Chapter

Nanoparticle Synthesis, Applications, and Toxicity

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Abstract

Nowadays production of different nanoparticles (NPs) with plausible biomedical benefits is tremendously increasing. NPs are of great interest in drug delivery systems, drug formulation, medical diagnostic, and biosensor production. Aside from the importance of NPs in medicine, their negative side effects including potential cytotoxicity, inflammatory response induction, and drug interruption should be carefully considered. Several molecular and physicochemical mechanisms are involved in toxicity induction of NPs. Finding the negative effects of NPs on human tissues and investigation of their mechanism of action are a way for preventing the happening of unpleasant event. Here in this work, we would describe the main way of NP production with special attention to green NP production, and then their application in medical diagnosis and disease treatment would be explored. Also the main toxicity effects of NPs on different tissues would be explored, and the parameters affecting the quality of NPs and their corresponding biological properties would be highlighted.

Keywords: nanoparticle, biomedical, drug delivery, cytotoxicity, medical diagnostic

1. Introduction

Nanotechnology is referring to the technology of production, characterization, and application of materials in nanoscale [1]. After the definition of this term by Norio [2], nanoparticle (NP) production and application in several different fields gain much attention. The small size and high surface area of NPs are the causes of their tunable physicochemical properties such as improved thermal conductivity, light absorbance, significant chemical stability, and high catalytic activity [3]. Furthermore the surface layer of NPs can be functionalized using chemical and biological agents like small molecules, surfactants, and polymers for enhancing their activity and specificity [4]. It is revealed that each NP, regarding its size, surface charge, shape, surface groups, and type of ions, shows unique biological and physicochemical properties [5, 6]. Owing to such diversity, these materials got immense biomedical applications such as drug delivery, radionuclide therapy, biosensors, cancer therapy, diagnostics, magnetic resonance imaging (MRI), and biological molecules purification [7, 8]. Although a wide variety of NPs with diverse ions and surface modifications are produced and preclinically tested, only a limited number of them gain approval for clinical uses. The long-term stability, general cytotoxicity and inflammatory response induction, and lack of guideline for relevant biological testing are the main reasons for low-approved NPs [6]. Due to the fast development of NPs, it is necessary to identify correlation between the physical and chemical

attributes of NPs and their corresponding biological effects. For instance, it is shown that although the positive charge of NPs enhances the efficacy of gene delivery, and imaging, it also enhances the cytotoxicity of corresponding constructs [9]. In this chapter we briefly introduce the main way of NP production and their applications in biological and medical studies. Also the mechanism of cytotoxicity induction and the main ways of detecting this toxicity are explained.

2. NP production methods

2.1 Physicochemical methods

NPs can be produced through two main approaches including top-down approach which is the production of NPs by making smaller and smaller structures by etching the bulk agents and bottom-up approaches which is the building up of NPs from atoms [6]. Physical, chemical, biological, and in some cases hybrid technique are the main ways of NP production. The physical methods of NP production include methods like laser ablation, high-energy irradiation, spray pyrolysis, and ion implantation, and the chemical one includes chemical reduction technique, sonochemical method, solgel process, microemulsion method, and electrochemistry. The biological method which is also called green NP biosynthesis involves application of plants extracts, microorganisms, enzymes, and even some agricultural wastes for NP production. Although the physical and chemical methods resulted in bulk amount of NPs a few times, application of chemical agents during the NP production in coordination with production of environmentally dangerous compounds simultaneously with NP production limited their applications [10]. For instance, thermolysis which is a chemical method for dissociation of organometallic precursors is performed at high temperatures by using organic solvents. Also in some cases, surfactant is added to the reaction medium for reducing coalescence of particles [11]. Chemical reduction technique is an adopted chemical method which used a wide range of reducing agents such as sodium borohydride, hydroxylamine, and N,N-dimethylformamide for production of zerovalent ions. Wave-assisted chemical method used ultrasonic waves in coordination with surfactant or reducing agent for production of NPs. The formation of micro cavities with high temperatures upon ultrasonic induction can start chemical reduction of substrates. The physical NP production methods are mainly energy intensive and need special devices. For instance, milling process is a way by which metallic microparticles are crush using high-energy ball mills. The gas-phase process or aerosol process which is divided into four main types (including flame reactor, plasma reactor, laser reactor and hot wall reactor, and chemical gas-phase deposition) is a particular way for the production of NPs like fullerenes and carbon nanotubes. All types of these methods need special devices and are mainly high energy consuming. NP production by wet chemical synthesis takes place at low temperature and is one of the most employed methods for NP production. Limitations in increasing batch reactor because of limited mixing and low heat transfer are mentioned as the main disadvantage of wet chemical synthesis method. The main advantages and disadvantages of NP production by physicochemical methods are summarized in **Table 1**.

