.

It is evident that biomolecule from natural sources stabilized and functionalized nanomaterials are promising candidate as antimicrobial agent. In order to take advantage of the noble parameter of antimicrobial agent, it is still required to explore more about the mechanism of biomolecule stabilized and functionalized nanomaterials, together with appropriate parameter will allow to create more suitable design functional nanomaterials as antimicrobial agent.

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