2.2 Green NP production methods

Green technology using biological systems like plants, microorganisms, and enzymes is rising fast as an alternative method for conventional chemical and

Method	Advantages	Disadvantages	Ref.
Chemical vapor deposition and chemical vapor condensation (CVD and CVC)	• High pure NPs production	• High temperature of procedure (above 300°C) • Uses of chemical agents	[12]
Gas condensation	• Production of ultrafine nanocrystalline metals and alloys	• Need for special devices • Extremely slow	[13]
Laser ablation	• High-purity NP production	• Need special devices • Difficult to control size, agglomeration, and crystal structures	[14]
Solgel	• Simple method • Production of large range of materials • Uses low temperature	• Using chemical agents • Undesirable agents production • The cost of materials may be high	[15]
Chemical reduction	• Cost-effective • Good production rate	• Application of toxic agents • Hazardous by product formation	[11]

Table 1.
The main physicochemical methods of NP production and their corresponding advantages and disadvantages.

physical. In contrast to physiochemical methods which mainly lead to environmental toxicity, the biological NP production methods are known as eco-friendly and nontoxic protocols [16]. The biologically produced NPs' special features including high catalytic activity, low toxicity contaminations, high stability, and plausible biocompatibility and biodegradability make them distinctive from NPs produced from other methods. The microorganism's related NP productions are classified into intracellular and extracellular synthesis methods [17]. In an intracellular way, ions of interest are transported into the microbial cell and then reduced in the presence of enzymatic processes, while the metal ions are entrapped and reduced at microorganism's surface in an extracellular way [18]. Microbial NP production regarding the ability of the majority of bacteria and fungi in tolerating ambient conditions of varying temperatures, pH, salt concentrations, and pressures makes this approach a safe, cost-effective, and environmental method. Several microbial species have been isolated from different environments and used for production of various NPs. Compared to microbial production method, plant NP production is more desirable because it does not need any special and multistep processes, it has faster production rate, and it has easy scaling up procedure and because of its cost-effectiveness [19]. Investigations have revealed that metals bioaccumulated in plants which sometimes are called phytomining are mainly in the form of NPs. For instance, high level of silver NP accumulation in *Brassica juncea* and *Medicago sativa* [20], gold NP production in *M. sativa* [21], and copper NP accumulation in *Iris pseudacorus* [22] has been reported. This type of NP production has several disadvantages including heterologous size and morphology of NPs, difficult extraction and isolation procedure, and low production yield [21]. The alternative approach is in vitro production method which is based on reduction of ions using plant extracts. This method is more controllable through making change in plant extract and ion concentration, time of reaction, temperature, and pH of reaction medium. The production rate of this method is much faster and easier than in vivo method [23, 24]. For example, the

extract of *Tectona grandis* seeds was used for reduction of AgNO3 to 10–30 nm Ag NPs with significant antibacterial properties [25], whereas Au NPs with an average size of about 3 nm have been synthesized using leaf extract of *Ziziphus zizyphus* [26]. Various plant extracts have been used for production of NPs from different ions with diverse sizes and shapes [27]. **Table 2** summarizes some examples of NP production through a biological way.

Biological entity	Type of NPs	Size (nm) and shape	Special characteristics	Ref.
Bacteria and fungi				
Delftia sp. SFG	Bi	Sphere/40–120	Antibiofilm activity against *P. aeruginosa*	[28]
Escherichia coli	CdS	Spherical/2–5		[29]
Botryococcus braunii	Cu, Ag	Sphere/10–100	Antibacterial and antifungal effects against *Pseudomonas aeruginosa (MTCC 441), Escherichia coli (MTCC 442), Klebsiella pneumoniae (MTCC 109) and Staphylococcus aureus (MTCC 96), Fusarium oxysporum*	[30]
Yeast strain MKY3	Ag	Hexagonal/2–5		[31]
Fusarium oxysporum	Ag	25–50		[32]
Bacillus mojavensis	Ag	105	High antibacterial activity against multidrug resistant pathogens	[33]
Aspergillus fumigatus BTCB10	Ag	41	Antibacterial and cytotoxic effects	[34]
Plants				
Apple extract	Ag	22–30	Great antibacterial effects against *Geobacillus stearothermophilus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*	[35]
Lavandula vera	Zn	60–80	Valuable antibacterial and anti-biofilm activity	[36, 37]
Psidium guajava	Se	Spherical/8–20	Antibacterial effects	[38]
Cassia alata	ZnO	60–80	Antibacterial effect against *Escherichia coli*	[39]
Gnidia glauca and Plumbago zeylanica	Cu	Spherical/1–5	Good antibacterial effects	[40]
Andrographis paniculata	Ag	54	Good antifungal activity	[41]
Cassia fistula	Au	55–98	Hypoglycemia treatment	[42]
Enzyme and other biological agents				
Melanin	Cu	Spherical/66	Good antibacterial activity against *E. coli* and *L. monocytogenes*	[43]
Horseradish peroxidase	Au	10	Detection of low concentrations of phenylhydrazine	[44]
Macerating enzymes	Ag	Hexagonal/38	High antibacterial effects	[45]

Table 2.
Some examples of biologically produced NPs and their corresponding special characteristics.

3. NP biomedical applications

With respect to special properties of NPs discussed before, they have various applications. Here we investigate some of these applications with special look at their uses in biomedical fields.

3.1 Drug delivery

NPs are of great interest for being used as a device for site-specific drug delivery with optimum dosage drug release. Current NP-based drug delivery approaches focused mainly on enhancing drug shelf life though improving drug uptake efficiency [46]. NP-based drug carriers are able to cross the blood-brain barrier and tight junctions of the skin epithelial tissue [47]. Also they improve hydrophobic molecule solubility and increase stability of biological therapeutic agents.

NPs enabled us to deliver drugs by various routes including nasal mucosa and oral administration, aerosol method, and topical vaccination. The aerosol technology is used for respiratory disorder drug delivery. Target drug delivery approaches using magnetic NPs are widely being used for cancer therapy, gene therapy, MRI, and cell sorting [48, 49]. For instance, Fe_3O_4, γ-Fe_2O_3, and super magnetic iron oxide NPs (SPIONs) are the main NPs used for site-specific drug delivery. The surface properties and particular shape of fullerenes and carbon nanotubes make them attractive for drug delivery. These particles are such small that can pass through cell membrane and deliver agents like DNA and protein into the cells [50, 51].

3.2 Antibacterial agent

The prevalence of antibiotic-resistant bacteria species becomes a threat for human health. NPs with significant antibacterial properties and no bacterial resistance are the best alternative for common antibiotics [52]. Ag NPs are the leading NP-based antibacterial agents with significant bactericidal effects on both Gram-negative and Gram-positive bacteria [53]. Every day various NPs with different physicochemical properties and bactericidal activities have been developed, and their mechanism of action and potential side effects are under investigations. Also application of common antibiotics such as ampicillin, chloramphenicol, and kanamycin in the presence of NPs demonstrated the positive effects of this combination. Previous studies showed that NPs can be used as a vehicle for antibiotic delivery. The attachment of NPs to the bacterial surface and induction of damages are reported as the main mechanism bacterial death with NPs [54, 55]. Interaction of NPs with bacterial cell membrane and disruption of its normal function are the most common way of NP bacterial killing. NPs are also able to hindrance bacterial biofilm formation. Furthermore, NPs are able to produce different types of ROS species. For example, Mg NPs are able to produce O^{2-}, and ZnO NPs produce H_2O_2 and OH. These ROS species interact with bacterial cells and cause acute stress reactions and finally lead to acute microbial death [56, 57].

3.3 Biosensor

The optical and electronic properties of NPs make them suitable for biosensor application. The size, type of ion, and shape of NPs are critical parameters affecting SPR peaks and line widths of sensor. The noble metals like Au, Ag, and Pt NPs showed special physicochemical features which make them the most popular components of NP-based biosensors [58]. NPs have different roles in any types

of biosensors. For instance, electrochemical biosensor is performed by fixing the potential at a suitable value and determining the current changes versus time. The role of NPs in this type of biosensor is to improve sensitivity and signal detection [59]. In optical biosensors, the free electron oscillation in conduction bands of some metals (Ag, Au, Cu) interacts with light photons and produces a polariton. Size tuning of plasmonic metals is a way for enhancing surface plasmon resonance and making the device suitable for biomedical applications. Using NPs leads to reaching to highest detection sensitivity. Au NPs because of their easy functionalization and showing different colors based on their size and shape are good choices for colorimetric biosensor, plasmonic sensing, immune sensors, and electrochemistry [60]. The Au NPs showed unique stability compared to other metals when used for bio-conjugation production and have valuable sensitive plasmon change which lead to their wide use in classic immunoassays. The stronger Raman and fluorescence enhancement of Ag NPs than Au NPs resulted to their broad uses in optical applications [61]. Also they can easily be oxidized and be used in electrochemical sensors. Ag NPs with ability to detect proteins have been used for cancer detection. Furthermore they were also used for detecting glucose, DNA, dopamine, ascorbic acid, and several other biological molecules. Magnetic NPs are used in sensors through three main approaches including pre-concentration of analyte, magnetic tags, and integration into transducer materials [62, 63].

3.4 Diagnostic agent

The special features of NPs such as fluorescence properties, optical scattering and electromagnetic field enhancement, and even transferring light energy to heat resulted in a wide application of these compounds in medical diagnostic field. Furthermore, NPs are excellent carriers for delivery of active biomedical agents. Biomedical imaging is one of the useful tools for human disease diagnosis. The NPs with special optical, magnetic, and radioactive properties can enhance the quality of imaging. It is possible to functionalize NPs with multiple modals and by this way minimize the interface between each modality and provide multimodal agent for better imaging [64]. The optical nano-probes can be designed for being used in linear optical imaging with high-emission quantum energy yield and expanded optical capacity. In the case of fluorescent imaging, the degradation of organic dyes (photo bleaching) and metal complexes under light exposure is alleviated with fluorophore-doped silica NPS. These NPs have been used for untargeted imaging of human epithelial cells of the cervix and targeted imaging of cells A549, HeLa, and HepG2 [65, 66]. Phosphorescence imaging using NPs produced images with lower background autofluorescence and scattered excitation light in the spectral range. Magnetic resonance imaging (MRI) used contrast agents for detection of small tumor and lesions in a normal tissue. The NPs with magnetic functionality are used in MRI, and the ones with larger magnetic moment are preferred [67].

3.5 Catalytic agent

NPs have been developed for various catalytic applications. The NP catalytic reactions have several advantages including low reaction temperature, light transparency, and easily immobilization on solid supports, for instance, the catalytic activity of Au NPs in degrading methylene blue demonstrated by Khan et al. [68]. Also the effect of geometrical parameters of supported Au NPs on its carbon monoxide oxidation has been evaluated. The NPs with an average diameter of 2 nm and height of six atomic monolayers showed optimum catalytic activity [69]. The Au NPs on amorphous silica support produced by Mukherjee et al. were able

to catalyze hydrogenation of cyclohexene [70]. The Ag NPs produced by lychee (*L. chinensis*) extract showed significant photocatalytic activity even after three times of reusing [71].

3.6 Wound healing activity

NPs are a suitable wound dressing agent because of their valuable antibacterial, anti-inflammatory effects and ability to accelerate skin reepithelialization. Reports of healing effects of Ag NPs indicated that these nanoscale materials decrease local matrix metalloproteinase and neutrophil apoptosis. Also they showed inhibitory effects on pro-inflammatory cytokines interferon gamma and tumor necrosis factor alpha [72]. The combination of Ag NPs and collagen results in the formation of component with suitable antibacterial activity. Au NPs do not have any antibacterial effects alone, but their combination with biological agents like collagen and gelatin improve their biocompatibility and biodegradability and make them suitable for wound dressing. Au NP antibacterial properties resulted from their interaction with cell membrane and inhibiting ATP synthase which consequently lead to ROS-independent cell death. The reports indicated that the combination of Au NPs, gallate, and epigallocatechin has positive effects in healing of mouse skin wounds through regulation of angiogenesis and anti-inflammatory effects [73]. Pd, Pt, Se, and ZnO are other promising NPs for regenerative medicine and wound healing. The PAPLAL® solution (Toyokose Pharmaceuticals, Japan) (Shibuya et al. 2014) which is a mixture of Pd NPs and Pt NPs showed protective effects against aging-related skin pathologies and normalized the gene expression levels of Mmp2, Has2, TNF-α, and IL-6 in the skin [74].

Zn NPs have valuable antibacterial effects, and its topical application leads to reduction of inflammation and improvement of skin reepithelialization. TiO_2 NP wound treatment enhances body fluid coagulation by making interaction with blood proteins. The formation of adherent crust of a nanocomposite improved healing of wound and inhibited infection and inflammation [75].

Also nanotechnology can be used for delivery of active agents with antimicrobial, anti-inflammatory, and healing effects. Curcumin treatment of diabetic wounds leads to significant enhancement in reepithelialization and an increase in fibroblast proliferation of injured tissue. Curcumin NPs not only have higher lifetime than curcumin but also showed valuable antibacterial effects against methicillin-resistant *Staphylococcus aureus* [76]. With respect to molecular chemistry and self-assembly approaches, it is possible to develop peptide NPs with a variety of medical applications.

Polymeric NPs using both biological and synthetic polymers are of great interest for the development of wound dressing compound. Polymeric NPs are able to stimulate cell proliferation through enhancing angiogenesis and reepithelialization. They are able to stimulate the infiltration of inflammatory cells in the initial phase of healing. Furthermore, they are suitable carriers for therapeutic agents including cytokines, growth factors, and antibiotics which make them suitable for being used in treatment of both normal and delayed infectious wounds [77, 78].

4. NP toxicological consideration

Regarding the extensive uses of NPs in foods, paper, drug delivery, biosensor, cancer therapy, and imaging, looking for possible toxicity and long-term exposure side effects and finding the mechanism underlying the adverse effects of NPs seem necessary.

Any toxicity induction of NPs is strongly related to NP base material, shape, size, and functional groups coated at their surface. The smaller NPs have a larger specific surface area which in turn leads to higher interaction cell components including nucleic acid, proteins, and carbohydrates. Also the smaller NPs can penetrate better into cells and interact higher with cells. Surface charge of NPs has strong correlation with their interaction with cells and absorbance. It is showed that the NPs with higher positive charge have higher cytotoxicity effects. Also NPs with positive charge are more toxic than negatively charged NPs [79]. The shape of NPs is the other critical parameter which largely affects their cytotoxicity and antiproliferative effects. For instance, the amorphous TiO_2 NPs produced higher levels of oxidative stress and cell surface defects than anatase TiO_2 NPs. Also the spherical Fe_2O_3 NPs had lower cytotoxic effects than rod-shaped ones. Also cytotoxicity is strongly dependent on the type of cells. For instance, although citrate-capped gold NPs were nontoxic to human liver carcinoma and hamster kidney cells, they were severely toxic to human carcinoma lung cells [80].

NPs can easily penetrate into the cells and interact with cells' normal functions. ROS formation and consequently oxidative stress induction are the common side effects of metal NPs. The produced ROS disrupt normal cell function through attacking essential biological molecules including DNA, enzymes, and lipids. Peroxidation of membrane lipids; enhancing calcium entrance; release of calcium from intracellular stores, protein kinase C, and mitogen-activated protein kinase activation; and DNA damages are some of the main changes that lead to cell death after interaction with NPs [81, 82]. Furthermore the risk of early apoptosis upon exposure to some NPs such as ZnO and TiO_2 has been demonstrated. Also CuO, NiO, TiO_2, Fe_3O_4, ZnO, and Al_2O_3 NPs can arrest cell cycle and induce apoptosis. It is demonstrated that the phase of cell arrest depends on the type of cell and NPs. G2/M phase arrest is the most common type of cell arrest induced by metallic NPs. The induction of P53 pathway in NCM460 cells and cyclin-dependent kinase 1 downregulation in HaCaT cells after exposure to ZnO and cyclin B1 downregulation in A549 cells by TiO_2 have been reported as the main causes of cell proliferation disruption [83, 84]. Many researches have been done on different cell lines and animal models for finding the mechanism of NP toxicity and physiological changes. The high absorption of gold NPs and their aggregation inside cells are probably the main cause of gold NP toxicity.

Argyria is a condition of the skin and other organs' blue-gray discoloration as a result of long-time exposure to high levels of Ag NPs. Irritation, stomach pain, allergic reactions, and inflammation are reported as the main side effects of body exposure to high levels of Ag NPs [85]. TiO_2 and ZnO NPs are widely being used in cream and lotions as sunscreen or materials for water- or stain-repellent properties. The cytotoxicity induction of TiO2 NPs through increasing reactive oxygen species and lactate dehydrogenase has been demonstrated [86]. It revealed that UV and visible light irradiation enhanced ZnO NP cytotoxicity power [87]. Also the Zn NPs were produced by microwave-assisted method, and its in vivo cytotoxicity and levels of distribution in different tissues have been evaluated. According to the obtained results, the produced Zn NPs were classified as nontoxic agents with highest distribution in the testis, liver, and brain [37].

Several assays have been developed for the determination of NP toxicity both in vitro and in vivo. Proliferation assay which measures the active cell metabolism is the most popular method for determining the antiproliferative potency of NPs. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), XTT, thymidine incorporation, alamar blue, and clonogenic assay are the most popular methods for determining cell proliferation rate [88, 89]. DNA damage and apoptosis induction of NPs which are mainly due to generation of free radicals can be

determined by methods like annexin V, comet assay, DNA laddering, and TdT-mediated dUTP-biotin nick end labeling (TUNEL) [90, 91]. NPs are able to interact with cell membrane and lead to cell integrity destruction and cell death. This phenomenon which is known as cell necrosis is mainly measured by neutral red and trypan blue exclusion assays [92, 93]. The in vivo assays including biochemical tests, histopathological analysis, hematology, and NP bio-distribution are also used for finding the effect of NPs on normal function of cells and tissues [94].

5. Conclusion

NPs are becoming the spreadable part of medicine, and their uses are increasing every day. They exhibited promising biomedical uses regarding their special redox potentials, small sizes, high surface area, optical scattering, and fluorescence. Due to special biological effects of these compounds including significantly high antibacterial and antiproliferative effects against a wide range cells, their production and surface modification are increasing for reaching more effective agents. Besides they are able to be used as delivery devices for dispensing drugs and biological agents to specific sites. Owing to the advances in generation of multifunctional NPs, application of NP-based platforms is significantly increasing. While all NPs showed some degree of success in laboratory tests and some of them are now on the market, considering their potent environmental and biological side effects is necessary. Although several researches demonstrated the toxicity of different NPs, the cause of toxicity is mainly unknown. Any NP has its special toxicological characteristics, and there is not a comprehensive method for calculation or grading different NP toxicity. Production of NPs through methods with lowest dangerous side products, optimizing the NP production protocols, and doing both in vitro and in vivo tests of toxicity are the main steps toward production of NPs with lowest negative effects on the environment and human health. Short- and long-term toxicities of NPs and their pharmacokinetic and pharmacodynamic tests should be evaluated for FDA approval.

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